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ORIGINAL ARTICLE

Effects of endurance training on combined goserelin acetate and doxorubicin treatment–induced cardiac dysfunction

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Abstract
Purpose Doxorubicin (DOX) and goserelin acetate (GA), when administered individually, can lead to impaired cardiac function via different mechanisms. Combining GA and DOX (GA + DOX), however, could potentially exacerbate cardiac dysfunction when compared to GA and DOX treatments administered individually. Therefore, the first purpose of this study was to investigate the effects of GA + DOX on cardiac function. Additionally, since exercise training has been shown to protect against GA- and DOX-induced cardiac dysfunction when administered individually, the second purpose of this study was to examine the effects of exercise during GA + DOX on cardiac function.
Methods Female rats were randomly assigned to control (CON), GA, DOX, GA + DOX, or exercise training during GA + DOX (EX GA + DOX). Following 56 days, cardiac function was analyzed in vivo using echocardiography and ex vivo using an isolated working heart model.
Results GA + DOX had significantly lower mitral valve maximal and mean blood flow velocities and aortic valve maximal blood flow velocity than CON (in vivo analysis, $P < 0.05$), but these differences were not observed between EX GA + DOX and CON. In the isolated working heart, GA + DOX hearts had significantly different left ventricular developed pressures and maximal rates of pressure development and decline than CON ($P < 0.05$), but these differences were not observed in EX GA + DOX.
Conclusions GA + DOX resulted in significantly impaired in vivo and ex vivo cardiac function, but exercise training during GA + DOX was cardioprotective.

Keywords Anthracycline · Cardiac function · Echocardiography · Estrogens · Ovariectomy · Physical activity

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Introduction

Although the anthracycline antibiotic doxorubicin (DOX, trade name Adriamycin®) is an effective chemotherapy drug used to treat a variety of solid tumors and systemic malignancies, one of its most commonly recognized side effects is cardiotoxicity, which limits its use clinically. This cardiotoxicity is most commonly attributed to myocardial oxidative stress that can lead to impaired cardiac performance observed immediately following, days following, weeks following, or even months to years following treatment (for review, see Ref [1]). Although DOX-induced cardiotoxicity is an overwhelming clinical concern, DOX has been shown to induce ovarian damage, which can perpetuate irreversible infertility [2–5]. In an attempt to combat DOX-induced ovarian damage, supplemental treatment with luteinizing hormone–releasing hormone (LHRH) agonists such as goserelin acetate (GA) has been shown to minimize DOX-induced sterility by suppressing gonadal function prior to and during DOX treatment [6, 7]. Consequently, once DOX treatment is complete, GA treatment is discontinued and gonadal function returns to normal [2, 8].

Although combined GA and DOX use is favorable for preserving ovarian function, there is evidence that this

combined therapy may exacerbate DOX-induced cardiotoxicity. It is well known that estrogens are cardioprotective [9–11], and sex hormone ablation using surgical castration is associated with impaired cardiac function [12, 13]. Additionally, GA treatment may be used to treat endocrine-responsive breast cancers by inhibiting sex hormone synthesis thereby inhibiting cancer cell growth and proliferation, but GA treatment has been shown to promote cardiac dysfunction in the female rat [14]. In addition, estrogens have been shown to play an important role in protecting against cardiac insults such as ischemia-reperfusion injury [9, 15, 16] and DOX administration [16, 17]; however, to our knowledge, there have been no studies examining the effects of a clinically relevant combined GA and DOX treatment on cardiac function. Therefore, the first purpose of this study was to investigate the effects of combined GA and DOX treatment on cardiac function. It was hypothesized that combining GA and DOX would result in a more severe form of cardiac dysfunction than GA treatment alone or DOX treatment alone.

In an attempt to minimize cancer treatment–induced cardiac dysfunction, our laboratory has previously reported that exercise training attenuates GA-induced cardiac dysfunction [14, 18] and DOX-induced cardiac dysfunction [19–22]. However, there have been no studies investigating the effects of exercise on cardiac function during combined GA and DOX treatment. Therefore, the second purpose of this study was to examine the effects of endurance exercise training during combined GA and DOX treatment on cardiac function. It was hypothesized that exercise training would protect against combined GA and DOX treatment–induced cardiac dysfunction.

Methods

Animals and animal care

All procedures were approved by the Institutional Animal Care and Use Committee and were in compliance with the Animal Welfare Act guidelines. Female Sprague-Dawley rats ($N = 49$, ~215 g) obtained from Harlan (Indianapolis, IN) were maintained on a 12:12 h light:dark cycle and allowed access to chow and distilled water ad libitum. Animals were randomly assigned to the combined GA and DOX group ($n = 20$), the GA only group (GA, $n = 11$), the DOX only group (DOX, $n = 9$), or the control group (CON, $n = 9$).

Exercise training and drug treatments

Combined GA and DOX–treated animals were further randomized to either remain sedentary (normal cage activity) throughout treatment (GA + DOX, $n = 8$) or be exercise trained throughout treatment (EX GA + DOX, $n = 12$). Animals undergoing combined GA and DOX treatment received one implant of Zoladex® (3.6 mg GA housed in a 5 mm long × 1 mm wide biodegradable cylinder, Astra Zeneca, Macclesfield, Cheshire, UK) on day one, implanted s.c. at the scruff of the neck. Each 3.6 mg GA formulation of Zoladex® is effective at reducing sex hormone synthesis for 28 days [23]. Beginning on day 15, animals received daily 1.5 mg/kg i.p injections of 2 mg/ml DOX HCl for 10 consecutive days (cumulative 15 mg/kg). On day 29, animals then received a second Zoladex® 3.6 mg GA implant. The total treatment time for combined GA and DOX animals was 56 days. Twenty-four hours following the first GA implant, EX GA + DOX animals began training on a motorized treadmill using a progressive training protocol (Table 1). Animals were trained 5 day/week throughout the entire treatment period (56 days, 8 weeks).

Table 1 Exercise training protocol

Week of training								
	1	2	3	4	5	6	7	8
Duration (min)	20	40	60	60	60	60	60	60
Speed (m/min)	25	25	25	25	18	18	18	18
% Grade	0	0	0	3	6	9	12	15

GA animals received a s.c Zoladex® implant at the scruff of the neck on day 1, and on day 15, they began receiving daily i.p. 0.9% NaCl injections for 10 consecutive days at an equivalent volume of DOX HCl to act as sham injections. Then, on day 29, GA animals received a second s.c. Zoladex® implant at the scruff of the neck. Animals assigned to the DOX group received a s.c. implant of biodegradable Silastic tubing (5 mm long × 1 mm wide, Dow Corning, Midland, MI) to act as a sham implant on day 1, and on day 15, they began receiving daily 1.5 mg/kg i.p injections of 2 mg/ml DOX HCl for 10 consecutive days (cumulative 15 mg/kg). On day 29, DOX animals then received a second sham Silastic tubing implant. CON animals received a s.c. Silastic tubing sham implant on day 1 and day 29, and on day 15, CON animals began receiving i.p. 0.9% NaCl injections for 10 consecutive days at an equivalent volume of DOX HCl to act as sham injections. GA, DOX, and CON animals remained sedentary (normal cage activity) for the entire 56-day experimental period.

In vivo cardiac function

On day 57, in vivo cardiac function was assessed by transthoracic echocardiography using a commercially available echocardiography system (Toshiba Nemio 30;

Table 2 Animal characteristics

	CON	GA	DOX	GA + DOX	EX GA + DOX
1st Implant BM (g)	209 ± 9	216 ± 4	218 ± 5	209 ± 9	216 ± 5
Injection BM (g)†	229 ± 10	262 ± 5*	239 ± 5	259 ± 6*	252 ± 5*
2nd Implant BM (g)†	220 ± 9	293 ± 4*	234 ± 5	253 ± 6*	247 ± 7*
Final BM (g)†	263 ± 10	327 ± 7*	295 ± 16	327 ± 6*	290 ± 11
Heart mass (g)†	1.21 ± 0.05	1.33 ± 0.05	1.12 ± 0.04	1.10 ± 0.03	1.18 ± 0.03

Data are means ± SEM

CON control, *GA* goserelin acetate, *DOX* doxorubicin, *EX* treadmill exercise trained, *BM* body mass

* $P < 0.05$ versus CON

† $P < 0.05$ significant between group difference

10 MHz pediatric transducer) as described previously by our laboratory [14, 24]. Briefly, the anterior and left lateral thoracic region of sedated animals (ketamine 40 mg/kg i.p.) was shaved, and the transducer was positioned to obtain left ventricular (LV) M-mode images for the determination of septal wall thickness at systole (SWs) and diastole (SWd), posterior wall thickness at systole (PWs) and diastole (PWd), LV end-systolic diameter (LVDs) and LV end-diastolic diameter (LVDd), and fractional shortening (FS).

Pulsed wave Doppler images were then acquired from an apical view to obtain mitral and aortic valve blood flow profiles. Measurements taken at the mitral valve yielded maximal blood flow velocity (MV_{max}) and mean blood flow velocity (MV_{mean}). Similarly, measurements taken at the aortic valve yielded maximal (AV_{max}) and mean blood flow velocities (AV_{mean}).

Ex vivo cardiac function

Upon conclusion of echocardiography, hearts from anesthetized animals (heparinized [500 U] sodium pentobarbital [50 mg/kg]) were excised and perfused using an isolated working heart preparation as described previously by our laboratory [14, 19]. Briefly, the aorta was cannulated, and retrograde perfusion ensued with Krebs-Hanseleit buffer (in mM: 120 NaCl, 5.9 KCl, 2.5 $CaCl_2$, 1.2 MgCl, 25 $NaHCO_3$, 17 glucose, and 0.5 EDTA, pH 7.4) aerated with 95% O_2/5% CO_2. The pulmonary vein was then isolated and cannulated, and flow was redirected from the aorta to the left atrium to initiate the working heart mode. Following stabilization, a standardized preload and afterload was obtained for each heart (10 cmH_2O and 100 cmH_2O, respectively). A microtip pressure transducer (Scisence Inc., London, Ontario, Canada) was inserted into the LV cavity for acquisition of developed pressure (LVDP), maximal rate of pressure development (+dP/dt), and maximal rate of pressure decline (−dP/dt). For all data collection, hearts were paced at 240 bpm using a stimulus isolator (ADInstruments, Colorado Springs, CO).

Statistical analysis

All results are expressed as mean ± SEM. A one-way analysis of variance was performed to identify group differences between CON, GA, DOX, GA + DOX, and EX GA + DOX. If a significant F value was observed, Dunnetts's *post hoc* testing was done to identify significant differences from CON. For all procedures, significance was set at the $\alpha = 0.05$ level.

Results

Animal characteristics

Table 2 contains body and heart mass data from CON, GA, DOX, GA + DOX, and EX GA + DOX. No significant between-group difference was observed on day 1 (1st implant body mass [BM]), $P > 0.05$). However, at the time of the first DOX injection (Injection BM), groups receiving GA implants (GA, GA + DOX, and EX GA + DOX) had significantly greater body masses than CON ($P < 0.05$). This difference continued on day 29 (2nd Implant BM) with GA, GA + DOX, and EX GA + DOX possessing significantly greater body masses than CON ($P < 0.05$). However, on day 57 (Final BM), only GA and GA + DOX possessed greater body masses than CON ($P < 0.05$). At the time of sacrifice, a significant between-group heart mass difference was observed ($P < 0.05$), but *post hoc* testing did not reveal where differences existed versus CON ($P > 0.05$).

In vivo cardiac function

Cardiac geometry data derived from M-mode echocardiography are presented in Table 3. A significant between-group

Table 3 Echocardiograhy-derived cardiac geometry

	CON	GA	DOX	GA + DOX	EX GA + DOX
SWs (mm)†	3.04 ± 0.07	2.98 ± 0.09	2.52 ± 0.14*	2.41 ± 0.10*	2.65 ± 0.09
SWd (mm)†	1.79 ± 0.06	1.92 ± 0.08	1.61 ± 0.12	1.64 ± 0.09	1.53 ± 0.06
PWs (mm)	3.07 ± 0.10	3.04 ± 0.17	2.78 ± 0.21	2.55 ± 0.18	2.95 ± 0.09
PWd (mm)	1.56 ± 0.06	1.61 ± 0.12	1.44 ± 0.12	1.52 ± 0.09	1.50 ± 0.07
LVDs (mm)†	2.28 ± 0.18	2.47 ± 0.21	2.72 ± 0.20	3.15 ± 0.26*	2.34 ± 0.18
LVDd (mm)	5.65 ± 0.05	6.01 ± 0.16	5.63 ± 0.21	6.15 ± 0.256	5.87 ± 0.12
FS (%)†	60 ± 3	59 ± 3	51 ± 3	49 ± 3	60 ± 3

Data are means ± SEM

CON control, *GA* goserelin acetate, *DOX* doxorubicin, *EX* treadmill exercise trained, *SWs* septal wall thickness at end systole, *SWd* septal wall thickness at end diastole, PWs, posterior wall thickness at end systole, *PWd* posterior wall thickness at end diastole, *LVDs* left ventricular dimension at end systole, *LVDd* left ventricular dimension at end diastole, *FS* fractional shortening

* $P < 0.05$ versus CON

† $P < 0.05$ significant between group difference

difference was observed with SWs, and *post hoc* testing showed that DOX and GA + DOX possessed significantly thinner SWs than CON ($P < 0.05$), but this difference was not observed in EX GA + DOX ($P > 0.05$). Although a significant between-group difference was found in SWd ($P < 0.05$), *post hoc* testing did not reveal where differences from CON existed ($P > 0.05$). No significant between-group differences in posterior wall thicknesses (PWs and PWd) were observed ($P > 0.05$), but LVDs were found to be significantly greater in GA + DOX when compared to CON ($P < 0.05$). This difference was not observed in any other group ($P > 0.05$). No LVDd differences were observed ($P > 0.05$), but a significant between-group FS difference was observed ($P < 0.05$). However, *post hoc* testing did not reveal any individual group differences when compared to CON ($P > 0.05$).

Figure 1 illustrates mitral valve blood flow velocities obtained from pulsed wave Doppler images. DOX and GA + DOX possessed significantly lower maximal blood flow velocities at the mitral valve when compared to CON (Fig. 1a, $P < 0.05$). However, EX GA + DOX mitral valve maximal blood flow velocity did not differ from CON ($P > 0.05$). A similar pattern was observed with mean blood flow velocity at the mitral valve (Fig. 1b) with DOX and GA + DOX showing significantly lower blood flow velocities than CON ($P < 0.05$), but no difference was observed between EX GA + DOX and CON ($P > 0.05$).

Regarding aortic blood flow velocities, a significantly lower maximal blood flow velocity was observed in DOX and GA + DOX when compared to CON (Fig. 2a, $P > 0.05$). This difference, however, was not observed in EX GA + DOX ($P > 0.05$). As for aortic valve mean blood flow velocity, only DOX significantly differed from CON (Fig. 2b, $P < 0.05$).

Ex vivo cardiac function

Pressure data obtained from the isolated perfused working heart preparation are illustrated in Fig. 3. The isolated working heart model allows for the determination of cardiac function without the influence of circulating and systemic factors, which could be altered by treatments (e.g., endocrine, neuronal, preload, and afterload). Left ventricular developed pressure was found to be significantly lower in hearts from GA + DOX when compared to CON (Fig. 3a, $P < 0.05$), but this difference was not observed in EX GA + DOX ($P > 0.05$). Likewise, left ventricular maximal rates of pressure development (Fig. 3b) and pressure decline (Fig. 3c) were found to be significantly different in hearts from GA + DOX when compared to CON ($P < 0.05$), but this difference was not observed in hearts from EX GA + DOX ($P > 0.05$).

Discussion

The present study is the first to report that the combined effects of GA and DOX impair cardiac function. For the most part, in vivo cardiac function assessed using echocardiography was found to be similar with combined GA and DOX treatment and DOX treatment alone. However, ex vivo cardiac function assessed using the isolated working heart was found to be significantly impaired with combined GA and DOX treatment when compared to DOX treatment alone. Additionally, the present study's design was consistent with clinical practice as GA treatment prior to and during DOX treatment has been shown to preserve gonadal function [6, 25]. Since combined treatment with GA and DOX may be a potential treatment option for female cancer patients, exacerbated cardiac dysfunction

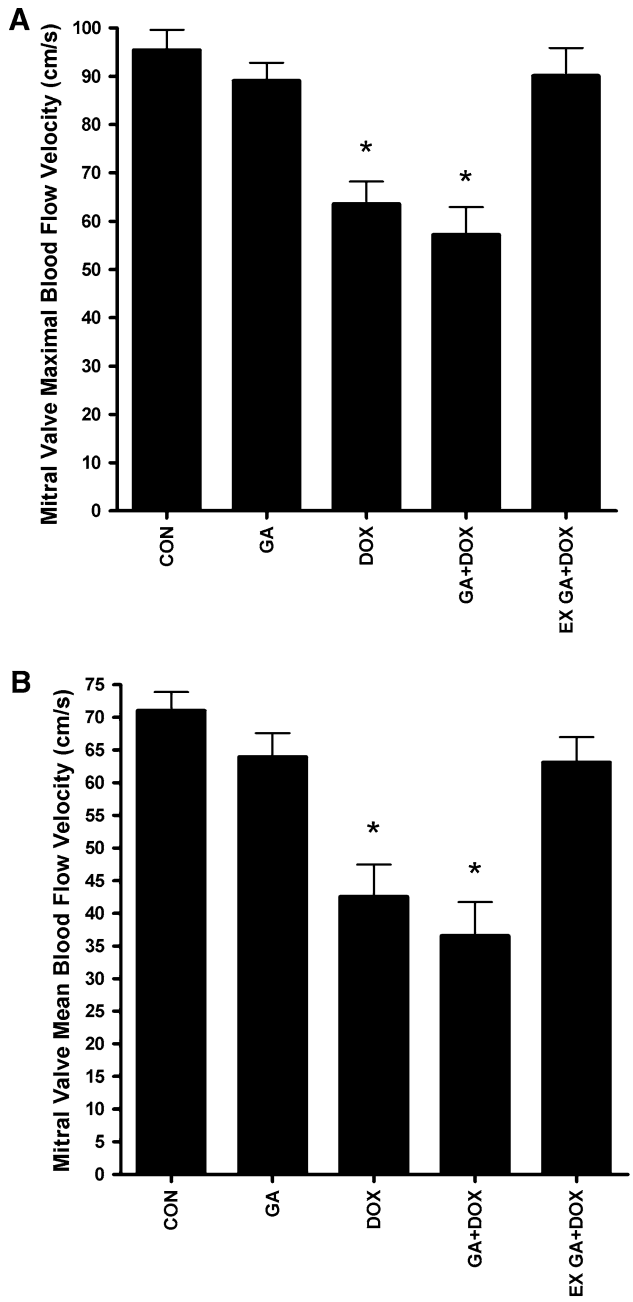


Fig. 1 Mitral valve blood inflow velocities of hearts from control, goserelin acetate, doxorubicin, combined goserelin acetate and doxorubicin, and exercise-trained combined goserelin acetate and doxorubicin–treated animals. **a** maximal blood flow velocity. **b** mean blood flow velocity. $^{*}P < 0.05$ versus CON; *CON* control, *GA* goserelin acetate, *DOX* doxorubicin, *GA + DOX* combined goserelin and doxorubicin, *EX GA + DOX* exercise-trained combined goserelin acetate and doxorubicin

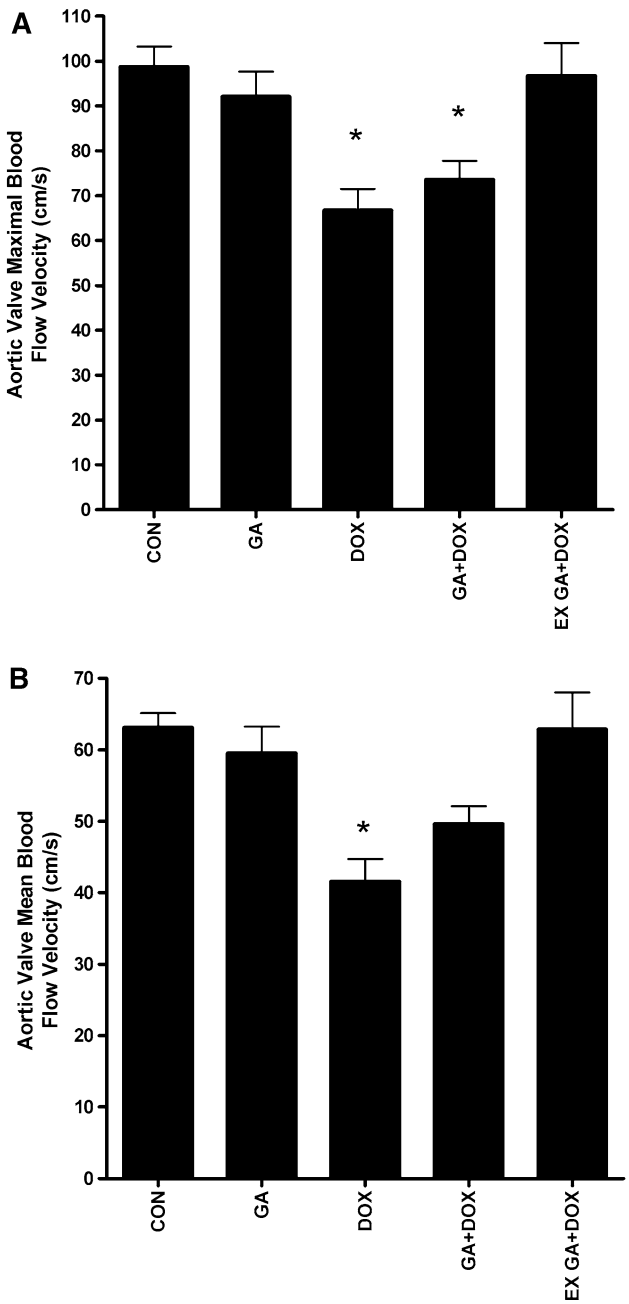
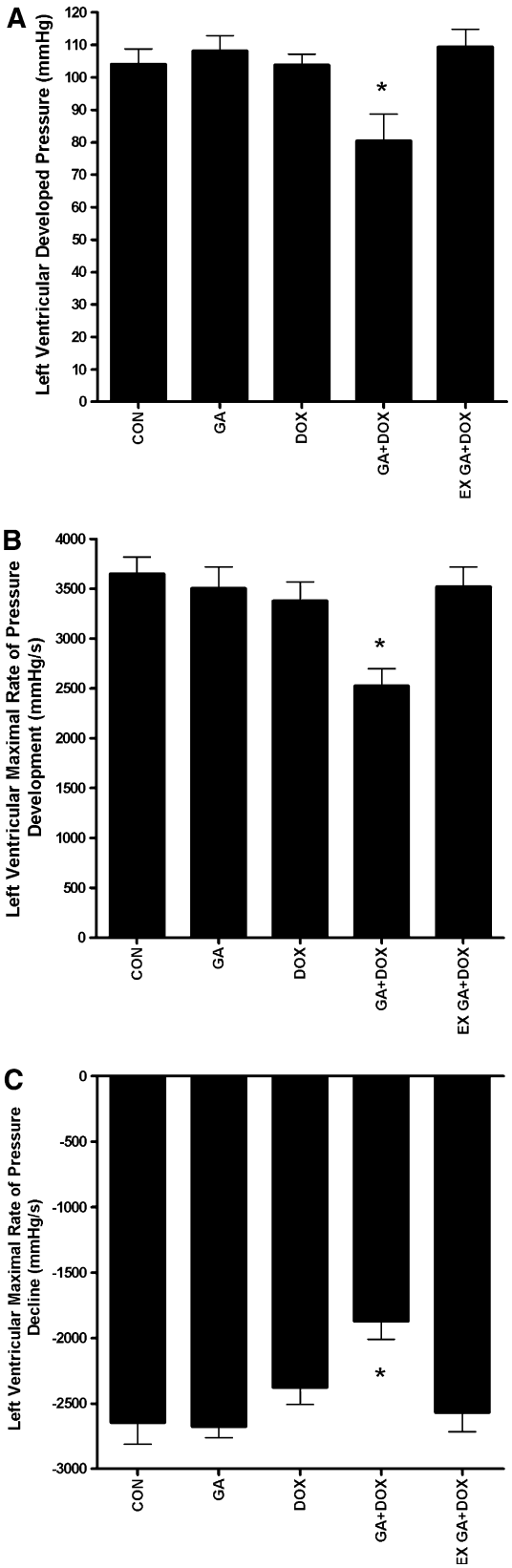


Fig. 2 Aortic valve blood inflow velocities of hearts from control, goserelin acetate, doxorubicin, combined goserelin acetate and doxorubicin, and exercise-trained combined goserelin acetate and doxorubicin–treated animals. **a** maximal blood flow velocity. **b** mean blood flow velocity. $^{*}P < 0.05$ versus CON; *CON* control, *GA* goserelin acetate, *DOX* doxorubicin, *GA + DOX* combined goserelin and doxorubicin, *EX GA + DOX* exercise-trained combined goserelin acetate and doxorubicin

(when compared to GA and DOX treatments alone) may be of clinical concern.

The female myocardium contains estrogen receptors [26], and surgical ovariectomy has been shown to have adverse effects on the cardiovascular system [12, 13]. However, the nature of estrogen withdrawal in surgical ovariectomy versus chemical ovariectomy (i.e., GA) is worth noting as the surgical approach results in immediate sex hormone withdrawal, whereas GA treatment is associated with sex hormone flare-up followed by withdrawal



◀ **Fig. 3** Isolated working heart left ventricular function of control, goserelin acetate, doxorubicin, combined goserelin acetate and doxorubicin, and exercise-trained combined goserelin acetate and doxorubicin–treated animals. **a** developed pressure. **b** maximal rate of pressure development (+dP/dt). C, Maximal rate of pressure decline (−dP/dt). *$P < 0.05$ versus CON; *CON* control, *GA* goserelin acetate, *DOX* doxorubicin; *GA + DOX* combined goserelin and doxorubicin, *EX GA + DOX* exercise-trained combined goserelin acetate and doxorubicin

[27]. Nonetheless, it is well known that estrogen plays a protective role on the cardiovascular system [28, 29], but the long-term effects of depressed estrogen levels on cardiac performance are beyond the scope of the present study. However, the positive effects of estrogen on the female myocardium may very well be associated with 17β-estradiol's antioxidant effects on the cardiovascular system [30–32], which is especially important when considering the combined effects of GA and DOX treatment on the heart as DOX cardiotoxicity is associated with oxidative stress [33].

It is well known that DOX cardiotoxicity is associated with free radical production and oxidative stress [1]. Doxorubicin undergoes redox cycling at complex I of the electron transport chain and/or forms a complex with cellular iron (DOX-Fe^{3+}), which eventuates to reactive oxygen species (ROS) production. These ROS induce myocardial oxidative stress and ultimately cellular damage in the form of lipid peroxidation [34] and protein carbonylation [35]. Reductions in antioxidant capacity have been shown to further exacerbate DOX cardiotoxicity [36], and the present study's observed exacerbated ex vivo cardiac dysfunction in hearts from animals receiving combined GA and DOX treatment may be due to a compromised antioxidant status from estrogen withdrawal (i.e., reduced 17β-estradiol) thereby leading to a greater susceptibility to DOX-induced cardiac damage.

Because of the expected cardiac dysfunction with combined GA and DOX treatment, we tested the hypothesis that exercise training would provide protection against GA + DOX-induced cardiac dysfunction. We have shown previously that exercise training protects against GA-induced cardiac alterations [14] and DOX-induced cardiotoxicity [19, 21] administered alone, but the effectiveness of exercise in combating the dual cardiac insult of combined GA and DOX treatment is unknown. The present study demonstrates that exercise training utilizing a motorized treadmill during combined GA and DOX treatment protected against cardiac dysfunction analyzed in vivo and ex vivo. In fact, all cardiac function parameters measured in EX GA + DOX did not differ statistically from CON.

Exercise training has been shown to improve antioxidant status [37], and it is possible that antioxidants were upregulated in the present study to a level capable of quenching the excessive free radicals produced by combined GA and DOX treatment. However, the protective effects of exercise extend beyond that of antioxidants. Exercise training has been shown to protect against DOX cardiac dysfunction without significant upregulation of key antioxidants [21, 34]. Estrogen ablation (GA and surgical ovariectomy) and DOX treatment alone have been shown to impair key mechanisms involved in cardiac contraction and relaxation including myosin heavy chain expression disruption [12, 20, 38] and calcium handling impairments [39, 40]. However, exercise training has been shown to protect against these aforementioned contributing factors not only in sex hormone ablation [13, 14] and DOX [19, 20] models but also in models of ischemia-reperfusion injury [41], hypertension [42], and diabetes [43]. Nonetheless, future investigations should focus on the molecular mechanisms contributing to combined GA and DOX treatment–induced cardiac dysfunction as well as the mechanisms behind exercise-induced cardioprotection.

In conclusion, combined GA and DOX treatment promoted cardiac dysfunction that was found to be more severe than GA and DOX treatment alone when analyzed ex vivo in the absence of circulating factors, which may impact myocardial performance. Exercise training, however, protected against combined GA and DOX treatment–induced cardiac dysfunction as in vivo and ex vivo measures of cardiac dysfunction did not differ between EX GA + DOX and CON. Although combined GA and DOX use to preserve ovarian function is still controversial [44, 45], these findings may help shed light on the potential increased cardiac risks. Previous studies examining the value of exercise in combating cancer treatment–related cardiac dysfunction have focused on each treatment administered singly (i.e., GA or DOX alone), and therefore, it is encouraging that exercise may be able to minimize the adverse side effects of combined treatments in cancer patients.

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Conflict of interest None.

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