Left ventricular remodeling with exercise in hypertension

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Kolwicz SC, MacDonnell SM, Renna BF, Reger PO, Seqqat R, Rafiq K, Kendrick ZV, Houser SR, Sabri A, Libonati JR. Left ventricular remodeling with exercise in hypertension. Am J Physiol Heart Circ Physiol 297: H1361–H1368, 2009. First published August 7, 2009; doi:10.1152/ajpheart.01253.2008.—We investigated how exercise training superimposed on chronic hypertension impacted left ventricular remodeling. Cardiomyocyte hypertrophy, apoptosis, and proliferation in hearts from female spontaneously hypertensive rats (SHRs) were examined. Four-month-old SHR animals were placed into a sedentary group (SHR-SED; n = 18) or a treadmill running group (SHR-TRD, 20 min/m, 1 h/day, 5 days/wk, 12 wk; n = 18). Age-matched, sedentary Wistar Kyoto (WKY) rats were controls (n = 18). Heart weight was greater in SHR-TRD vs. both WKY (P < 0.01) and SHR-SED (P < 0.05). Morphometrically derived left ventricular anterior, posterior, and septal wall thickness were increased in SHR-SED relative to WKY and augmented in SHR-TRD. Cardiomyocyte surface area, length, and width were increased in SHR-SED relative to WKY and further increased in SHR-TRD. Calcineurin abundance was increased in SHR-SED vs. WKY (P < 0.001) and attenuated in SHR-TRD relative to SHR-SED (P < 0.05). Protein abundance and mRNA of Akt was not different among groups. The rate of apoptosis was increased in SHR-SED relative to WKY and mitigated in SHR-TRD. The abundance of Ki-67+ cells across groups was not statistically different across groups. The abundance of cardiac progenitor cells (c-Kit+ cells) was increased in SHR-TRD relative to WKY. These data suggest that exercise training superimposed on hypertension augmented cardiomyocyte hypertrophy, despite attenuating calcineurin abundance. Exercise training also mitigated apoptosis in hypertension and showed a tendency to enhance the abundance of cardiac progenitor cells, resulting in more favorable cardiomyocyte number in the exercise-trained hypertensive heart.

hypertrophy; myocytes; apoptosis; proliferation

CHRONIC HYPERTENSION INDUCES overall cardiac enlargement, which is, in part, due to cardiomyocyte hypertrophy. This is a significant health issue, since pathological cardiac enlargement increases the risk for the development of congestive heart failure (4). Increased apoptosis has also been noted in the hypertensive heart, which may be an integral substrate in overall remodeling and progression to heart failure (33). Several studies have reported that cardiac myocytes are capable of mitotic division and proliferation (13, 14). While the control mechanisms for the induction of cardiomyocyte proliferation remain unclear, one theory purports the involvement of a resident population of cardiac progenitor cells (1, 30), which have been shown to increase their activity in stress-induced pathological conditions (1, 28, 31). In the hypertensive heart, cardiomyocyte proliferation may counteract apoptosis, thus reducing the progressive loss of cardiomyocytes.

Recent studies from our laboratory, as well as others, have shown an overall phenotypical improvement for the myocardium with exercise training in hypertension (3, 17, 24, 34, 35, 42). However, the precise putative mechanisms associated with the observed adaptations with exercise training remain unclear. Our present hypothesis is that exercise training in hypertension alters the balance between cardiomyocyte apoptosis and proliferation. This hypothesis is based on a small number of studies in rodents, which generally report that apoptosis is attenuated with training (9, 15, 16, 20, 39, 40), with the caveat that no study to date has examined the impact of exercise training on cardiomyocyte proliferation in the spontaneously hypertensive rat (SHR) model. The importance of these questions is underscored by the recent report by Schultz et al. (38), who showed that excessive levels of voluntary wheel running in aging spontaneously hypertensive heart failure rats increased myocardial fibrosis, worsened left ventricular (LV) function, and hastened the progression to heart failure. Thus the purpose of the present study was to determine how exercise training impacts myocardial remodeling in hypertension, specifically examining the relative roles of cardiomyocyte hypertrophy, apoptosis, and proliferation. We also examined how exercise training superimposed on hypertension impacted myocardial calcineurin and Akt abundance.

METHODS

Animal care and model. Sixteen-week-old female, Wistar Kyoto (WKY, n = 18) rats and SHRs (n = 36), weighing 150–175 g at the initiation of the experiment, were obtained from Charles River Laboratories (St. Constant, Quebec, Canada). WKY animals were assigned to a sedentary, control group (WKY-SED, n = 18), while animals within the SHR group were assigned to a sedentary (SHR-SED, n = 18) or an exercise training group (SHR-TRD, n = 18). Rats were housed three to five per cage and maintained on a 12:12-h light-dark cycle and fed ad libitum (Harlan Teklad Global Diets, 18% Protein Diet, Madison, WI). All animals received humane care in compliance with Temple University Standards and the “Principles of Laboratory Animal Care” formulated by the National Society for Medical Research and the “Guide for the Care and Use of Laboratory Animals” prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication no. 85-23, revised 1985). All experimental procedures were reviewed and approved by the Intitutional Animal Care and Use Committee at Temple University.

Exercise training. Rats that underwent exercise training were provided a 1-wk acclimatization period before the initiation of standardized exercise training. During the first week, rats were placed on a motor-driven treadmill at a speed of 20–25 m/min for 15 min. Duration was gradually increased to 60 min during the first week. At...
the beginning of the second week, the training protocol consisted of a speed of 20–25 m/min for 60 min, 5 days/wk, over 12 consecutive weeks.

**Myocardial morphometry.** Some hearts were arrested with the LV balloon placed in the LV with the balloon volume set to yield an end-diastolic pressure of 5 mmHg. These hearts were fixed by retrograde perfusion with 4% paraformaldehyde for 20 min. Hearts were then cross sectioned at the level of the mitral valve and embedded in paraffin. Transverse sections of 4 µm were obtained from the midventricular region of the heart. The histological sections were stained for the detection of cardiac myocyte apoptosis [terminal deoxynucleotidyl transferase (TdT) dUTP-mediated nick-end labeling (TUNEL)], proliferation (Ki-67), and progenitor cells (c-kit).

**TUNEL.** The TUNEL method was used for the identification of apoptotic cardiomyocytes by identifying DNA breaks in the cell nuclei. Specifically, it involved the binding of TdT to 3'-OH ends of DNA, which resulted in the formation of a polydeoxyribonucleotide polymer. After proteolytic treatment, TdT incorporated biotinylated deoxyuridine at the site of DNA breaks. After the addition of avidin-peroxidase, histochemical identification was made via microscopy. To determine rates of apoptosis, slides were analyzed using an epifluorescence microscope and camera system (E800 Eclipse, Nikon). Five fields were sampled from the AW, PW, SW, and FW of each heart. Only longitudinal fibers from the midmyocardium were included. The total number of TUNEL positive (TUNEL+) and total nuclei (DAPI) were counted from each image. Apoptotic index was determined by dividing the number of TUNEL+ nuclei by the total number of sampled cardiomyocyte nuclei.

**Ki-67.** The application of the nuclear protein, Ki-67, was used to measure cardiomyocyte cell proliferation. Ki-67 expression occurs throughout all phases of the cell cycle, except for the G0 phase. Using an antibody specific to the Ki-67 antigen, the proliferative activity of cardiomyocytes (60–100 nuclei per field) were determined. A micrometer was utilized to set the scale (micrometer-to-pixel ratio) before the analysis of each segment. For this analysis, 18–20 fields were analyzed with 80–100 cardiomyocytes per field. All pictures were analyzed using the Scion NIH Image program (version 1.62).
cells was determined. Paraffin-embedded sections were submerged in xylene, washed in ethyl alcohol, and rehydrated in double distilled water. Slides were incubated for 10 min in 20% goat serum in PBS to minimize nonspecific binding and then in 0.1% Triton X-100 in 20% goat serum for 30 min at room temperature. Slides were then incubated overnight at 4°C with primary antibody. The next day, slides were washed 3× with PBS; the secondary antibody (AlexaFluor 594) was added and incubated at room temperature for 1 h. DAPI was added for 5 min at the end of the second incubation. Slides were washed twice with PBS and then covered with mounting medium and a cover slip. Slides were stored at 4°C. To verify that cells undergoing mitotic division were cardiac myocytes, an antibody to α-sarcinomic actin was added. To determine proliferation rates, each slide was analyzed using a confocal microscope (C1 D-Eclipse, Nikon). Digital images were created from 20 fields from the AW, PW, SW, and FW of each heart. The total number Ki-67+ total nuclei were counted from each image. Proliferation index was determined by dividing the number of Ki-67+ nuclei by the total number of sampled nuclei.

c-kit. For identification of cardiac progenitor cells, specimens were fixed and labeled with a c-kit antibody (R&D Systems, Minneapolis, MN). Paraffin-embedded sections were submerged in xylene, washed in ethyl alcohol, and rehydrated in double distilled water. Slides were incubated for 20 min at 4°C with 1–5 μg/100 μl of primary antibody against c-kit. Slides were washed twice in PBS. DAPI was added for 5 min at the end of the second incubation period. To exclude mast cells from the quantification, slides were also stained for sarcomeric actin. For identification of cardiac stem cells, each slide was analyzed using a bright-field microscope (E600, Nikon). Digital images were created of c-kit+ cells throughout the entire myocardium. The total number of c-kit+ cells was counted in the AW, PW, SW, and FW of each heart.

Calcineurin abundance. Paraffin-embedded sections were submerged in xylene, washed in ethyl alcohol, and rehydrated in double distilled water. Slides were incubated for 20 min at 4°C with 1–5 μg/100 μl of primary antibody against c-kit. Slides were washed twice in PBS. DAPI was added for 5 min at the end of the second incubation period. To exclude mast cells from the quantification, slides were also stained for sarcomeric actin. For identification of cardiac stem cells, each slide was analyzed using a bright-field microscope (E600, Nikon). Digital images were created of c-kit+ cells throughout the entire myocardium. The total number of c-kit+ cells was counted in the AW, PW, SW, and FW of each heart.

Calcineurin immunofluorescent-stained sections were analyzed by measuring the area expressing fluorescence above a defined threshold. Before acquisition, the camera’s f-stop was maintained at a constant level for each acquired image. Measurements were made in a 0.0768-mm2 or smaller field using the irregular region of interest option of the Bioquant software (Bioquant TCW 98; BIOQUANT, Nashville, TN). The video count area array option of the Bioquant software was also utilized for these measurements. Video count area was defined as the number of pixels in a field that meet a user-defined color threshold of staining multiplied by the area of a pixel at the selected magnification (×100 in our analyses). The threshold value for immunostaining of calcineurin was stored in the computer program for consistent automated measurement of the immunostained slides. The area fractions of immunoreaction product for calcineurin were calculated by dividing the video count area containing pixels at or above the defined background threshold by the video count area of the total number of pixels in the chosen field (region of interest). This determination was made from three fields for each heart.

RT-PCR for Akt. Analysis of mRNA expression levels from untreated (basal) tissue (WKY: n = 6; SHR-SED: n = 6; SHR-TRD: n = 6) for Akt was performed with primers designed to detect rat gene products: Akt used primers, FW: GAGGACGCCCATGGATTA-CAAG and REV: GACAGCTACCTCATCATCTCAGA. RT-PCR reactions were performed with 1 μg of cDNA created from RNA using iScript (BioRad), followed by 22 cycles of PCR amplification (annealing temperature 62.5°C) using a BioRad iCycler. Appropriate melt curves were also run to ensure specificity of the product. The expression levels were compared with, and normalized to, levels of 28S FWD: 5'-TTGAAAATCCGGGGAGAG and REV: 5'-ACAT-TGTTCCAACATGCCAG.

Western blots for Akt. Snap-frozen LV tissue samples were prepared by homogenization in lysis buffer containing 150 mmol/l NaCl, 1.2 mmol/l MgCl2, 1 mmol/l EGTA, 1 mmol/l Na3VO4, 10 mmol/l sodium-pyrophosphate, 100 mmol/l NaF, 50 mmol/l HEPES, pH 7.4,
1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 10 μg/ml leupeptin, 10 mg/ml aprotinin, and 1 mmol/l phenylmethylsulfonyl fluoride. Protein concentrations were assessed with use of a bicinechonic acid assay (Pierce), and equal amounts of protein (75 μg) were separated by SDS-PAGE and transferred to nitrocellulose membranes (Hybond, Amersham). Active and total form of Akt accumulation was detected by Western blot analysis with use of an anti-Akt antibody, and a horseradish peroxidase-conjugated goat anti-mouse secondary antibody (BioRad). Bands were visualized by enhanced chemiluminescence (Amersham) and quantified by laser densitometry.

Statistical analysis. ANOVA, followed by a Tukey post hoc analysis, was used to test significant differences in physical characteristics, gross myocardial morphometry, myocyte size, calcineurin abundance, Akt, TUNEL+ cardiomyocytes, c-kit+, and Ki-67+ cardiomyocytes. All data are presented as means ± SE. All results were tested at the P < 0.05 level of significance.

RESULTS

Physical characteristics. The physical characteristics of each group are presented in Table 1. The body weight of SHR-SED was significantly less than that of WKY. SHR-TRD had increased heart mass relative to WKY and SHR-SED (P < 0.05). Heart weight-to-body weight ratio was greater in SHR-SED and SHR-TRD compared with WKY (P < 0.05). Tibial length was greater in both SHR groups relative to WKY (P < 0.001).

Myocardial morphometry. Hematoxylin and eosin-stained 4-μm cross sections of each heart were analyzed to obtain myocardial wall thickness (Fig. 1). The LV AW thickness was increased in both SHR groups compared with WKY and augmented with training (P < 0.05). PW thickness was greatest with training in hypertension (P < 0.01; SHR-TRD vs. WKY). SW thickness was increased in both groups of SHR relative to WKY. LV FW and the right ventricular wall thicknesses were similar among groups.

CSA. As depicted in Fig. 2, CSA was increased in both SHR groups compared with WKY (P < 0.001). Similar to the gross morphometry results, training superimposed on hypertension (SHR-TRD) further augmented CSA relative to SHR-SED (P < 0.01). The volume fraction of myocytes was similar between groups (WKY, 79 ± 3%; SHR-SED, 81 ± 5%; SHR-TRD, 80 ± 3%, P = nonsignificant).

Fig. 3. Cardiomyocyte morphometry. Cell area, width, and length from the anterior, posterior, and septal walls are illustrated. A: anterior and posterior wall cell areas were greater in both hypertensive groups compared with WKY. The anterior, posterior, and septal wall cell areas were greater in SHR-TRD compared with both WKY and SHR-SED. B: posterior cell width was increased in SHR-SED vs. WKY and greatest in SHR-TRD. Additionally, anterior cell width was greater in SHR-TRD vs. WKY. C: cell length was greater in SHR-TRD vs. WKY in all identified walls. An increase in posterior cell length was observed in SHR-SED vs. WKY. Exercise training further increased septal wall cell length in hypertension. Representative photomicrographs are displayed for each group (×40). Values are means ± SE. *P < 0.05, †P < 0.001, ‡P < 0.01.
Cardiomyocyte morphometry. Cell area, width, and length are illustrated in Fig. 3. In parallel with the wall thickness and the histological CSA measurements, isolated cardiomyocytes from the AW and PW had greater areas in both hypertensive groups compared with WKY (Fig. 3A). Exercise training superimposed on hypertension (SHR-TRD) augmented the hypertrophic response observed in the sedentary group (SHR-SED). The AW, PW, and SW cell areas were greater in SHR-TRD compared with both WKY and SHR-SED (Fig. 3A). As depicted in Fig. 3B, posterior cell width was increased in SHR-SED vs. WKY (P < 0.05) and was greatest in SHR-TRD (P < 0.001). Additionally, cardiomyocyte width in cells harvested from the AW was greater in SHR-TRD vs. WKY (P < 0.05). Cardiomyocyte length was greater in SHR-TRD vs. WKY within all identified walls (Fig. 3C). An increase in posterior cell length was observed in SHR-SED vs. WKY (P < 0.05). Cardiomyocyte length was greater in SHR-TRD vs. WKY within all identified walls (Fig. 3C). An increase in posterior cell length was observed in SHR-SED vs. WKY (P < 0.001). Exercise training further increased SW cell length in hypertension (P < 0.01).

TUNEL, Ki-67, and c-kit. Apoptosis was heterogeneously dispersed throughout the LV cross section, occurring most frequently in anterolateral wall of the LV. As depicted in Fig. 4A, SHR-SED had a significantly greater apoptotic index relative to WKY-SED. The apoptotic index was not increased in SHR-TRD relative to WKY-SED, suggesting an anti-apoptotic effect of exercise training in hypertension. The identification of Ki-67+ nuclei was rare in all groups, with only 11 total Ki-67+ identified in ~75,200 sampled nuclei. Although SHR-TRD resulted in the highest proliferation rate, no statistically significant differences were observed between the groups (Fig. 4B). The presence of c-kit+ cells was identified across the myocardium, and, as was the case with Ki-67+ cells, the overall abundance of c-kit+ cells was low. We found 9 c-kit+ cells in WKY, 17 c-kit+ cells in SHR-SED, and 31 c-kit+ cells in SHR-TRD. The relative increases (as percentage of WKY-SED) for each group are presented in Fig. 4C. There were no significant differences between the groups for c-kit+ cells.

Akt and calcineurin abundance. As Fig. 5 illustrates, both Akt mRNA (A) and Akt protein abundance (B) were not statistically different between groups. Calcineurin abundance was significantly greater in both SHR-SED and SHR-TRD compared with the normotensive WKY controls (P < 0.001) (C). Exercise training in hypertension failed to entirely normalize calcineurin abundance; however, a reduced abundance was observed when SHR-TRD was compared with SHR-SED (P < 0.05).

DISCUSSION

In this study, we report that exercise training induced LV remodeling in SHR, primarily by augmenting cardiomyocyte hypertrophy. The hypertrophy associated with the superimposition of exercise training on hypertension was generally dispersed across the LV myocardium, i.e., AW, PW, and SW, and appeared to be both concentric and eccentric in nature. One important finding in the present study was that exercise training induced cardiomyocyte hypertrophy, despite offsetting the increase in calcineurin abundance associated with hypertension. Moreover, Akt abundance was not different between groups, suggesting that calcineurin- and Akt-mediated signaling were not the exclusive hypertrophic pathways involved in exercise-induced cardiomyocyte hypertrophy in hypertension. In the

Fig. 4. Cardiomyocyte apoptosis, proliferation, and progenitor cells. A: apoptotic index, defined as the percentage of terminal deoxynucleotidyl transferase dUTP-mediated nick-end labeling positive cells per total nuclei sampled. Apoptosis was increased in SHR-SED and attenuated in SHR-TRD. B: cardiomyocyte proliferation index was defined as the percentage of Ki-67+ nuclei per total nuclei sampled. C: cardiac progenitor cells. The percentage of c-kit+ cells are expressed relative to WKY-SED. D: representative photomicrograph of c-kit+ cells with green representing c-kit+ cells, blue (4',6-diamidino-2-phenylindole) representing nuclei, and red representing α-sarcomeric actin. *P < 0.05 vs. WKY-SED.
Exercise training reduced calcineurin expression in hypertension. A: gene expression of Akt from the hearts of WKY, SHR-SED, and SHR-TRD. No differences were noted between the groups. B: representative Western blots of Akt protein abundance from WKY, SHR-SED, and SHR-TRD hearts. No significant differences were noted. C: calcineurin immunofluorescent-stained sections were analyzed by measuring the area expressing fluorescence above a defined threshold. Calcineurin expression was significantly greater in both SHR-SED and SHR-TRD compared with the WKY. Exercise training reduced calcineurin expression in hypertension.

In the present study, we also report that the rate of cardiomyocyte apoptosis greatly exceeded cardiomyocyte proliferation in hypertension, leading to a net loss of cardiomyocytes at this relatively young age in both WKY and SHR. Exercise training in hypertension improved the overall profile of cardiomyocyte balance in hypertensive hearts via an attenuation of cardiomyocyte apoptosis and a slight increase in the frequency of cardiomyocyte proliferation.

Myocardial hypertrophy secondary to hypertension is associated with a parallel addition of sarcomeres that characteristically increases cardiomyocyte cell size and width. From a cellular perspective, concentric hypertrophy differs from eccentric hypertrophy in that, with eccentric hypertrophy, cardiomyocytes adapt by increasing sarcomeres in series, thereby inducing an increase in cell length. Recently, specific signaling cascades have been associated with concentric and eccentric hypertrophic phenotypes, i.e., IGF and calcineurin (26, 27). While compensatory concentric hypertrophy is often regarded as an adaptation to normalize wall stress in hypertension, it is frequently manifest with abnormal cardiac function (17, 24, 34, 35). While recent reports have questioned the necessity of wall stress normalization (10), the mechanisms associated with the dichotomous adaptive and maladaptive aspects of myocardial hypertrophy are important to understand.

Few data exist with respect to how exercise training superimposed on hypertension impacts LV remodeling. Our finding that exercise training increases whole heart hypertrophy is similar to previous reports (36). In our study, we observed that exercise training in hypertension increased both cardiomyocyte length and width to a greater degree than sedentary counterparts, suggesting a further induction of concentric and eccentric hypertrophy in this model. Wilkins et al. (43) noted that physiological hypertrophy in mice subjected to both wheel running and swim training failed to significantly increase calcineurin-nuclear factor of activated T cells coupling in hearts. Additionally, Konhilas et al. (18) reported wheel running in mice decreased calcineurin expression while increasing Akt and p70 S6 phosphorylation. In the present study, we found Akt mRNA and protein abundance were similar across groups. Thus the observed hypertrophic response with exercise training was fully explained by neither calcineurin abundance nor Akt abundance.

Calcineurin-dependent signaling appears to be centrally involved in pressure overload cardiac hypertrophy (2, 21, 27, 44). Both cyclosporine A and FK506 have been successfully used to attenuate the development of cardiac hypertrophy by suppressing calcineurin abundance (25, 44). Of interest, recent data in animals have shown that hypertrophic growth could be attenuated by calcineurin inhibition, despite the persistence of the pressure-overload stimulus. Arresting hypertrophy in the face of pressure overload has been shown to not induce short-term hemodynamic compromise in hypertensive hearts (10), suggesting that calcineurin-mediated hypertrophic growth may not be a required compensatory response in hypertension.

To our knowledge we are the first to report that exercise training lessened calcineurin abundance in SHR animals, an effect of interest, since recent work has shown that calcineurin activity was increased with training in both normotensive and cardiomyopathic animals (7). Functionally, calcineurin appears to modulate Ca$^{2+}$ cycling via dephosphorylation of the sarcoplasmic reticulum Ca$^{2+}$-ATPase-2a inhibitor phospholamban (29). The reduced phospholamban phosphorylation likely reduces sarcoplasmic reticulum Ca$^{2+}$ loading and subsequent release affecting both myocardial inotropy and lusitropy. In this present work, the reduced calcineurin abundance in SHR-TRD mechanistically add to our laboratory’s previous report, which showed that exercise training improved LV inotropic and lusitropic β-adrenergic receptor responsiveness in SHR (24).

The primary purpose of the present study was to determine the impact of exercise training on the balance of cardiomyocyte apoptosis and proliferation in the SHR model. Apoptosis is a strong overall determinant of the cardiac phenotype and is thought to significantly contribute to the progression of heart failure (33). Increased apoptosis has been observed in the myocardium of SHR (8, 23) and in other models of hypertrophy (6, 11, 41). Results of the present study are in accord with these previous reports, as we showed a 100% increase in apoptotic rates in SHR-SED relative to WKY-SED controls (6, 8, 11, 23, 41).

Several studies demonstrated improvements in the apoptotic profile in rodent myocardium with exercise training (9, 12, 15, 19, 39, 40). However, there are a limited number of studies on exercise training and apoptosis in hypertension, particularly in the SHR model (20, 22). One such study showed an increased expression of the proapoptotic protein, Bax, in the myocardium of trained SHR animals (20). Although this finding suggests
that exercise training increases apoptotic activity, increases in antiapoptotic proteins, Bcl-2 and heat shock protein-72, were also observed (20). Conversely, Lee et al. (22) showed decreased overall rates of apoptosis and a reduction in Bax. In accordance with Lajoie, et al. (20), increased levels of the antiapoptotic protein, Bcl-2, were also found in the hearts of trained SHR animals. In the present study, the rate of apoptosis was ~30% lower in SHR-TRD relative to SHR-SED.

In the present study, cardiac myocyte proliferation was determined using the Ki-67 antigen, since it is expressed during active phases of the mitotic cell cycle (37). Studies in rodent cardiomyopathy and human heart failure (13, 14, 32) have reported increased cardiac myocyte proliferation using Ki-67, as well as other techniques. In the present study, no statistically significant group differences were found for Ki-67+. These statistical differences are reflective of the extremely rare occurrence of cardiomyocyte proliferation in the SHR model. When the phenomena of proliferation is considered concomitantly with apoptosis, the results of our study suggest that exercise training in hypertension increases the overall number of functional cardiomyocytes. This finding may, in part, explain the enhanced myocardial phenotype associated with exercise training in hypertension.

Several reports in both human and animal models have identified a population of cardiac progenitor cells within the myocardium (28, 30). In the present study, although the numbers of c-kit+ cells per heart were few, the presence of c-kit+ cells in SHR-TRD was ~200% greater than in WKY-SED. Relative to the normotensive sedentary condition, the combination of exercise training and hypertension may promote a homing stimulus for primitive cells.

Afterload is a crucial determinant of myocardial remodeling. Thus it should be noted that shifts in systemic blood pressure are not likely to fully explain our observations in exercise-trained, hypertensive myocardium. In our laboratory’s previous studies using the identical exercise paradigm in SHR, we have shown only modest, statistically insignificant, training-induced decrements in systemic blood pressure, i.e., ~4 mmHg (17, 24, 34, 35). In the present study, blood pressure was determined in a subset of animals (data not shown), and once again demonstrated a minimal training effect. Our data in female SHRs are consistent with the work of Coimbra et al. (5), who showed that exercise training was less effective in reducing blood pressure in female vs. male SHR animals. Thus, while our conclusions are sex specific, chronic afterload alterations do not appear to underlie our observations.

There are several limitations to our study. First, we determined cardiac proliferation and progenitor cell abundance in fixed LV sections, as opposed to establishing these phenomena with isolated cardiomyocyte sorting techniques. Second, we did not incorporate an exercise-trained WKY group in our study. Third, our conclusions are limited only to the relatively young, female SHR model. More prolific cardiac differences may be observed later in the time course of the disease. However, despite these limitations, data from the present study suggest that, in hypertension, net cardiomyocyte loss is pronounced. Exercise training in the hypertensive myocardium improves cardiomyocyte balance, largely by attenuating apoptosis. Exercise training in hypertension also increases cardiomyocyte hypertrophy, despite mitigating calcineurin abundance, without altering Akt abundance. Further studies are needed to understand how cardiac remodeling with exercise in hypertension impacts myocardial phenotype.

GRANTS

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