Regular exercise improves cardiac contractile activation by modulating MHC isoforms and SERCA activity in orchidectomized rats

Pavaran P, Wattanapermpool J. Regular exercise improves cardiac contractile activation by modulating MHC isoforms and SERCA activity in orchidectomized rats. J Appl Physiol 119: 831–839, 2015. First published August 13, 2015; doi:10.1152/japplphysiol.00224.2015.—Data from the trial known as Testosterone in Older Men with Mobility Limitations (TOM) has indicated an association between testosterone administration and a greater risk for adverse cardiovascular events. We therefore propose that regular exercise is a cardioprotective alternative that prevents detrimental changes in contractile activation when a deficiency in male sex hormones exists. Ten-week-old orchidectomized (ORX) rats were subjected to a 9-wk treadmill running program at moderate intensity starting 1 wk after surgery. Although exercise-induced cardiac hypertrophy was observed both in rats that underwent ORX and sham surgery, regular exercise enhanced cardiac myofilament Ca\(^{2+}\) sensitivity and myosin light-chain 2 phosphorylation only in rats that underwent a sham operation. Although the rats that had sham surgery and and given exercise exhibited no change in maximum developed tension, regular running prevented the suppression of maximum active tension in the hearts of ORX rats. Regular exercise also prevented a shift in myosin heavy chain (MHC) isoforms toward β-MHC, a reduction in sarco(endo)plasmic reticulum Ca\(^{2+}\)-ATPase (SERCA) activity, and an increase in SERCA sensitivity in the hearts of ORX rats. Neither SERCA content nor its modulating component, phospholamban (PLB), was altered by exercise in either sham-operated or ORX rats. However, decreases in the phosphorylated Thr\(^{17}\) form of PLB and the phosphorylated Thr\(^{287}\) form of Ca\(^{2+}\)/calmodulin-dependent kinase II in the hearts of ORX rats were abolished after regular exercise. These results thus support the use of regular running as a cardioprotective alternative to testosterone replacement in hypogonadal conditions.

treadmill running; myofilament Ca\(^{2+}\) activation; phospholamban; myosin light chain 2; CaMKII

GENDER DIFFERENCES IN HEART disease incidence suggest that sex hormones may play a significant role in cardiac function. A lower incidence of heart disease in young women compared with age-matched men has been documented (3, 32). After menopause, cardiac disease incidence in women is similar to that observed in men, and studies have demonstrated a beneficial role for female sex hormones in cardiac performance (1, 19, 57). However, less is known about the effects of male sex hormones on cardiac function (29, 50-52). Despite the well-known higher risk of coronary heart disease in men than women (40), a higher incidence of heart disease was, surprisingly, observed in hypogonadal men (16; 33). Results from studies of patients with androgen deficiency have implied that male sex hormones may serve in a cardio-protective role (23, 54). Moreover, the presence of androgen receptors in cardio-

myocytes suggests that male sex hormones have a direct effect on cardiac function (35, 41).

In animal models, male sex hormone deficiency and orchidectomy induces prolonged repolarization, decreased intracellular Ca\(^{2+}\) mobilization, and reduced maximum myosin ATPase activity of cardiomyocytes (9, 21). Studies in our laboratory have also shown decreases in maximum active tension and activity of sarco(endo)plasmic reticulum Ca\(^{2+}\)-ATPase (SERCA) in the hearts of orchidectomized (ORX) rats, which could be prevented by testosterone supplementation (60). These observations point to a significant cardio-regulatory role for male sex hormones in contractile function and suggest that male sex hormones may have use in heart disease prevention and treatment in cases of male hypogonadism. Despite many favorable outcomes for testosterone replacement, its use is limited in some patients, such as those with a high risk of prostate cancer. Moreover, results from the trial known as Testosterone in Older Men with Mobility Limitations (TOM) indicated a significantly higher incidence of adverse cardiovascular events in the group that received testosterone supplementation compared with the group that received a placebo, resulting in the discontinuation of the study (4). Thus low-risk alternatives for preventing cardiac dysfunction induced by androgen-deficient conditions should be explored.

Regular exercise is well known to enhance many aspects of cardiovascular performance (6, 14). In patients with heart disease, exercise training can improve cardiac function and longevity (49). In addition, defects in myofilament activation and intracellular Ca\(^{2+}\) handling in female sex hormone-deprived rats can be suppressed by exercise training (10, 11). Endurance exercise also protects cardiac contractile dysfunction in rats subjected to LHRH agonist-induced androgen deficiency (27). These studies suggest a high potential for regular exercise in serving as an alternative strategy for heart disease prevention or treatment in patients with hypogonadism.

We hypothesize that regular exercise can serve as an effective alternative to current therapies in protecting cardiac changes induced by male sex hormone deprivation. In this study, the cardiac contractile functions of sedentary and exercised ORX rats were compared with those of rats that underwent a sham operation and served as controls. Exercised rats were subjected to a moderate-intensity, 9-wk running regimen using a motor-driven treadmill. Changes in cardiac contractile function were analyzed using skinned fiber preparations. SERCA activity was also measured using vesicles prepared from cardiac sarcoplasmic reticulum (SR) (8). Our results support the use of regular exercise as an intervention to prevent cardiac changes of the myofilament and SR Ca\(^{2+}\) activations induced by male sex hormone deprivation.

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MATERIALS AND METHODS

Materials. All chemicals were purchased from Merck (Darmstadt, Germany) or Sigma Chemical (St. Louis, MO). Electrophoresis reagents were from Amersham Pharmacia Biotech (Buckinghamshire, UK) or BioRad (Hercules, CA). Peroxidase-conjugated affinity pure donkey anti-mouse IgG (H+L) was obtained from Fitzgerald (Acton, MA), and horseradish peroxidase-conjugated goat anti-rabbit IgG (H+L) was from Zymed (San Francisco, CA). Antibodies against SERCA2a, phospholamban (PLB), and Ca\(^{2+}\)/calmodulin-dependent kinase II (CaMKII) were from Badrilla (Leeds, UK) and Thermo Scientific (IL, USA). Thapsigargin was from Alomone (Jerusalem, Israel).

Animals. Male Sprague-Dawley rats (8 wk old) weighing between 250 and 280 g underwent a sham operation or orchidectomy as previously described (60). Rats were randomly divided into sedentary and exercise training groups. Animals were housed individually in standard cages with ad libitum access to rat chow and water in a humidity-controlled room with a 12-h light-dark cycle. One week following surgery, the exercise groups began a 9-wk running program on a motor-driven treadmill, with exercise 5 days/wk. Rats were pretrained during the first week of the program at a fixed speed of 21 m/min with 0% grade. Exercise consisted of two periods of running with a 10-min resting interval. Running time was increased from 2 × 5 min on day 1 until reaching 2 × 25 min on day 5. From the second week to the end of the program, rats were subjected to 2 × 30 min of running at a fixed speed of 21 m/min with 5% grade in both sham-operated and ORX groups. Exercise intensities were calculated for a work rate of 65–75% maximum oxygen consumption on the basis of body weight as previously described (5). Adequacy of the running program was verified by analysis of citrate synthase activity in plantaris muscle dissected on the day the rat was killed. Lack of androgen was indirectly verified by measuring the decrease in seminal vesicular weight on the day the rat was killed. Animal protocols were approved by the Experimental Animal Committee of the Faculty of Science of Mahidol University, in accordance with guidelines of the National Laboratory Animal Centre of Thailand.

SERCA measurement. SR membrane vesicles were prepared from left ventricular homogenates as previously described (12). Briefly, the left ventricle was minced and homogenized in ice-cold 10 mM NaHCO\(_3\) buffer (pH 6.8) containing a cocktail of protease inhibitors. The homogenate was centrifuged twice at 10,000 g for 10 and 20 min, respectively, to precipitate myofilaments and nuclei. KCl (0.6 M) was added to the resulting supernatant, which was then centrifuged for 60 min at 100,000 g to obtain the sedimentary pellet. The final SR-enriched membrane vesicles were purified using sucrose-gradient centrifugation at 100,000 g for 60 min. Suspensions of the SR-enriched membranes (pellet) in Tris-HEPES buffer containing 0.4 M sucrose were immediately stored at −80°C and assayed within 1 wk. Activity of SERCA was determined using a trienzyme-coupled assay with various concentrations of Ca\(^{2+}\) ranging from pCa 8.0 to 5.0, at pH 7.0 and 37°C as previously described (15). In brief, 5 μg of SERCA protein was added into 1 ml of reaction mixture containing the following (in mM): 21 MOPS, 4.9 NaN\(_3\), 0.06 EGTA, 100 KCl, 3 MgCl\(_2\), 0.2 NADH, and 1 phospho(enol)pyruvate, with 8.4 units of pyruvate kinase and 12 units of lactate dehydrogenase. ATP at a final concentration of 1 mM was added to initiate ATPase activity. Total activity of SERCA was determined from the linear kinetics of NADH degradation monitored at 340 nm using a spectrophotometer (UV 2550, Shimadzu, Japan). Nonspecific ATPase activity was measured in the reaction mixture of pCa 5.0 containing 0.1 μM thapsigargin, a SERCA inhibitor.

Measurement of myofilament Ca\(^{2+}\) activation. The left ventricular papillary muscle was dissected and cut longitudinally into a small fiber bundle (150–250 μm in diameter) for measurement of myofilament Ca\(^{2+}\) activation using the method described by Janssen et al. (28). Briefly, stripped papillary fibers from the left ventricle were skinned in high-relaxing buffer containing 1% Triton X-100 for 1 h at 25°C. The skinned fiber bundle was attached to a displacement generator at one end and to a force transducer (KG-7) at the other using aluminum T-clips (28). The cross-sectional area of the fiber bundle was calculated based on an elliptical model. Active tension was measured at a sarcomere length of 2.2 μm in solution containing various Ca\(^{2+}\) concentrations ranging from pCa 7.0 to 4.5 at pH 7.0 and 15°C.

Immunoblot analysis. A small portion of left ventricular tissue was homogenized in extracting buffer containing protease inhibitors. Protein concentrations were determined using the bichinchoninic acid assay. Monoclonal antibodies against SERCA2a (1:1,000 dilution) and PLB (1:5,000) were used for immunonochemical staining of SERCA and PLB, respectively. Polyclonal antibodies against CaMKII (1:5,000), phosphorylated-Ser\(^{16}\) PLB (1:5,000), phosphorylated-Thr\(^{17}\) PLB (1:5,000), and phosphorylated-Thr\(^{187}\) CaMKII (1:5,000) were used for CaMKII, phospho-Ser\(^{16}\) PLB, phospho-Thr\(^{17}\) PLB, and phospho-Thr\(^{187}\) CaMKII, respectively. Levels of β-actin were determined using polyclonal antibodies (1:10,000) to normalize gel loading. Density of the protein bands was measured using Image Master Labscan version 3.01 and Image Master TotalLab version 1.0 (Amerham Pharmacia Biotech).

Analysis of phosphorylation level of myofilament proteins. ProQ Diamond stain (Invitrogen P3 3300) was used to detect changes in phosphorylation level of myofilament proteins. Briefly, a small portion of left ventricular muscle was homogenized (50 mg/ml) in a buffer solution (60 mM KCl, 30 mM imidazole, 2.5 mM MgCl\(_2\), and 1 cocktail of protease and phosphatase inhibitors) at 4°C, and then centrifuged at 12,000 g, 4°C for 10 min. The pellet was resuspended in 1 ml of the buffer solution containing 1% Triton X-100, homogenized, and centrifuged at 3,000 g at 4°C for 10 min. The pellet was again homogenized in 1 ml of buffer solution without Triton X-100 and centrifuged as described above. The final pellet was mixed with the buffer solution described above containing 1% Triton X-100, and myofilament proteins were separated using 12.5% SDS-PAGE. The gel was fixed, stained with ProQ Diamond, and destained according to the manufacturer’s recommendations before imaging with a fluorescence image scanner (Typhoon 9400; Amersham Biosciences). Coomassie blue staining was used to normalize protein load to actin. Optical density of the proteins was determined using ImageQuant TL (GE Healthcare) software.

General method and statistical analysis. MHC isoforms of left ventricular homogenate were electrophoretically separated as previously described (42). The relationships of pCa-active tension and pCa-SERCA activity were fitted to the Hill equation using nonlinear least-squares regression analysis (GraphPad Inplot, ISI software PRISM4) to determine half-maximal activating Ca\(^{2+}\) concentration values (pCa\(_{50}\)) and Hill coefficients. Data are presented as means ± SE. Significance of difference among groups was analyzed using one-way ANOVA followed by a Student-Newman-Keuls test for multiple comparisons, with values of P < 0.05 being considered significantly different.

RESULTS

The effectiveness of the exercise training program used in the study was verified by significant increases in both the percent heart-to-body weight ratio and the oxidative enzyme activity of plantaris muscle (Table 1). Despite no changes in body weight or tibial length among the experimental rats, heart weights were significantly higher in exercised animals compared with sedentary controls. Thus both the heart-to-body weight and heart-to-tibial length ratios were significantly greater in exercise-trained rats compared with sedentary controls, indicating a cardiac hypertrophic effect of efficient regular aerobic exercise. Citrate synthase activities of plantaris...
myofilament Ca\(^{2+}\) (5.61 ± 0.03), which was not observed in exercised ORX rats (5.62 ± 0.01) (Fig. 1, B and D).

Parallel to orchidectomy-induced reduction of maximum force development, analysis of MHC isoforms indicated a 22.0% lower ratio of \(\alpha\)-MHC/total MHC in the heart of ORX rats compared with sham-operated controls (Fig. 2). The lower ratio of \(\alpha\)-MHC/total MHC in the hearts of ORX rats could also be prevented by regular exercise. The effect of myosin isoform can also be different depending on differences in posttranslational modifications of myofilament proteins. Overall protein phosphorylation level of the myofilaments was then analyzed using ProQ Diamond stain and results demonstrated no effect of orchidectomy on the state of myofilament protein phosphorylation compared with that of sham-operated controls (Fig. 3). On the other hand, regular exercise induced a significant increase in myosin light chain 2 (MLC2) phosphorylation (Fig. 3B). This series of data demonstrates differential effects of male sex hormone deficiency and regular exercise on cardiac myofilament activation. Regular exercise prevents the isoform

![Image](http://jap.physiology.org/)

**Fig. 1.** Effects of orchidectomy and regular exercise on maximum force contraction and myofilament Ca\(^{2+}\) sensitivity in rat hearts. A and B: pCa-activity tension relationships (A) and pCa-percent maximum active tension (B) of skinned left ventricular fibers from rats that underwent sham surgery (SHAM) or were orchidectomized (ORX) in sedentary and exercise (EX) groups. C and D: bar graphs summarizing maximum active tension (C) and the half-maximal activating Ca\(^{2+}\) concentration values (pCa\(_{50}\)) (D) from each group. Data are means ± SE of 16–25 fibers from 6–9 hearts/group. *Significantly different from other groups (\(P < 0.05\)) using a Student-Newman-Keuls test after one way ANOVA.
switching of MHC and preserves the suppressive effect of male sex hormone deficiency on maximum force development of the heart.

The effect of regular exercise on the intracellular Ca\textsuperscript{2+} handling activity of cardiac SERCA might also underlie the contractile change of the heart in conditions of male sex hormone deprivation. SERCA activity was evaluated using a triple-enzyme assay and, as expected, SERCA activity (Fig. 4, A and C) was significantly lower in hearts of ORX rats (0.57 ± 0.04 μmole Pi·mg\textsuperscript{-1}·min\textsuperscript{-1}) compared with sham-operated controls (0.87 ± 0.10 μmole Pi·mg\textsuperscript{-1}·min\textsuperscript{-1}). In addition, the sensitivity of SERCA activation was significantly enhanced in hearts of ORX rats (pCa\textsubscript{50} = 6.69 ± 0.03) compared with those of sham-operated control animals (pCa\textsubscript{50} = 6.51 ± 0.04) (Fig. 4, B and D). Despite no effect of regular exercise on SERCA activation in sham-operated control animals, regular exercise prevented both the suppressed maximum activity and the enhanced sensitivity of SERCA in hearts of ORX rats (Fig. 4, C and D). To determine whether regular exercise prevented changes in SERCA activity in hearts of ORX rats through alterations in SERCA and/or its major regulatory protein PLB, the amounts of SERCA and PLB were then determined by immunoblot analysis. Results indicated no change in the content of either SERCA or PLB among the experimental groups (Fig. 5). Monomeric and pentameric levels of PLB were also unchanged among the experimental groups (Fig. 6). However, the phosphorylation level of PLB at Thr\textsuperscript{17} but not Ser\textsuperscript{16} was significantly lower in the hearts of sedentary ORX rats. This lower phosho-Thr\textsuperscript{17} PLB was not detected in hearts of exercised ORX rats (Fig. 7). Consistent with the lower level of phosphorylation of Thr\textsuperscript{17} PLB, CaMKII activity as assessed by its phosphorylation was also significantly lower in the hearts of sedentary ORX rats. In contrast, hearts of exercised ORX rats showed no change in either CaMKII activity or protein expression (Fig. 8).

**DISCUSSION**

We have previously reported a regulatory role for male sex hormones in modulating SERCA activity, which affects cardiac muscle contraction and relaxation. Results from the present study in ORX rats provide further important and novel evidence for regular exercise as a cardioprotective alternative in conditions of male sex hormone deficiency. Our study has illustrated that regular, moderate-intensity running can prevent the cardiac dysfunctions associated with orchidectomy, including suppression of myofilament Ca\textsuperscript{2+} activation, a shift in MHC isoforms toward β-MHC, a reduction in SERCA activity, and decreases in phosphorylation of CaMKII and Thr\textsuperscript{17} PLB. Thus, our study further supports the importance of regular exercise as a cost-effective alternative to testosterone therapy in hypogonadal conditions.

Results from our previous study and others have reported a significant role for male sex hormones in cardiac contractile function, supporting the possible use of testosterone in the prevention and treatment of heart disease in conditions of male sex hormone deficiency (17, 20, 60). It is also a well-established fact that testosterone replacement therapy is required for various conditions when remarkably low levels of testosterone are present due to castration or other primary testicular impairment, such as those induced by chemotherapy, radiation therapy, or androgen deprivation therapy. In conclusion, our study further supports the potential of regular exercise as a cost-effective alternative to testosterone therapy in conditions of male sex hormone deficiency.
apy, and congenital problems. In addition, testosterone replacement has been prescribed for older men with low testosterone levels and clinical disorders associated with androgen deficiency. Although testosterone therapy would be expected to provide the best risk-benefit profile for signs and symptoms associated with deficiency, a significantly greater risk of prostate events and of hematocrit has been demonstrated for middle-aged and older men with testosterone replacement (13). Moreover, a significantly higher incidence of adverse cardiovascular events was reported in the testosterone group compared with the placebo group in the TOM trial (4). The potential adverse effects of testosterone replacement highlight the importance of regular exercise, observed in our analysis, as a cost-effective alternative strategy in conditions of male sex hormone deficiency.

The mechanism through which regular exercise improves contractile activation in the heart of ORX rats has remained elusive, although it is a well accepted model for cardiac rehabilitation and prevention of heart disease (2). Results presented here extend our understanding of the cellular/molecular adaptations in the male sex hormone-deprived rat heart in response to regular, moderate-intensity running. Generally, quality and quantity of myofilament sliding is dependent on the interaction of myosin and actin (the major proteins on the thick filament and thin filament, respectively) through a process governed by the troponin complex and tropomyosin in response to Ca\(^{2+}\) signals. Changes in myofilament proteins or intracellular Ca\(^{2+}\) mobilization could alter the contractile function of the heart. The present results obtained from androgen deprivation-induced MHC switching toward -MHC were consistent with those of previous reports (22, 26, 38, 39, 43). We have further demonstrated that in ORX rats, regular running led to a normalization of the contractile force of the cardiac myocyte and reversed the MHC isoform. Although it

Fig. 4. Effects of orchidectomy and regular exercise on sarcoplasmic reticulum (SR) Ca\(^{2+}\)-ATPase (SERCA) activity in rat hearts. A and B: pCa-SERCA relationship (A) and pCa-per cent maximum SERCA activity (B) of cardiac SR vesicles from SHAM and ORX rats in sedentary and exercise groups. C and D: bar graphs summarizing maximum SERCA activity (C) and pCa50 (D) from each group. Data are means ± SE from 12–14 preparations/group. *Significantly different from other groups (P < 0.05) using a Student-Newman-Keuls test after ANOVA.

Fig. 5. Effects of orchidectomy and regular exercise on SERCA and phospholamban (PLB) protein levels in rat hearts. A and B: immunoblot analysis of SERCA2a and actin (A), and PLB and actin (B) from left ventricular homogenates from SHAM and ORX rats in sedentary and exercise groups. C: bar graph showing the ratio of SERCA2a to PLB from each group. Data are means ± SE from 5–8 hearts/group.
was previously shown that both MHC isoforms generated similar maximal Ca\(^{2+}\)-activated force (24, 46), different MHC isoforms could still affect cardiac contractile activation differently through modifications of myofilament proteins. Because the ProQ Diamond stain data demonstrated unaltered myofilament protein phosphorylation, the reduction in maximum tension in cardiac myocytes of ORX rats was not involved in myofilament phosphorylation. Thus the underlying mechanism of testosterone action on myofilament protein function needs to be further explored.

In line with previous reports, the exercise-induced myofilament Ca\(^{2+}\) hypersensitivity observed in the healthy heart of exercised control rats (Fig. 1, B and D) suggests other physiological adaptations to the heart, in addition to cardiac hypertrophy, in improving contractile performance after training (18, 25, 47, 58, 59). Regardless of experimental variables such as types, strains and sexes of animal, protocols for exercise training, preparations of cardiac fiber, and measurement techniques, the exercise-induced Ca\(^{2+}\) hypersensitivity of the cardiac myofilament is remarkably similar among studies. Association of MLC2 phosphorylation with myofilament Ca\(^{2+}\) sensitivity has been previously documented (44, 48). A significant increase in MLC2 phosphorylation only in hearts of rats that underwent a sham operation and exercise demonstrated in our study (Fig. 3B) then suggests a possible mechanism underlying the exercise-induced Ca\(^{2+}\) hypersensitivity of the cardiac myofilament in testosterone-intact rats. An interesting finding in the present study was the demonstration that the cellular mechanism of exercise-enhanced contractile performance, a consequence of increased myofilament Ca\(^{2+}\) sensitivity, was not apparent in ORX rats. In contrast, exercise-induced hypertrophy of hearts in ORX rats was still clearly observed to the same degree as that in exercised rats that served as controls. The results of this study thus suggest that regular exercise enhances cardiac contractile performance by both quantitative and qualitative adaptive responses through muscle hypertrophy and myofilament Ca\(^{2+}\) hypersensitivity, respectively, using differential signaling processes.

Besides myofilament proteins, changes in intracellular Ca\(^{2+}\) handling represent another potential mechanism that may confer altered contractile properties to the myocardium. Testosterone has been reported to directly regulate intracellular Ca\(^{2+}\) handling by inducing an increase in L-type Ca\(^{2+}\) current in cardiomyocytes (56), which can be attributed to a reduction in the intracellular Ca\(^{2+}\) transient amplitude induced by male sex hormone deprivation (17). The reduced intracellular Ca\(^{2+}\) transient amplitude has also been shown to involve a decrease in the Ca\(^{2+}\) release activity of ryanodine receptors in the heart.

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Fig. 6. Effects of orchidectomy and regular exercise on monomeric and pentameric PLB in rat hearts. A: immunoblot analysis of PLB from left ventricular homogenates from SHAM and ORX rats in sedentary and exercise groups. B and C: bar graphs summarizing the percent of monomeric (B) and pentameric (C) PLB from each group. Data are means ± SE from 8 hearts in each group.

Fig. 7. Effects of orchidectomy and regular exercise on phosphorylation of PLB at Ser\(^{16}\) and Thr\(^{17}\) in rat hearts. A: immunoblot analysis of phospho-Ser\(^{16}\) PLB and actin (top) from left ventricular homogenates and ratio of phospho-Ser\(^{16}\) PLB to actin (bottom). B: immunoblot analysis of phospho-Thr\(^{17}\) PLB and actin (top) and ratio of phospho-Thr\(^{17}\) PLB to actin (bottom). Data are means ± SE from 6 hearts each group. *Significantly different from other groups (P < 0.05) using a Student-Newman-Keuls test after ANOVA.
of ORX rats (53). In previous studies, we have reported that chronic deprivation of male sex hormones induces a reduction in SERCA activity, which is completely reversed by testosterone supplementation (60). Impaired SR Ca\(^{2+}\) content due to altered SR Ca\(^{2+}\) uptake activity has been suggested as another mechanism by which testosterone affects intracellular Ca\(^{2+}\) handling. Our current study has advanced the field by demonstrating a mechanistic potential of an ORX-induced reduction in CaMKII activity and the consequent decrease in phospho-Thr\(^{17}\) PLB, which can all be abolished by regular treadmill running (Fig. 8).

In conclusion, results from the present study support the protective effects of regular running on reducing contractile dysfunction in the hearts of ORX rats by maintaining homeostasis of both myofilament proteins and intracellular Ca\(^{2+}\) mobilization. Together with the protective role of male sex hormones, regular running at moderate intensity appears to enhance myocardial performance by inducing hypertrophy without affecting MHC isoform switching or Ca\(^{2+}\) uptake activity. Thus regular running may serve as an alternative to the use of male sex hormones in reducing the risk for heart disease in hypogonadal conditions.

ACKNOWLEDGMENTS

We thank Dr. Pieter de Tombe for assistance with force measurement techniques and Dr. Thanat Chookajorn for assistance with fluorescence image scanning. We also thank Drs. Laran T. Jensen and Nateetip Krishnamra for their critical reading of the manuscript.

GRANTS

This work was supported by a Mahidol University grant to J. Wattanapermpool and, in part, by the Faculty of Science of Mahidol University. P. Vutthasathien received support from The Institutional Strengthening Program of the Faculty of Science of Mahidol University.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

J.W., conception and design of research; P.V., performed experiments; P.V., and J.W., analyzed data; P.V. and J.W., interpreted results of experiments; P.V.,...
and J.W. prepared figures; P.V. and J.W. drafted manuscript; J.W. edited and revised manuscript; P.V. and J.W. approved final version of manuscript.

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Regular Exercise Improves Orchidectomized Rat Hearts • Vatthasathien P et al. • J Appl Physiol • doi:10.1152/japplphysiol.00224.2015 • www.jappl.org


