Modulation of Anthracycline-Induced Cardiotoxicity by Aerobic Exercise in Breast Cancer
Current Evidence and Underlying Mechanisms

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Anthracycline-containing chemotherapy (eg, doxorubicin) is well known to cause dose-dependent, progressive cardiac damage clinically manifest as decreased left ventricular (LV) ejection fraction and, ultimately, heart failure (HF) (Table 1).1,2 Unfortunately, the only clinically accepted method to minimize injury is dose modification and/or therapy discontinuation.3 An important current challenge in breast cancer management is therefore to maximize the benefits of doxorubicin while minimizing cardiac damage. Identification and examination of new interventions to prevent and/or treat doxorubicin-induced cardiotoxicity are urgently required.

Aerobic exercise is a nonpharmacological therapy that promises to attenuate doxorubicin-induced cardiotoxicity. Aerobic exercise is well documented to improve systolic and diastolic function and attenuate pathological cardiac remodeling, resulting in improved exercise tolerance and resistance to fatigue during exertion in patients with HF.4-5 The cardioprotective properties of aerobic exercise in the context of doxorubicin have, in contrast, received scant attention. It is not generally used in cancer patients despite its lack of “side effects” and the paucity of alternative strategies to prevent/treat doxorubicin-associated cardiac damage.

As a first step in the possible use of exercise in cancer patients, we reviewed the mechanisms of doxorubicin-induced cardiotoxicity and the available evidence supporting the utility of aerobic exercise to prevent/treat cardiac injury. We also explored the molecular mechanisms that may underlie the cardioprotective properties of aerobic exercise. These findings have implications for future research regarding the application and effectiveness of exercise and doxorubicin treatment in humans.

Mechanisms of Anthracycline Cardiotoxicity

The mechanisms underlying the antitumor function of anthracyclines have been described previously.6-8 Among the proposed mechanisms of cardiac injury, doxorubicin-induced generation of reactive oxygen species (ROS)9,10 is a central mediator of numerous direct and indirect cardiac adverse consequences (for review, see Minotti et al11). In the present report, we will briefly review doxorubicin-induced oxidative injury relevant to aerobic exercise and relating to (1) accelerated myofilament apoptosis,12 (2) suppression of myofilament protein synthesis,13 (3) alterations in cardiac energy metabolism,14 and (4) ultrastructural changes to myocytes15 (Figure 1).

Myofilament Apoptosis
Myocyte cell death stems from the doxorubicin-induced generation of ROS, which, in turn, activate a multiplicity of signaling pathways that determine cell fate. A key pathway involves activation of the tumor suppressor protein p53.16 p53-dependent apoptosis involves the transcriptional activation or inhibition of certain target gene pathways such as mitogen-activated protein kinases (MAPK). Zhu et al17 found that 24 hours of doxorubicin exposure induced apoptosis in cardiac myocytes through p38-MAPK-dependent activation, whereas Yamamoto et al18 reported that p38-MAPK and c-Jun N-terminal kinases, but not extracellular signal-regulated kinases, were activated by doxorubicin in cardiomyocytes. Inhibitors of p53,19 p38-MAPK, and c-Jun N-terminal kinases20 have all been shown to prevent doxorubicin-induced apoptosis, suggesting that interventions targeting p53 or its downstream pathways could attenuate LV systolic dysfunction and decrease myocyte apoptosis.

Suppression of Myofilament Protein Synthesis
Suppression of sarcomere protein synthesis through depletion of cardiac progenitor cells (CPCs) or GATA-4-dependent gene expression is also postulated to contribute to doxorubicin-induced cardiac injury.21 De Angelis et al22 reported that doxorubicin exposure significantly reduced the population of CPCs, raising the possibility that CPC death may represent a primary event responsible for impaired myocyte turnover, accumulation of senescent cells, and the onset of ventricular dysfunction. Interestingly, delivery of
syngeneic CPCs into doxorubicin-induced failing hearts caused regeneration of cardiomyocytes, leading to improved LV performance and overall survival. Doxorubicin also downregulates GATA-4, a CPC regulatory transcription factor and an essential survival factor for postnatal cardiomyocytes. Decreased GATA-4 levels after doxorubicin exposure may, in turn, inhibit sarcomere protein synthesis, thus contributing to LV dysfunction. Accordingly, restoring or preventing GATA-4 and/or CPC depletion could be exploited as a novel means to ablate doxorubicin-induced cardiac toxicity.

Ultrastructural Changes to Myocytes
ROS also cause alterations in calcium homeostasis leading to systolic (contractile) and diastolic (lusitropic) dysfunction. Doxorubicin stimulates calcium release and inhibits sarcoplasmic reticulum calcium reuptake, resulting in cytosolic calcium overload. This calcium overload may contribute to impaired contractile function by (1) promoting release of the proapoptotic factor cytochrome c and/or (2) activating the cysteine protease calpain. Calpains initiate turnover of both regulatory and structural myofibrillar proteins through cleavage and release of large polypeptide fragments. To our knowledge, the role of calpain in doxorubicin cardiac injury has not been investigated.

Alterations in Cardiac Energy Metabolism
The heart requires ATP to sustain contraction and relaxation. As such, deficiencies in cellular homeostasis are important factors in the development of cardiomyopathy. Doxorubicin reduces cardiac energy reserves by lowering ATP and phosphocreatine levels as well as the phosphocreatine/ATP ratio. In the normal heart, AMP-activated protein kinase (AMPK) plays a crucial role in protecting cardiac cells from perturbations in energy homeostasis via activation of catabolic pathways to generate ATP. Doxorubicin reduces the level of both AMPK protein and its basic activation state, which leads to decreased phosphorylation of anti-acetyl-CoA carboxylase (ACC), an AMPK downstream target. Lack of ACC inhibition results in impairment of fatty acid oxidation; thus, interventions that increase AMPK expression and/or normalize myocyte metabolism may be an effective strategy to offset cardiotoxicity. The mechanisms underlying inhibition of AMPK are not clear; alterations in gene expression and upstream signaling require further investigation. Other important factors activated in response to metabolic perturbations include hypoxia-inducible factor-1 and peroxisome proliferator-activated receptor gamma coactivator 1. Doxorubicin has been shown to inhibit hypoxia-inducible factor-1 activity in human hematoma and prostate cancer cells, and therefore it appears biologically plausible that it may also modulate hypoxia-inducible factor-1 in the heart, although, to our knowledge, no study to date has investigated this question.

Doxorubicin also modulates structural proteins such as titin. Lim et al reported that short-term doxorubicin exposure leads to significant degradation of titin in cultured adult rat cardiomyocytes. This degradation occurred in concert with impaired relaxation as measured by τ. Intriguingly, pretreatment of myocytes with calpain inhibitors for 1 hour before doxorubicin treatment preserved the ratio of titin to myosin heavy chain similar to that of control levels and reduced the doxorubicin-induced myofibrillar disarray.

Table 1. Stages of Doxorubicin-Induced Cardiotoxicity

<table>
<thead>
<tr>
<th>Cardiotoxicity Stage</th>
<th>Time</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute</td>
<td>Within 1 wk of therapy</td>
<td>Supraventricular and ventricular arrhythmias, acute coronary syndromes, acute pericarditis/myocarditis</td>
</tr>
<tr>
<td>Early</td>
<td>Within 1 y of therapy</td>
<td>Progressive LV dilation, LV wall thinning, decreased contractility</td>
</tr>
<tr>
<td>Late</td>
<td>After 1 y of therapy</td>
<td>LV dysfunction, arrhythmias, heart failure</td>
</tr>
</tbody>
</table>

LV indicates left ventricular.
Role of Aerobic Exercise in Modulation of Cardiac Function in Health and Disease

As summarized in Table 2, aerobic exercise training improves diastolic filling and increases stroke volume, leading to augmentation of cardiac output and maximal oxygen uptake (VO$_{2}$max) in healthy women.\textsuperscript{40–42} In addition to the wealth of evidence in healthy individuals, exercise has been the cornerstone of clinical rehabilitation in HF for $>$15 years.\textsuperscript{43} Aerobic exercise attenuates pathological LV remodeling, which is associated with LV dilatation.\textsuperscript{44} The effects of endurance exercise in producing cardiac physiological hypertrophy in murine and human models are well described.\textsuperscript{45} The beneficial properties of endurance exercise are mediated, in part, through the phosphoinositide 3-kinase/serine-threonine protein kinase signaling axis via growth factors such as insulin-like growth factor-1 and insulin,\textsuperscript{46} although recent work demonstrated that reduction of the transcription factor C/EBP\textbeta also plays a critical role.\textsuperscript{47} These improvements have been shown to directly translate to increases in VO$_{2}$max, overall quality of life, and prognosis in individuals with HF.\textsuperscript{43,48} However, the largest HF exercise trial to date found a nonsignificant reduction in rehospitalization or death in patients randomized to aerobic exercise training, although such findings may be explained in part by an insufficient exercise training stimulus required to significantly modulate clinical outcomes.\textsuperscript{49} Indeed, the median duration of exercise training in the intervention group was only 95 min/wk. A recent meta-analysis reported that exercise training is associated with significant improvements in ejection fraction among patients with HF,\textsuperscript{49} suggesting that exercise can induce significant benefits for patients with cardiomyopathy. Higher-volume (increased duration and/or intensity) exercise training interventions are likely required to significantly modulate clinical outcomes such as rehospitalization or death.

Despite exercise-based rehabilitation being an integral component of clinical cardiac disease management, the molecular mechanisms underlying the effects of exercise remain incompletely understood. At the level of the cardiomyocyte, aerobic exercise improves the maximal amplitude of shortening in unloaded cells\textsuperscript{51–54} and increases the steepness of the sarcomere tension-length relationship, suggesting that the Frank-Starling mechanism is improved at the level of the single cardiomyocyte.\textsuperscript{55} Thus, improved contractile capacity of the cardiac myocyte forms a cellular basis for improved systolic function and diastolic filling.

### Exercise and Prevention of Doxorubicin-Induced Cardiotoxicity

Ten studies investigated the protective effects of aerobic exercise training before doxorubicin administration. Overall, findings indicate that exercise prevents doxorubicin-induced impairments in LV systolic and diastolic function. For example, Chicco et al\textsuperscript{62} found that maximal developed pressure velocity (dP/dt$_{\text{max}}$), a measure of LV systolic contractility, was 39% higher in the hearts of aerobically trained rats than in sedentary controls; LV relaxation (minimal developed pressure velocity [dP/dt$_{\text{min}}$]) rates also were 46% faster in endurance-trained animals. Four studies examined the effects of aerobic exercise during concurrent doxorubicin therapy. Kanter and colleagues\textsuperscript{69} demonstrated that swim training in mice decreased the histopathological myocyte damage induced by doxorubicin, and Hydock et al\textsuperscript{64} reported that aerobic exercise training protected against changes in heart rate, dP/dt$_{\text{max}}$, and dP/dt$_{\text{min}}$. Finally, our group recently found that aerobic exercise (treadmill running) in mice attenuated LV dysfunction after doxorubicin exposure as well as ablated a doxorubicin-induced increase in atrial natriuretic peptide and cardiac sarcoplasmic reticulum calcium transporter. We also explored whether these findings extended to women with newly diagnosed histologically confirmed operable breast cancer receiving doxorubicin-cyclophosphamide neoadjuvant chemotherapy. In a phase II randomized design, patients were randomized to (1) doxorubicin-cyclophosphamide alone or (2) doxorubicin-cyclophosphamide in combination with supervised endurance exercise training (cycle ergometry, 3 times per week, 60% to 100% of baseline VO$_{2}$peak) for 12 weeks. Contrary to prior reports and our preclinical data, there were no significant changes in any echocardiographic measures in either group from baseline to 12 weeks, although...
the doxorubicin-cyclophosphamide–induced increase in atrial natriuretic peptide was attenuated by exercise training.67

### Exercise and Treatment of Doxorubicin-Induced Cardiotoxicity

Two studies investigated the efficacy of aerobic exercise to treat or mitigate established cardiac injury caused by doxorubicin exposure. Specifically, Combs et al69 hypothesized that an acute bout of aerobic exercise after doxorubicin treatment would exacerbate drug-induced cardiotoxicity as evidenced by a decrease in survival rate. Results indicated that the survival rate was actually increased in exercised mice. Heon and colleagues63 also demonstrated that cardiac proapoptotic markers were decreased in rats exercised for 2 weeks after doxorubicin exposure. However, because cardiac assessments were not performed in these studies, it is not possible to determine whether exercise-induced improvements in cardiac function mediated the improvements in survival.

### Potential Molecular Mechanisms Mediating Exercise-Induced Protection of Doxorubicin Injury

Several putative gene pathways have been postulated to mediate the protective properties of the exercise–doxorubicin injury relationship (Table 4; Figure 2). These pathways are described briefly herein.

### Exercise, Reactive Oxygen Species Damage, and Heat Shock Proteins

The central role of doxorubicin-induced ROS in activation of the major pathways leading to cardiac injury suggests that exploitation of ROS generation and/or activity holds considerable therapeutic promise (Figure 1). Aerobic training protects the heart against ROS by enhancing the endogenous antioxidant protective machinery (for an extensive review on the topic, see Ascensao et al72). Exercise has also been shown to mitigate doxorubicin-induced ROS release (measured via cardiac tissue hydrogen peroxide as an indicator of superox-
ide production). Indeed, moderate-intensity treadmill running before doxorubicin treatment not only blunts ROS production from cardiac mitochondria in murine models but also significantly increases expression of antioxidant enzymes such as glutathione peroxidase 1, catalase, and manganese superoxide dismutase in cardiac tissue. Exercise-induced increase in heat shock proteins 60 and 72 may also contribute, at least partially, to myocardial protection from doxorubicin. The putative mechanisms by which heat shock proteins can protect cardiomyocytes include control of protein folding, prevention of denaturation and aggregation of intracellular proteins, and acceleration of the breakdown of damaged proteins.

Exercise and Myofilament Apoptosis
Aerobic training may be cardioprotective by regulating pro-apoptotic signaling. Ascensao et al and Chicco and colleagues reported that exercise prevents doxorubicin-induced increases in the activities of the proapoptotic mediators Bax and tissue caspase-3. In an elegant study, Werner et al demonstrated that short-term running (21 days) reduced cardiac expression of p53 (an apoptotic mediator) by one half in doxorubicin-treated mice and potently decreased apoptotic cardiomyocytes by 5-fold.

Exercise and Suppression of Myofilament Protein Synthesis
Aerobic training may stimulate mobilization and homing of CPCs and consequently limit myocyte turnover after doxorubicin treatment. This notion has not been examined in cancer models; however, Kolwicz et al found that exercise attenuated cardiomyocyte apoptosis, increased CPC proliferation by ~200%, and augmented the presence of KIT-positive cells (a stem cell factor crucial for the mobilization of progenitor cells to sites of injury) in the heart. Together, this led to a higher abundance of cardiomyocytes in exercised relative to the sedentary animals. Thus, aerobic exercise could increase the release, mobilization, and homing of CPCs after doxorubicin insult to facilitate the reparative response. Identification of the nuclear effectors of exercise may provide insight into the regulatory pathways of CPC release and myocyte survival. Intriguingly, genetic or pharmacological enhancement of GATA-4 prevents cardiomyocyte apoptosis and drug-induced cardiotoxicity. Bostrom and colleagues demonstrated that aerobic exercise significantly increases GATA-4 mRNA, suggesting that exercise regulation of this factor is plausible given that GATA-4 levels are regulated by 1-adrenergic agonists, which are modulated by exercise.

Exercise and Ultrastructural Changes to Myocytes
Aerobic exercise elicits favorable adaptations in myocardial calcium handling, which could limit cardiotoxicity by preventing calcium overload in the myocyte. Indeed, exercise reverses systolic and diastolic dysfunction by improving calcium handling (sarcoplasmic reticulum calcium release, diastolic sarcoplasmic reticulum calcium leak, and sarcoplasmic reticulum calcium sequestration). Modulation of calpain activation may also be implicated. French et al found that acute aerobic exercise attenuated ischemia/
reperfusion-induced calpain activation in rats. Importantly, these events were associated with protection against ischemia and cardiomyocyte apoptosis after an ischemia/reperfusion insult; it is not known whether such events translate to the setting of doxorubicin-induced injury.

Exercise and Alterations in Cardiac Energy Metabolism
AMPK is activated in response to changes in cellular energy, primarily a rise in AMP and a decrease in ATP or phosphocreatine. Exercise is a potent activator of AMPK activity in skeletal muscle via phosphorylation of ACC and a consequent decrease in malonyl-CoA, leading to acute stimulation of fatty acid oxidation.81,82 Aerobic training is also a potent modulator of AMPK activity in cardiac tissue. Coven et al83 demonstrated that acute exercise increased total AMPK activity as well as both α-isoforms of the catalytic subunit of AMPK and all AMPK downstream targets (eg, ACC phosphorylation). It therefore appears biologically plausible that aerobic exercise, as a potent activator of cardiac AMPK, may protect cardiac cells against doxorubicin-induced toxicity, although no study to date has investigated this.

Future Directions: The Role of Aerobic Exercise to Modulate Cardiotoxicity
Timing, Intensity, and Volume of Aerobic Exercise
More cardiotoxicity studies are required during and after doxorubicin therapy in both clinically relevant murine models and human trials. Two thirds of the literature examined the impact of exercise before doxorubicin exposure. Because the time between cancer diagnosis and initiation of anthracycline chemotherapy is generally very short, the translational relevance of these findings is limited; therefore, particular attention should be given to exercise and treatment of doxorubicin-induced cardiotoxicity. Investigation of the effects of different exercise intensities and volumes for the modulation of doxorubicin injury should be included. Most appropriate and efficacious exercise prescription for preventing and/or treating anthracycline-induced cardiotoxicity is not known. In addition to mechanistically driven investigations, human studies are required to compare the effects of different aerobic exercise prescriptions on cardiac function after an acute bout of aerobic exercise in rodents and after doxorubicin. One intriguing area is the oxidative, metabolic, and functional cardiac consequences of high-intensity aerobic exercise. These translational approaches will lead to better understanding of the role of aerobic exercise in preventing and/or treating cardiotoxicity.

Molecular Mechanisms of Aerobic Exercise Cardioprotection
Elucidation of the potential molecular mechanisms by which aerobic exercise reduces doxorubicin-induced intrinsic cardiac dysfunction and improves inotropy and lusitropy is needed. For instance, the relationship between exercise, proapoptotic mediators, and cardiac function in preclinical and clinical models would establish whether aerobic exercise can prevent doxorubicin-induced death of myocytes. Work is also needed to characterize not only the contribution of exercise-induced generation of CPCs in improving repair of damaged myocardium and limiting cardiotoxicity but also the underlying molecular mechanisms resulting in upregulation of cardiomyocyte proliferation (eg, GATA-4). Given the doxorubicin-induced downregulation of AMPK, future investigations should consider the effects of exercise on AMPK activation and its downstream targets on cardiac function. Furthermore, on the basis of experimental work in rat84 and human skeletal muscle,85 exercise-induced neuregulin signaling could be cardioprotective.86 Increasing activity of neuregulin has importantly been shown to promote myocardial regeneration after injury87 and to improve survival and cardiac function after doxorubicin-induced HF.88 Confirmation of these underlying mechanisms could be established via exploitation of novel animal models such as transgenic models and other techniques such as organ-targeted ribonucleic acid interference, which may be of use to identify exercise-induced gene functions.

Novel Markers of Cardiotoxicity
Sensitive cardiac imaging techniques need to be integrated into future studies. Specifically, newer techniques that combine detailed quantification of myocardial function (eg, strain, twisting, and untwisting)89,90 and metabolic (eg, ATP/phosphocreatine) evaluation of the heart with the use of cardiac magnetic resonance imaging hold promise for early detection of changes in cardiac performance as a result of exercise and/or cancer therapy. The use of plasma (circulating) biomarkers, in conjunction with imaging modalities, may identify signatures associated with injury phenotypes and may also be helpful in identifying those patients who would benefit most from exercise training.

Looking Past the Heart
Cardiac function is a component of a highly integrated system responsible for the transport of O2 (pulmonary-cardiac-vascular-muscular function axis) for ATP resynthesis in the skeletal muscle. As such, when cardiac injury is evaluated, it is also important to consider injury to other O2 transport organs. Exercise is one of a limited number of interventions that can augment the reserve capacity of several O2 transport organs and therefore may have tremendous clinical benefits for patients receiving doxorubicin for solid malignancies. Indeed, we found recently that doxorubicin-containing chemotherapy caused a significant decline in VO2peak despite normal LV function in women with breast cancer.67 In contrast, supervised aerobic exercise training caused significant improvements in VO2peak during concurrent doxorubicin therapy, despite decreased hemoglobin and negligible changes in LV function.

Other Patient Populations
The modulation of doxorubicin-induced cardiotoxicity by aerobic exercise in other patient groups should be investigated. In particular, cardiovascular disease is a major cause of morbidity and possibly premature mortality in adult survivors of childhood cancers.91,92 It is possible that normal physiological hypertrophy is significantly blunted in children undergoing chemotherapy via mechanisms discussed in previ-
ous sections. Given that exercise activates physiological hypertrophy of the heart, aerobic training in pediatric cancer patients could act as a crucial modulator of anthracycline-induced cardiotoxicity, thus preventing late-occurring cardiac effects.

Conclusions

Cardiotoxicity is a frequent and devastating adverse complication of doxorubicin therapy leading to morbidity, poor quality of life, and premature mortality. Evidence reviewed here indicates that aerobic exercise is a promising strategy to prevent and/or treat doxorubicin-induced cardiac injury. Future studies are required to further elucidate the molecular mechanisms underlying the cardioprotective properties of exercise before, during, and after doxorubicin exposure. Collectively, hypothesis-driven translational studies are required to define the nature and magnitude of the cardioprotective effects of exercise in the setting of anthracycline chemotherapy. Such research will lead to mechanistically driven clinical trials, which, in turn, will inform exercise prescription rehabilitation guidelines for patients with breast cancer and patients with other solid anthracycline-sensitive malignancies.

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Disclosures

None.

References


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