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SYSTEMATIC REVIEW AND META-ANALYSIS

Efficacy of Physical Exercise to Offset Anthracycline-Induced Cardiotoxicity: A Systematic Review and Meta-Analysis of Clinical and Preclinical Studies

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BACKGROUND: Physical exercise is an intervention that might protect against doxorubicin-induced cardiotoxicity. In this meta-analysis and systematic review, we aimed to estimate the effect of exercise on doxorubicin-induced cardiotoxicity and to evaluate mechanisms underlying exercise-mediated cardioprotection using (pre)clinical evidence.

METHODS AND RESULTS: We conducted a systematic search in PubMed, Embase, and Cochrane Central Register of Controlled Trials (CENTRAL) databases. Cochrane's and Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) risk-of-bias tools were used to assess the validity of human and animal studies, respectively. Cardiotoxicity outcomes reported by ≥3 studies were pooled and structured around the type of exercise intervention. Forty articles were included, of which 3 were clinical studies. Overall, in humans (sample sizes ranging from 24 to 61), results were indicative of exercise-mediated cardioprotection, yet they were not sufficient to establish whether physical exercise protects against doxorubicin-induced cardiotoxicity. In animal studies (n=37), a pooled analysis demonstrated that forced exercise interventions significantly mitigated in vivo and ex vivo doxorubicin-induced cardiotoxicity compared with nonexercised controls. Similar yet slightly smaller effects were found for voluntary exercise interventions. We identified oxidative stress and related pathways, and less doxorubicin accumulation as mechanisms underlying exercise-induced cardioprotection, of which the latter could act as an overarching mechanism.

CONCLUSIONS: Animal studies indicate that various exercise interventions can protect against doxorubicin-induced cardiotoxicity in rodents. Less doxorubicin accumulation in cardiac tissue could be a key underlying mechanism. Given the preclinical evidence and limited availability of clinical data, larger and methodologically rigorous clinical studies are needed to clarify the role of physical exercise in preventing cardiotoxicity in patients with cancer.

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Anthracyclines are a group of antineoplastic antibiotics that play an important role in the treatment of a wide variety of cancers. However, use of anthracyclines in clinical practice is associated with the development of severe side effects, of which irreversible, dose-dependent cardiotoxicity is among the most important.[1,2] In a pooled analysis of nearly 50 000 patients with cancer treated with contemporary

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CLINICAL PERSPECTIVE

What Is New?

- This systematic review and meta-analysis indicates that various exercise interventions can protect against doxorubicin-induced cardiotoxicity in rodents, with less cardiac doxorubicin accumulation as a key underlying mechanism.
- Evidence from clinical studies is limited, yet the observed effects are congruent with the hypothesis that physical exercise can yield cardioprotection in patients with cancer.

What Are the Clinical Implications?

- The preclinical evidence and lack of availability of clinical data warrants larger, methodological rigorous clinical studies to clarify the role of physical exercise in preventing cardiotoxicity in patients with cancer.

Nonstandard Abbreviations and Acronyms

DIC	doxorubicin-induced cardiotoxicity
FS	fractional shortening
FTM	forced treadmill
HSP	heat shock protein
LVP	left ventricular pressure
MD	mean difference
MHC	myosin heavy chain
mPTP	mitochondrial permeability transition pore
PE	physical exercise
PGC-1α	peroxisome proliferator-activated receptor-γ coactivator 1α
SERCA2a	sarcoendoplasmic reticulum calcium ATPase 2a
VWR	voluntary wheel running

anthracycline-based chemotherapy regimens, the incidence of clinical and subclinical cardiotoxicity after a median follow-up of 9 years was 6% (95% CI, 3%–9%) and 18% (95% CI, 12%–24%), respectively.[3] Cardiotoxicity of anthracyclines has been most extensively studied for doxorubicin, which is currently also the most commonly used anthracycline.

The pathogenesis of doxorubicin-induced cardiotoxicity (DIC), although not fully elucidated, is presumably a multifactorial complex with key roles for topoisomerase-IIβ and generation of oxidative stress. This eventually results in double-strand DNA breaks and mitochondrial dysfunction, leading to cardiomyocyte apoptosis and necrosis, with loss of functional cardiomyocytes as a result.[4-6] Since the myocardium has no regenerative capacities, this damage is irreversible. Compared with other cardiomyopathies, doxorubicin-induced heart failure has a particularly poor prognosis, with more than half of patients dying within 2 years after diagnosis.[7] As a result of the increased incidence as well as survival of patients with cancer,[8] DIC still poses a real clinical challenge.

There is growing awareness of the need to develop effective strategies to reduce DIC, of which physical exercise (PE) interventions could be a promising nonpharmacological method.[9] The potential of PE interventions to reduce DIC has been demonstrated in numerous preclinical studies.[10] Nevertheless, an estimate of the effect of exercise on cardiotoxicity has never been quantified. Moreover, preclinical studies often focus on single pathways or molecular/histological components, thereby targeting only a small fraction of the multifaceted pathogenesis of DIC. To fully understand the relative contribution of these mechanisms, a comprehensive overview incorporating all of the hypothesized pathways is necessary.

The aim of this meta-analysis and systematic review is 2-fold: (1) to provide a pooled estimate of the effect of PE on preventing DIC, and (2) to provide an overview of mechanisms underlying exercise-mediated cardioprotection in patients receiving doxorubicin-based chemotherapy in (pre)clinical studies.

METHODS

The authors declare that all supporting data are available within the article and its online supplementary files. This review was prospectively registered in the PROSPERO register (registration number: CRD42019118218), and the requirements for the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement were followed.[11] The systematic search, selection of articles, internal validity assessment, and data extraction were performed by 2 independent researchers (W. R. N. and D. B.). In cases where no consensus was reached, a third reviewer (A. M. M.) was consulted.

Search Strategy and Study Selection

A systematic search was conducted in PubMed, Embase, and Cochrane Central Register of Controlled Trials (CENTRAL) databases on August 14, 2020. The search string was a combination of search terms for anthracyclines, cardiotoxicity, and exercise and was developed in collaboration with an information specialist from the University Medical Center Utrecht, Utrecht, the Netherlands (Data S1). The search was limited to the English language, without a date restriction.

References of included full-text articles were checked to identify potentially relevant articles not found through the initial search.

Eligible studies compared any type of PE intervention, both single acute bouts and chronic exercise, in combination with anthracyclines with no intervention (ie, anthracyclines only). Outcomes included any parameters of cardiotoxicity, such as biomarkers, imaging parameters, histopathology, and clinical end points (ie, heart failure). Studies with both humans and animal subjects (with or without cancer) were eligible for inclusion. Studies in humans, however, had to be either randomized controlled trials with cardiotoxicity as one of the outcomes, or mechanistic studies focusing on underlying pathways in order to be deemed eligible. Since the effect of PE on anthracycline-induced cardiotoxicity has been previously[12] documented and might not be the same in children as in adults, given the relevant differences that exist between children and adults in anthracycline-induced cardiotoxicity (eg, different pharmacokinetics of anthracyclines)[13] and an increased susceptibility for cardiotoxicity in patients at a younger age[14] we excluded children from our study population. We also excluded studies combining doxorubicin with any other drug or substance, and conference abstracts.

Data Extraction and Analysis

We collected data using a pretested extraction form including information about the study population (type of patients, sex, age), study characteristics (number of patients per arm, duration and timing of intervention), characteristics of the anthracycline administration (timing, dose, and number of doses). Cardiotoxicity outcomes, along with their corresponding group averages, measures of variability or spread (SD or SE), and group size (number), were extracted and grouped into in vivo or ex vivo analysis and human or animal studies. In cases where the results were only reported by means of graphs, we contacted authors for numeric data. If no response was obtained, the study was excluded for the quantitative analysis.

We compared outcomes between exercising and nonexercising doxorubicin-treated patients. Parameters indicative of cardiac function that were described in ≥3 studies and were considered sufficiently clinically and statistically homogeneous were pooled. Random effects models were used to allow for heterogeneous underlying treatment effects, yet results were reanalyzed using fixed-effect models to explore whether this yielded differences regarding the summary inferences. Funnel plots with Egger test were used to detect publication for outcomes that were reported by ≥10 studies.[15] Statistical heterogeneity among studies was assessed via forest plots in combination with the I^2 statistics before undertaking the meta-analysis and presenting pooled results. Outcomes were structured around the type of intervention (forced treadmill [FTM] versus voluntary wheel running [VWR]). If a study had both an FTM and a VWR intervention arm, a single pairwise comparison (ie, FTM or VWR versus control) was entered in the appropriate analysis. Continuous outcomes were presented as mean differences (MDs) with corresponding 95% CIs and the variance of the effect size (T^2).

In a subanalysis, we tested the effect of timing of the PE intervention with respect to doxorubicin administration (ie, before [preconditioning] or concomitant with doxorubicin treatment). If a study had multiple intervention arms, the number of patients in the control group was divided approximately evenly among the comparisons (forced or voluntary versus control), which were entered as single pairwise comparisons into the analysis. Data were analyzed with R version 3.5.1 and RStudio version 1.1.456 (RStudio Inc.). A narrative synthesis was used to systematically describe the underlying mechanism for exercise-induced cardioprotection.

Risk-of-Bias Assessment

We assessed risk of bias for animal studies using the Systematic Review Centre for Laboratory animal Experimentation (SYRCLE) risk-of-bias tool.[16] We used the Cochrane risk-of-bias tool (version 1) for human studies.[17] Per category, studies could score "high," "low," or "unclear" for risk of bias. Authors were contacted for further details in case of an unclear score. Further internal validity was assessed via the reporting of quality indicators. The quality indicators were scored as "yes" or "no," corresponding to reported or unreported, respectively.

RESULTS

The search yielded 1224 original articles (Figure 1). One additional article[18] was identified through other sources. After full-text screening, 40 articles were considered eligible and included in this review. References for articles that were excluded on the basis of full-text (n=14) are provided in Data S2. Of the 40 studies included in the analysis, 3 were human randomized controlled trials[19-21] and the remaining 37 were conducted in rodents. Details of all study protocols are summarized in Table 1.

Characteristics of the Clinical Studies

The 3 clinical studies were all conducted in patients with breast cancer. Two studies by Kirkham et al[19] used the same population (n=24), describing the effects of the first exercise bout before the start of doxorubicin and cyclophosphamide–based chemotherapy, and

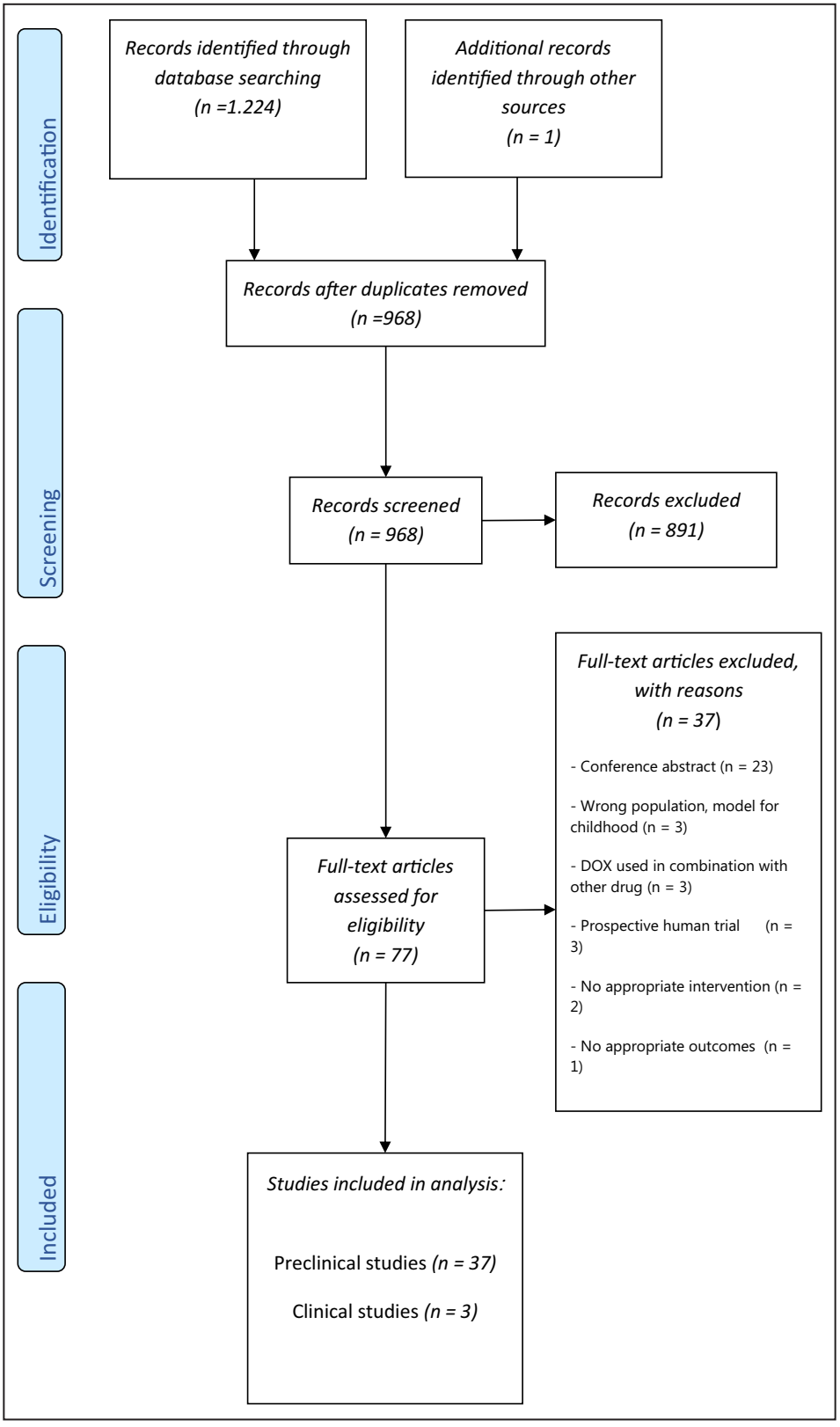


Figure 1. Flow diagram depicting the search process.
The format provided by Moger et al[11] in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement was used.

Table 1. Characteristics of the Study Protocols

Reference	Study population			Study characteristics					Doxorubicin characteristics		
	Patients/ animals*	Sex	Age	Experimental groups (n)	Exercise specifications	Exercise timing with respect to doxorubicin infusion	Control groups (n)	Timing of cardiotoxicity assessment	Timing of doxorubicin	Dose, mg/kg	No. of doses (time)
Clinical studies											
Kirkham, 2017[19]	Patients with stage I–III breast cancer, scheduled for doxorubicin-containing therapy	Female	50 y	Treadmill (13)	Acute: single bout of 30 min at 70% HRmax	Preconditioning	No vigorous exercise for 72 h before and 48 h after treatment (11)	Before first treatment (baseline) and 24–48 h After doxorubicin treatment	24 h After treadmill	60 mg/ m^2	1
Kirkham, 2018[20]	Patients with stage I–III breast cancer, scheduled for doxorubicin-containing therapy	Female	50 y	Treadmill (13)	Chronic: 4 bouts of 30 min across 6–9 wk before each doxorubicin administration, 70% HRmax	Concomitant	No vigorous exercise for 72 h before and 48 h after treatment (11)	Before first treatment (baseline) and 7–14 d after last treatment	24 h After each treadmill	Mean total 236 mg/ m^2	4 (6–9 wk)
Ma, 2018[21]	Patients with breast cancer, after operation	Female	43.1 y	Treadmill (31)	Chronic: 3 d/wk for 16 wk, 70% HRmax	Concomitant	No guidance in sports, performed normal daily activities (33)	After last exercise bout, time NS	NS	NS	4 (16 wk)
Animal studies											
Ahmadian, 2018[22]	Wistar rats	Male	3 mo, 16 mo, and 32 mo	Treadmill (8 per group)	Chronic : 5 d/wk for 3 wk, 15–17 m/min, 25–39 min/d	Preconditioning	Sedentary+doxorubicin (8 per group) Treadmill+saline (8 per group)	24 h After doxorubicin	24 h After treadmill	20	1
Alihemmati, 2019[23]	Wister rats	Male	NS	Treadmill (6)	Chronic: 5 d/wk for 6 wk, intensity 40%–90% VO_{2max}, 60 min/d	Preconditioning	Sedentary+saline (6) Sedentary+doxorubicin (6) Treadmill+saline (6)	72 h After doxorubicin	After exercise	20	1
Ascensão, 2006[24]	Wistar rats	Male	6–8 wk	Treadmill (6)	Chronic: 5 d/wk for 14 wk. Building up to 90 min/d, 30 m/min (grade 6%) by wk 5	Preconditioning	Sedentary+saline (6) Sedentary+doxorubicin (6) Treadmill+saline (6)	24 h After doxorubicin	24 h After treadmill	20	1
Ascensão, 2011[25]	Wistar rats	Male	6 wk	Treadmill (5)	Acute: single bout of 60 min, ≈5 min at 15 m/ min 0% gradient, ≈10 min 23 m/min 0% gradient, ≈45 min 25 m/min 5% gradient	Preconditioning	Sedentary+saline (5) Sedentary+doxorubicin (5) Treadmill+saline (5)	5 d After doxorubicin	24 h After treadmill	20	1
Ascensão, 2005[26]	Wistar rats	Male	6–8 wk	Treadmill (10)	Chronic: 5 d/wk for 14 wk. Building up to 30 m/min (6% grade), 90 min/d	Preconditioning	Sedentary+saline (10) Sedentary+doxorubicin (10) Treadmill+saline (10)	24 h After doxorubicin	24 h After treadmill	20	1

(*Continued*)

Table 1. (Continued)

Reference	Study population			Study characteristics					Doxorubicin characteristics		
	Patients/ animals*	Sex	Age	Experimental groups (n)	Exercise specifications	Exercise timing with respect to doxorubicin infusion	Control groups (n)	Timing of cardiotoxicity assessment	Timing of doxorubicin	Dose, mg/kg	No. of doses (time)
Ascensão, 2005[27]	Charles River CD1 mice	Male	6–8 wk	ST (11)	Chronic 5 d/wk for 14 wk. 1 h/d	Preconditioning	Sedentary+saline (11) Sedentary+doxorubicin (11) ST+saline (11)	24 h After doxorubicin	24 h After ST	20	1
Ashrafi, 2012[28]	Wistar rats	Male	8 wk	Treadmill+doxorubicin 10 mg/kg (8) Treadmill+doxorubicin 20 mg/kg (8)	Chronic: 5 d/wk for 3 wk. 15–17 m/min, 25–39 min/d	Preconditioning	Sedentary+saline (8) Sedentary+doxorubicin 10 mg/kg (8) Sedentary+doxorubicin 20 mg/kg (8) Treadmill+saline (8)	24 h After doxorubicin	24 h After treadmill	1. 10 2. 20	1
Chicco, 2005[29]	Sprague–Dawley rats	Female	NS	WR (7)	Chronic:voluntary for 8 wk	Preconditioning	Sedentary+saline (6) Sedentary+doxorubicin (7) WR+saline (8)	During and after perfusion	Directly after WR, ex vivo perfusion	10 μM	For 1 h
Chicco, 2006[30]	Sprague-Dawley rats	Male	NS	Treadmill (8)	Chronic: 5 d/wk for 2 wk. 15 m/min, 20 min/d	Concomitant	Sedentary+saline (6) Sedentary+doxorubicin (8) Treadmill+saline (6)	5 d After exercise	During treadmill	2.5	6 (2 wk)
Chicco, 2006[31]	Sprague-Dawley rats	Male	NS	Treadmill (15)	Chronic: 5 d/wk for 12 wk. Building up to 15–27 m/min (0–5% gradient), 20–60 min/d	Preconditioning	Sedentary+saline (6) Sedentary+doxorubicin (15) Treadmill+saline (6)	5 d After doxorubicin	24 h After treadmill	15	1
Dolinsky, 2013[32]	C57BL6 mice	Female	10 wk	Treadmill (9–11)	Chronic: 5 d/wk for 8 wk, building up to 18 m/min, 45 min/d	Concomitant	Sedentary+saline (9–11) Sedentary+doxorubicin (9–11) Sedentary+doxorubicin+resveratrol (9–11)	48 h After exercise	During treadmill	8	4 (4 wk)
Farzanegi, 2019[33]	Wistar rats	NS	40–50wk	ST (6)	Chronic: 3 d/wk for 8 wk. Building up from 5 to 30 min/d	Concomitant	Sedentary+saline (6) Sedentary+doxorubicin (6) Sedentary+doxorubicin+saline (6) Sedentary+doxorubicin+GA (6) ST+doxorubicin+GA (6)	Directly after completion of exercise	During ST in week 1	8.5	1
Hall, 2019[34]	Sprague-Dawley rats	Female	10 wk	WR (8)	Chronic: voluntary for 17 wk	Preconditioning	Sedentary+saline (6) Sedentary+CR (6) Saline+WR (6) Saline+CR+WR (8) Doxorubicin (8) CR+doxorubicin (8) CR+doxorubicin+WR (8)	5 d After doxorubicin	5 d After WR	15	1
Hydock, 2008[35]	Sprague-Dawley rats	Male	NS	Treadmill (24) WR (21)	Chronic: 1. 5 d/wk for 10 wk. 20–60 min/d, 20–30 m/min (0–18% grade). 2. voluntary for 10 wk	Preconditioning	Sedentary+saline (30) Sedentary+doxorubicin (28) Treadmill+saline (24) WR+saline (20)	5 Or 10 d after doxorubicin	24 h After treadmill/WR	10	1

(*Continued*)

Table 1. (Continued)

Reference	Study population			Study characteristics					Doxorubicin characteristics		
	Patients/ animals*	Sex	Age	Experimental groups (n)	Exercise specifications	Exercise timing with respect to doxorubicin infusion	Control groups (n)	Timing of cardiotoxicity assessment	Timing of doxorubicin	Dose, mg/kg	No. of doses (time)
Hydock, 2009[36]	Sprague-Dawley rats	Female	NS	WR (9)	Chronic: voluntary for 7 wk	Concomitant	Sedentary (10) Sedentary+doxorubicin (8)	7 d After doxorubicin	During WR, after wk 1	2.5	6 (6 wk)
Hydock, 2011[37]	Sprague-Dawley rats	Male	NS	Treadmill (17) WR (23)	Chronic: 5 d/wk for 10 wk. 30 m/min, 60 min/d. Voluntary for 10 wk	Preconditioning	Sedentary+saline (11) Sedentary+doxorubicin (14) Treadmill+saline (12) WR+saline (17)	4 wk After doxorubicin	24 h After treadmill/WR	1	10 (10 d)
Hydock, 2012[38]	Sprague-Dawley rats	Female	NS	WR+doxorubicin$_{dly}$ (9) WR+doxorubicin$_{wk}$ (10)	Chronic: voluntary for 10 wk	Concomitant	Sedentary+saline$_{dly}$ (8) Sedentary+saline$_{wk}$ (7) Sedentary+doxorubicin$_{dly}$ (15) Sedentary+doxorubicin$_{wk}$ (10) WR+saline$_{dly}$ (8) WR+saline$_{wk}$ (8)	Directly after completion of exercise	During WR	1$_{dly}$ 2.5$_{wk}$	15 (15 d) 6 (6 wk)
Jensen, 2013[39]	Sprague-Dawley rats	Female	10–11 wk	Treadmill (47–58) WR (40–51)	Chronic: 5 d/wk for 10 wk. 13–30 m/min (5%–18% grade), 60 min/d. Voluntary for 10 wk	Preconditioning	Sedentary+saline (5–9) Sedentary+doxorubicin (38–61)	1, 3, 5, 7 Or 9 d after doxorubicin	24 h After treadmill/WR	10	1
Ji, 1994[40]	Sprague-Dawley rats	Female	6 mo	Treadmill (7) Treadmill+REC (7)	Acute: single bout Until exhaustion Treadmill+REC=30 min recovery	Postconditioning	Sedentary+saline (13) Sedentary+doxorubicin (7) Treadmill+saline (6) Treadmill+REC+saline (7)	Directly after exercise	24 h And 30 min before treadmill/ treadmill+REC	4	2
Kanter, 1985[41]	Swiss White mice	Male	5 wk	ST (20)	Chronic: 5 d/wk for 21 wk. Building up to 1 h/d	Concomitant	Sedentary (20) Sedentary+doxorubicin (22) ST (21)	After 9 wk of exercise and after 21 wk. Histology only assessed after 21 wk	During treadmill, starting from wk 9	4	10 (7 wk)
Kavazis, 2010[42]	Sprague-Dawley rats	Male	4–6 mo	Treadmill (7) Treadmill$_{cold}$ (6)	Chronic: 5 d/wk for 5 d. 30 m/min, 60 min/d. Both in cold (4°C) and normal temperature	Preconditioning	Sedentary+saline (8+7) Sedentary+doxorubicin (6) Treadmill+saline (7) Treadmill$_{cold}$+saline (6)	24 h After doxorubicin	Directly after treadmill	20	1
Kavazis, 2014[18]	Sprague-Dawley rats	Male	6 mo	Treadmill (6)	Chronic: 1 time per d for 5 d. 30 m/min, 60 min/d	Preconditioning	Sedentary+saline (6) Sedentary+doxorubicin (6) Treadmill+saline (6)	24 h After doxorubicin	24 h After treadmill	20	1
Lee, 2020[43]	C57BL6 mice	Male	8 wk	Treadmill (10)	Chronic: 5 d/wk for 4 wk. 13 m/min, 60 min/d	Postconditioning	Sedentary+saline (10) Sedentary+doxorubicin (10)	24 h After exercise	24 Before exerciseE	20	4 (4 wk)

(*Continued*)

Table 1. (Continued)

Reference	Study population			Study characteristics					Doxorubicin characteristics		
	Patients/ animals*	Sex	Age	Experimental groups (n)	Exercise specifications	Exercise timing with respect to doxorubicin infusion	Control groups (n)	Timing of cardiotoxicity assessment	Timing of doxorubicin	Dose, mg/kg	No. of doses (time)
Lien, 2015[44]	Spraque-Dawley rats	Male	10 wk	Treadmill+ doxorubicin 10 mg/kg (10) Treadmill+ doxorubicin 15 mg/kg (13) WR+doxorubicin 10 mg/kg (10) WR+doxorubicin 15 mg/kg (12)	Chronic: 1 time per d for 5 d. 24 m/min, 60 min/d.	Preconditioning	Sedentary+saline (14) Sedentary+doxorubicin 10 mg/kg (10) Sedentary+doxorubicin 15 mg/kg (13) WR+saline (13) Treadmill+saline (13)	5 d After doxorubicin	24 h After treadmill/WR	10 15	1
Mackay, 2019[45]	C57BL6 mice	Male	5 wk	Treadmill (8)	Chronic: 1 time per d for 5 d. On 70% of max speed, 60 min/d	Concomitant	Sedentary+saline (9) Sedentary+doxorubicin (8) Treadmill+saline (11) MET+sedentary+saline (13) MET+doxorubicin (7)	3 d After doxorubicin	1 h After treadmill	15	1
Marques-Aleixo, 2015[46]	Spraque-Dawley rats	Male	6 wk	Treadmill (6) WR (6)	Chronic: 5 d/wk for 12 wk, 18–27 m/min, 60 min/d. Voluntary: 12 wk	Concomitant	Sedentary+saline (6) Sedentary+doxorubicin (6) Treadmill+saline (6) WR+saline (6)	48 h After exercise	During treadmill/WR, starting from wk 5	2	7 (7 wk)
Marques-Aleixo, 2018[47]	Spraque-Dawley rats	Male	6 wk	Treadmill (NS) WR (NS)	Chronic:. 5 d/wk for 14 wk, 18–27 m/min, 60 min/d. Voluntary: 14 wk	Concomitant	Sedentary+saline (NS) Sedentary+doxorubicin (NS) Treadmill+saline (NS) WR+saline (NS)	48 h After exercise	During treadmill/WR, starting from wk 5	2	7 (7 wk)
Morton, 2019[48]	Spraque-Dawley rats	Female	6 mo	Treadmill (10)	Chronic: 5 d/wk for 2 wk. 30 m/min, 60 min/d	Preconditioning	Sedentary+saline (10) Sedentary+doxorubicin (10) Treadmill+saline (10)	48 h After doxorubicin	24 h After treadmill	20	1
Parry, 2015[49]	Fischer 344 rats (inoculated with tumor cells after wk 11)	Female	12 wk	WR (36)	Chronic: voluntary for 12–13 wk	Preconditioning	Sedentary+saline (30) Sedentary+doxorubicin (36) WR+saline (30)	1, 3, Or 5 d after doxorubicin	24 h After tumor reached 1 cm	12	1
Pfannenstiel, 2018[50]	Spraque-Dawley rats	Male	10 wk	RT (15)	Chronic: RT for 12 wk by encouraging rats to stand up heightening the food/water supply	Preconditioning	Sedentary+saline (9) Sedentary+doxorubicin (15) RT+saline (9)	5 d After doxorubicin	24 h After RT	12.5	1
Phungphong, 2020[51]	Sprague-Dawley rats	Female	9 wk	Treadmill	Chronic: 5 d/wk for 14 d, 21 m/min, 2x 10–30 min/d	Concomitant	Sedentary/sham operated (11) OVX (12) OVX+doxorubicin (11) OVX+doxorubicin+estrogen (12) OVX+doxorubicin+mast cell stabilizer (13)	48 h After exercise	During treadmill	2.5	6 (2 d)

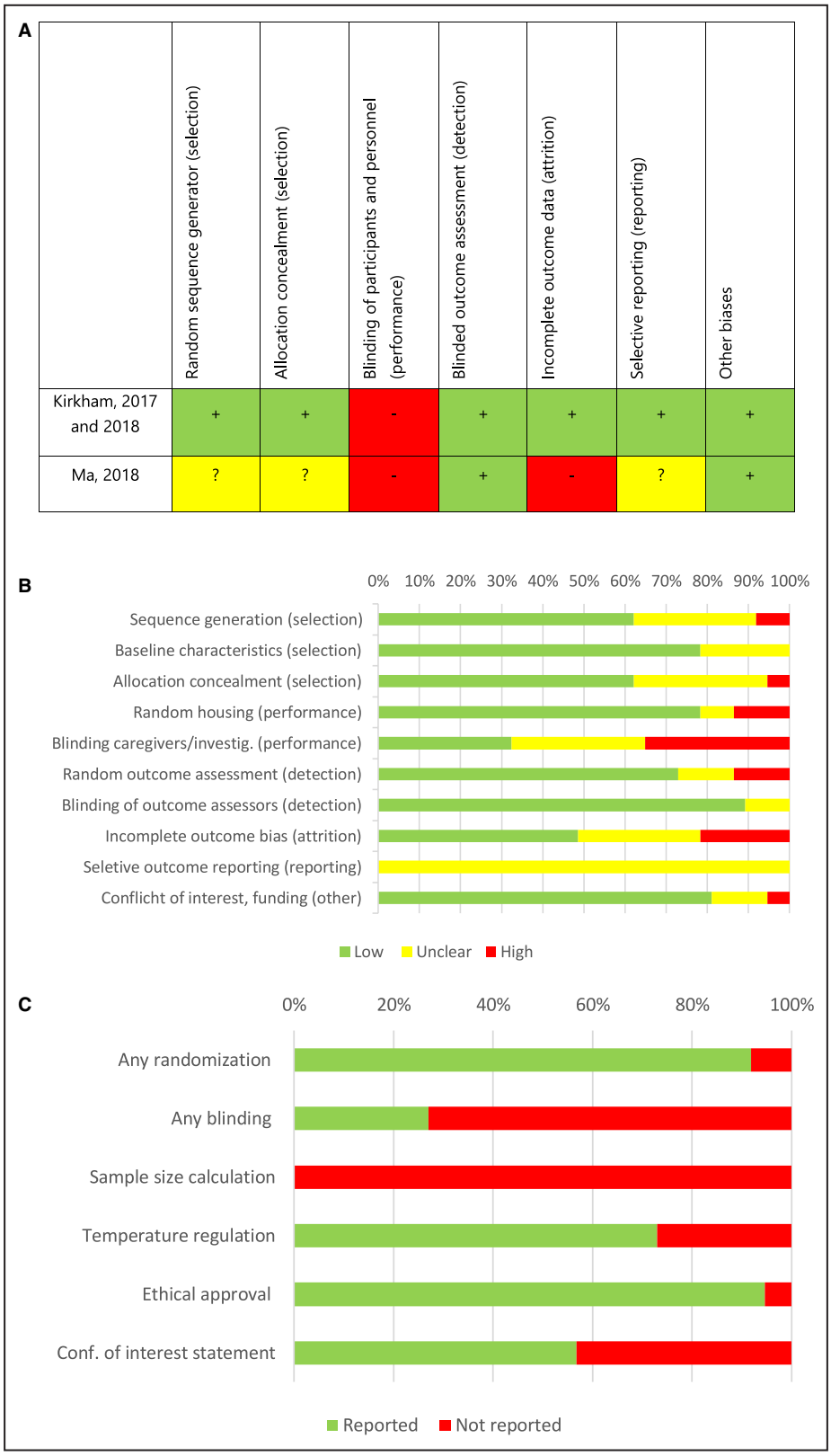
(*Continued*)

Table 1. (Continued)

Reference	Study population			Study characteristics					Doxorubicin characteristics		
	Patients/ animals*	Sex	Age	Experimental groups (n)	Exercise specifications	Exercise timing with respect to doxorubicin infusion	Control groups (n)	Timing of cardiotoxicity assessment	Timing of doxorubicin	Dose, mg/kg	No. of doses (time)
Shirinbayan, 2012[52]	Wistar rats	Male	10 wk	Treadmill (8)	Chronic: 5 d/wk for 3 wk, 15–17 m/min, 23–39 min/d	Preconditioning	Sedentary+saline (8) Sedentary+doxorubicin 10 mg/kg (8) Sedentary+doxorubicin 20 mg/kg (8) Treadmill+saline (8)	24 h After doxorubicin	24 h After treadmill	10 20	1
Smuder, 2013[53]	Sprague-Dawley rats	Male	6 mo	Treadmill (6)	Chronic: 1 time per d for 10 d, building up to 30 m/min, 60 min/d	Preconditioning	Sedentary+saline (6) Sedentary+doxorubicin (6) Treadmill+saline (6)	24 h After doxorubicin	Directly after treadmill	20	1
Sturgeon, 2014[54]	C57BL6 mice (injected with melanoma cells)	Male	6–8 wk	Treadmill (9)	Chronic: 5 d/w for 2 wk, 10 m/min, 45 min/d	Concomitant	Sedentary+saline (7) Sedentary+doxorubicin (8) Treadmill+saline (8)	48 h After exercise	During treadmill	2	2 (2 wk)
Werner, 2008[55]	C57BL6 mice eNOS–/– micecmar TERT–/– mice	Male	8 wk	WR (6–12)	Chronic: voluntary for 3 wk	Preconditioning	Sedentary+doxorubicin (8–12) Sedentary (8–12) $WR_{6\,mo}$ $Sedentary_{6\,mo}$	NS	After WR (further NS)	22.5	1 (24 h)
Wonders,2008[56]	Sprague-Dawley rats	Male	NS	Treadmill (NS)	Acute: single bout of 60 min, ≈5 min at 15 m/min 0% gradient, ≈10 min 23 m/min 0% gradient, ≈45 min 25 m/min 5% gradient	Preconditioning	Sedentary+saline (NS) Sedentary+doxorubicin (NS) Treadmill+saline (NS)	5 d After doxorubicin	24 h after treadmill	15	1
Yang, 2020[57]	Sprague-Dawley rats	Male	NS	Treadmill (8)	Chronic: 3 d/wk for 4 wk, 12 m/min, 60 min/d	Concomitant	Sedentary+saline (8) Sedentary+doxorubicin (8)	24 h After exercise	24 After doxorubicin	20	15 (3/wk, 5 wk)

Experimental groups refers to the intervention these groups underwent. All of the experimental groups also underwent doxorubicin administration. Details regarding doxorubicin are shown in the Doxorubicin characteristics columns. Doxorubicin dosages were specified in other columns in case multiple dosage groups were used. For studies reporting different numbers regarding the study populations, the largest number reported is shown. Doxorubicin administration during exercise was started in the first week of exercise, unless otherwise stated. For Hydock, 2012, "dly" and "wk" in subscript refers to the drug administration schemes, which were respectively daily in 15 consecutive days and weekly in 6 weeks. CR indicates calorie restriction; GA, garlic extract; HRmax, maximum heart rate; MET, metformin; NS, not specified; OVX, ovariectomized; REC, 30 minutes of recovering after exercise; RT, resistance training; ST, swimming training; VO_{2max}, maxium oxygen consumption; and WR, voluntary wheel running.

*The study population in the preclinical studies are animals without cancer, unless otherwise stated.



of a total of 4 bouts on subclinical cardiotoxicity (ie, strain and biomarkers) in the first and second report, respectively. The PE intervention consisted of four 30-minute vigorous-intensity treadmill exercise bouts before each administration of doxorubicin. The study by Ma[21] (n=64) evaluated the effect of a 16-week PE program during chemotherapy. Women allocated to the intervention grouped attended three 50-minute

Figure 2. Results of the risk of bias assessment for human studies (A), animal studies (B), and quality indicators for animal studies (C).

A, Results of the risk of bias assessment for human studies. The risk of bias was assessed using the Cochrane risk of bias tool. The color of the cells depicts the estimated risk of bias for the studies shown on the y-axis in the categories shown on the x-axis. Green, yellow, and red cells represent a low, unclear, and high risk of bias, respectively. Blinding of participants was not possible because of the nature of the intervention. **B**, Results of the risk of bias assessment for animal studies. The risk of bias was assessed using the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) risk of bias tool. Shown are the percentages of studies that were judged to have an "unclear," "low," or "high" risk of bias in the categories depicted on the y-axis. **C**, The reporting of quality indicators for animal studies. Shown are the percentages of studies that did or did not report (reported or not reported, respectively) the quality indicators depicted on the y-axis.

supervised treadmill sessions per week, while control group participants were asked to maintain their normal activity pattern. Cardiac function was assessed with echocardiography after 4 cycles of anthracyclines.

Characteristics of the Preclinical Studies

In the 37 animal studies, conducted in mice or rats, the type and duration of PE interventions widely varied. The majority (n=21) used FTM interventions, 5 studies used a VWR protocol,[29,34,36,38,49,55] and 6 studies included both FTM and VWR.[35,37,39,44,46,47] Three studies used a swimming training protocol,[27,33,41] and 1 study used a protocol to mimic resistance training.[50] The duration varied from a single bout[40,56] to a PE program that lasted 21 weeks.[41] Doxorubicin was the only anthracycline administrated with variation in cumulative dose, number and frequency of doses, and timing with respect to the exercise intervention (ie, preconditioning, concomitant with doxorubicin treatment, or postconditioning). Cumulative doxorubicin dose varied between 4 mg/kg and 100 mg/kg, with 20 mg/kg being the most used dosage (n=14). The majority of the studies (n=23) used a preconditioning protocol, where doxorubicin was administrated up to 24 hours after completion of the intervention. Twelve studies had protocols in which, at least to some extent, doxorubicin treatment ran parallel to the PE intervention, and 2 studies used a postconditioning protocol.[40,43]

Last, cardiotoxicity was described via various outcomes, including left ventricular (LV) function and morphology, histopathology, and biochemical analysis. The former was assessed in vivo as well as ex vivo using echocardiography or isolated heart perfusion, respectively.

Risk-of-bias assessment is presented in Figure 2 and Data S3. In brief, the 2 clinical reports by Kirkham et al were overall scored as low risk of bias, while the study by Ma was rated as having relatively low methodological quality. The animal studies widely varied in terms of risk of bias, with most studies scoring low on the items of selection and attrition bias, and relatively high on the risk of performance bias. Risk of publication bias, ie, detected by asymmetry in the funnel plots, was not assessed since no outcomes were reported by ≥10 studies.

Effect of Exercise on DIC

Clinical Studies

A total of 3 studies reported about PE on anthracycline-induced cardiotoxicity in patients with cancer. However, results were not pooled since 2 reports made use of the same study population and had substantial clinical differences (eg, duration of PE intervention and timing of outcome assessment) compared with the third study. The first study by Kirkham et al[19] showed that a single treadmill session mitigated an increase in NT-proBNP (N-terminal pro-B-type natriuretic peptide) 24 to 48 hours after the first anthracycline treatment. Nevertheless, echocardiographic parameters, including strain, were comparable between the exercise (n=13) and control group (n=10). In the second report,[20] the 4 exercise bouts did not prevent a rise in cardiac biomarkers (NT-proBNP and cardiac troponin). Longitudinal strain and LV ejection fraction remained unchanged in both groups before and after chemotherapy. However, the authors reported that the PE group had fewer changes in hemodynamics than the control group. The last study reported that, in the PE group (n=31), LV ejection fraction significantly increased after chemotherapy (from 55%±3.5% to 60%±2.9%), while a decrease (51%±5.6% to 47%±2.6%, $P<0.05$) was observed in the control group (n=33). However, between-group differences were not presented and risk of bias was high.[21]

Animal Studies: Pooled Analysis on In Vivo Cardiotoxicity

All animal studies that assessed in vivo parameters of cardiotoxicity (n=13),‡ used echocardiographic-derived fractional shortening (FS) as a marker for systolic LV function. All studies used a treadmill intervention as PE program, except for 1 study that used a protocol to mimic resistance training.[50] Results of this study, as well as those from a study where no numeric data

‡References 32,34,35,37–39,44,48,49,50,51,54,57

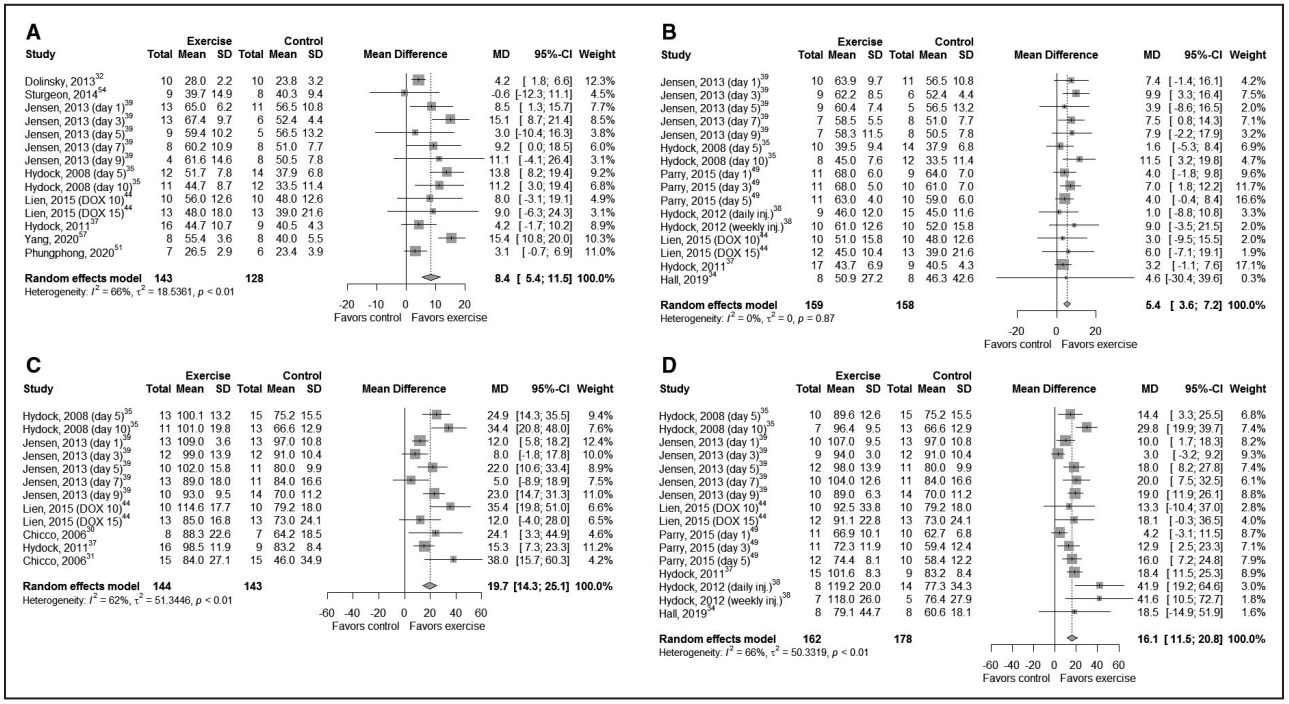


Figure 3. Forest plot of forced (A) or voluntary (B) physical exercise (PE) interventions on fractional shortening on echocardiography (in vivo) and of forced (C) or voluntary (D) PE interventions on isolated heart perfusion (ex vivo), compared with nonexercised controls in animal studies.
Results are presented as mean difference (MD) with 95% CI.

could be obtained,[48] were excluded from quantitative analyses. Both of these studies found an absolute improvement in FS in exercised rodents as compared with controls of 13% and ≈15%, respectively.[48,50]

Overall, the results of these studies demonstrated that PE interventions are able to mitigate doxorubicin-induced impairment in FS. For studies using FTM interventions (n=8),[32,35,37,39,44,51,54,57] FS was significantly higher in exercised versus nonexercised rodents (8.4%; 95% CI, 5.4–11.5 [T^2=18.5]) (Figure 3A). A pooled analysis of the 7 studies that used a VWR intervention revealed a slightly lower MD (5.4%; 95% CI, 3.6–7.2 [T^2=0])[34,35,37,38,39,44,49] (Figure 3B).

In a subanalysis regarding the timing of the PE intervention, ie, preconditioning (n=6)[34,35,37,39,44,49] or concomitant with doxorubicin administration (n=5),[32,38,51,54,57] the MD was 7.0% (95% CI, 5.2–8.7; T^2=0) and 5.9% (95% CI, 1.0–10.7; T^2=23.8) respectively. Heterogeneity was substantial in the latter subanalysis (I^2=78%, chi-square test: P<0.01) (Data S4, Figures S1 and S2).

Ex Vivo Parameter: Pooled Analysis on LV Pressure

Ex vivo cardiotoxicity was assessed in 12 animal studies via retrograde isolated heart perfusion,[§] in which the myocardium is perfused via the coronary system to assess cardiac function in controlled loading conditions. LV pressure (LVP), the maximum developed pressure per time unit (dP/dt-max), and the maximum rate decline per time unit (dP/dt-min) were measured. LVP represents cardiac function as a whole, whereas the latter are indicators of systolic and diastolic function, respectively.[58] Protocols for isolated heart perfusion were mostly comparable. One exception was a study by Chicco et al,[29] where doxorubicin was administrated ex vivo (ie, the hearts were transplanted before doxorubicin administration). This study, as well as a study that used only a single exercise bout[56] and a study that used a resistance intervention,[50] were therefore excluded from the pooled analyses.

For LVP, all studies reported results significantly favoring PE (Figure 3C and 3D). For studies using FTM interventions (n=6),[30,31,35,37,39,44] our meta-analysis yielded an MD of 19.7 mm Hg (95% CI, 14.3–25.1; T^2=51.3) compared with the nonexercised rodents. The MD was 16.1 mm Hg (95% CI, 11.5–20.8; T^2=50.3) for studies with VWR interventions versus controls (n=7).[34,35,37,38,39,44,49] Results of dP/dt-max and dP/dt-min were comparable to those of LVP and are presented in Data S5 and Figures S3 through S6.

In addition to isolated heart perfusion, 5 studies used histology or electron microscopy to establish exercise-mediated cardioprotection ex vivo.[26,33,41,46]

[§]References 29–31,34,35,37–39,44,49,50,56

Although these studies were too clinically heterogeneous for meta-analysis, all of them showed that microscopically established cardiac abnormalities induced by doxorubicin were mitigated through exercise, via swimming training,[33,41] FTM,[23,26,43,46,51,57] or VWR[46] interventions.

The analyses of echocardiography and perfusion parameters were repeated using fixed-effect models and yielded no relevant differences from the results based on the random effects models (data not shown).

Mechanisms Underlying Exercise-Mediated Cardioprotection

Multiple pathways were addressed by the included studies (Table 2) and were grouped into those associated with increased oxidative stress and doxorubicin accumulation. An overview of the available studies per pathway is presented in Table 3.

Exercise and Oxidative Stress

The induction of oxidative stress through inciting overproduction of reactive oxygen species is believed to play an important role in the pathogenesis of DIC, supported by increased levels of markers of oxidative stress, such as malondialdehyde, after doxorubicin administration.[59-61] Increased oxidative stress is related to a variety of other pathways or proteins, including upregulation of antioxidants or heat-shock proteins, inflammation, disruption of calcium homeostasis, autophagy, and apoptosis. Oxidative stress, or one of the related pathways, is the most commonly reported mechanism, with 21 studies focusing on how PE influences oxidative stress.[3]

Eighteen studies investigated the effects of PE on antioxidants, which can counterbalance oxidative stress.[22,23,24,25,26,27,28,29,30,31,32,33,40,41,42,43,46,51,52] The majority of these studies observed a beneficial effect of PE on antioxidants, meaning upregulation of antioxidants in the PE groups compared with controls. The most frequently studied antioxidants were superoxide dismutase and catalase. Although the effect sizes widely varied, the relative increase observed most often was in the range of 30% to 50%. In 4 of these studies, upregulation of antioxidants coincided with a reduction of DIC on either echocardiography[32] or histopathology.[27,33,41] Nonetheless, the role of antioxidants is ambiguous, as not all studies reported upregulation of the same antioxidants and some studies reported conflicting results. For superoxide dismutase, for example, no attenuation was found in 5 studies,[25,27,29,30,31] while 1 of these studies reported upregulation of catalase[27] and 3 studies found a cardioprotective effect of PE against DIC via isolated heart perfusion.[29-31] In contrast, in the study by Phungphong et al using ovariectomized rats, attenuation of oxidative stress markers by PE was observed but without preservation of LV function on echocardiography.[51]

Heat shock proteins (HSPs) are proteins that increase in various situations of cellular stress, eg, heat shock and ischemia.[62] From the family of HSPs, HSP60, HSP70, and HSP72 were investigated by 10 studies that yielded conflicting results. While the majority described significant upregulation of HSPs by a PE intervention,[22,24,26,29,31,52] 1 study reported upregulation of some, but not all HSPs2[27] or even a decrease in HSP following exercise.[33] Moreover, Kavazis et al[42] found that a swimming intervention, performed under cold conditions, which prevented upregulation of HSP72, still yielded cardioprotection in doxorubicin-treated rodents.

Cardiac inflammation and remodeling as a result of oxidative stress and impaired mitochondrial function[63] is hypothesized to be an important pathway in the development of DIC and may eventually lead to increased myocardial fibrosis.[57] Ahmadian et al[22] demonstrated that a 3-week preconditioning program resulted in lower levels of C-reactive protein in animals <3 months of age compared with controls. Similarly, a treadmill program concomitant with doxorubicin administration counteracted activation of an inflammatory response (interleukin 8, tumor nectrosis factor α) and upregulation of fibrotic markers (transforming growth factor β1), which was supported by reduced cardiac fibrosis and preserved systolic function on histology and echocardiography, respectively.[57] Phungphong et al,[51] using a 2-week treadmill program during doxorubicin treatment, found no attenuation of inflammatory markers (interleukin 6 and interleukin 10), but reported significantly less myocardial fibrosis in the exercise group compared with controls (7.0%±0.13% versus 8.0%±0.27% collagen deposition, respectively).

Deregulation of intracellular calcium homeostasis has also been proposed as a mechanism underlying DIC. This is often attributed to downregulation of sarcoendoplasmic reticulum calcium ATPase 2a (SERCA2a).[64] SERCA2a is the most often expressed isoform of sarcoendoplasmic reticulum calcium ATPase in cardiomyocytes and is responsible for pumping calcium from the cytosol into the sarcoplasmatic reticulum.[37] Calcium is of key importance in many cardiac functions and an interruption could result in a variety of diseases, including systolic and diastolic dysfunction and arrhythmias.[65] In 2 studies, treadmill interventions of 5 days and 8 weeks, respectively, before and during doxorubicin treatment partially prevented downregulation of SERCA2a.[32,44] Lien et al,[44] investigating doxorubicin dosages of 10 mg/kg and 15 mg/kg in forced and voluntary exercise groups, suggested that both exercise modalities can preserve SERCA2a, although FTM appeared more effective with the higher

‖References 22–24,26–33,40,42,43,45,46,50–52,56,57

Table 2. Overview of Pathways Studied by the Animal Studies and Their Main Results

Reference of animal study	Pathway(s)	Effect of PE intervention	Summary of main results
Ahmadian, 2018[22]	Marker of oxidative stress (malondialdehyde), antioxidants (SOD), HSP (HSP70), inflammation markers (IL-10, CRP)	Yes	A preconditioning exercise program had a beneficial effect on antioxidant capacity in all 3 age groups, yet the strongest effect was observed in the group of young rats
Alihemmati, 2019[23]	Apoptosis (Bax, BCL2, caspase 6, and gene and microRNA expression)	Yes	Preconditioning high-intensity interval training attenuated expression proapoptotic and apoptotic factors and microRNA, counteracting myocardial apoptosis
Ascensão, 2006[24]	Markers of oxidative stress (gluthatione analysis), Antioxidants (SOD), HSPs (HSP60, HSP70), mitochondrial respiratory functioning	Yes	Heart mitochondria of DOX-treated animals submitted to an endurance training protocol seemed less susceptible to in vitro anoxia-reoxygenation compared to DOX-treated sedentary controls
Ascensão, 2011[25]	Antioxidants (SOD), mPTP, apoptosis (eg, Bax, Bcl2, caspase), mitochondrial functioning	Yes	A single exercise bout mitigated doxorubicin-induced mPTP susceptibility and mitochondrial dysfunction and altered apoptotic signaling compared with nonexercised controls
Ascensão, 2005[26]	Markers of oxidative stress (malondialdehyde, aconitase), antioxidants (SOD), HSPs (HSP60, HSP70), mPTP, apoptosis (Bax, Ncl-2), mitochondrial functioning	Yes	An endurance treadmill exercise intervention improved antioxidant capacity and attenuated myocardial apoptosis. Histopathology confirmed significant attenuation of cardiotoxic changes in the exercise vs the control group
Ascensão, 2005[27]	Markers of oxidative stress (gluthatione analysis), antioxidants (SOD, catalase), HSPs (HSP60, HSP70), cardiac troponin I	Yes	An endurance swimming exercise program mitigated doxorubicin-induced oxidative damage compared with controls with positive effects on the glutathione system and HSP60
Ashrafi, 2012[28]	Markers of oxidative stress (malondialdehyde, NO), antioxidants (SOD, apelin)	Yes	A short-term PE preconditioning program counteracted doxorubicin-induced oxidative stress and upregulated oxidative capacity compared with nonexercised controls
Chicco, 2005[29]	Marker of oxidative stress (malondialdehyde), antioxidants (SOD), HSP (HSP72)	Yes	A voluntary wheel-running preconditioning protocol attenuated doxorubicin-induced alterations in lipid peroxidation compared with nonexercised controls. In addition, higher levels of HSP72 were observed in the intervention group. Cardiac function tended to be less impaired in the trained group
Chicco, 2006[30]	Marker of oxidative stress (malondialdehyde), antioxidants (SOD), HSP (HSP72), apoptosis (caspase 3), MHC distribution isoforms	Yes	A low-intensity treadmill exercise protocol mitigated doxorubicin-induced cardiac dysfunction, HSP72 and apoptotic signaling compared with nonexercised controls. No significant effect on lipid peroxidation, SOD, or MHC distribution was observed
Chicco, 2006[31]	Marker of oxidative stress (malondialdehyde), antioxidants (SOD), HSP (HSP72)	Yes	A preconditioning exercise program significantly mitigated doxorubicin-induced impairments in cardiac function compared with nonexercised controls. In addition, an increase in lipid peroxidation and greater expression of HSP72 following exercise was observed
Dolinsky, 2013[32]	Marker of oxidative stress (HNE), antioxidants (SOD, glutathione, catalase), SERCA2a expression	Yes	A preconditioning treadmill program counteracted doxorubicin-induced LV dysfunction, lowered lipid peroxidation, and increased the expression of SER2CA and SOD compared with nonexercised controls
Farzanegi, 2019[33]	Marker of oxidative stress (malondialdehyde), antioxidants (SOD, catalase), HSP (HSP70), inflammation marker (TNF-α)	Yes	A swimming program concomitant with doxorubicin treatment decreased inflammatory markers (TNF-a), HSP70, and lipid peroxidation, while improving antioxidant enzymatic activity compared with nonexercised controls
Hall, 2019[34]	Doxorubicin accumulation	Yes	Voluntary wheel running partially prevented doxorubicin-induced LV dysfunction in vivo and ex vivo and doxorubicin accumulation in cardiac tissue. PE combined with caloric restriction yielded the most cardioprotection
Hydock, 2008[35]	MHC distribution isoforms	Yes	A preconditioning forced and voluntary treadmill program prevented doxorubicin-induced LV dysfunction in vivo and ex vivo. MHC isoform distribution was preserved following exercise in doxorubicin-treated animals
Hydock, 2009[36]	MHC distribution isoforms	Yes	Access to voluntary wheel running before doxorubicin treatment significantly increased expression of α-MHC isoform compared with nonexercised controls

(*Continued*)

Table 2. Continued

Reference of animal study	Pathway(s)	Effect of PE intervention	Summary of main results
Hydock, 2011[37]	SERCA2a, MHC isoform distribution	Yes	Both forced and voluntary exercise interventions before doxorubicin treatment prevented decline in doxorubicin-induced LV dysfunction in vivo and ex vivo. The exercise interventions led to a preservation of MHC isoform distribution. No effect of PE on SER2CA was observed
Hydock, 2012[38]	MHC distribution isoforms	Yes	Compared with nonexercised controls, voluntary wheel running prevented in vivo and ex vivo doxorubicin-induced impairments in LV function and preserved MHC isoform distribution
Jensen, 2013[39]	Doxorubicin accumulation	Yes	Both forced and voluntary PE interventions preserved LV function (in vivo and ex vivo) and reduced doxorubicin accumulation in cardiac tissue compared with nonexercised controls. No difference was observed between the 2 exercise programs
Ji, 1993[40]	Marker of oxidative stress (malondialdehyde), antioxidants (SOD, catalase, glutathione)	No	Low-dose doxorubicin administration did not substantially impair oxidative functioning in cardiomyocytes, both at rest and during PE
Kanter, 1985[41]	Antioxidants (SOD, catalase, glutathione)	Yes	An endurance swimming protocol concomitant with doxorubicin administration mitigated doxorubicin-induced histopathological changes compared with nonexercised controls. No significant differences in antioxidants between exercise and nonexercised doxorubicin-treated animals were found
Kavazis, 2010[42]	Marker of oxidative stress (HNE), antioxidants (SOD, glutathione, catalase), HSP apoptosis (caspase 3, ubiquitine, calpain, TUNEL)	Yes	A short-term preconditioning PE program increased antioxidant capacity and HSP72 and against mitochondrial damage and apoptosis. Exercise-induced cardioprotection occurred independently of HSP72
Kavazis, 2014[18]	Gene expression (FoxO target genes), mitochondrial biogenesis (PGC-1α receptor)	Yes	Compared with nonexercised controls, the short-term PE intervention before doxorubicin administration attenuated doxorubicin-induced alteration in gene expression and protein abundance (PGC-1α receptor)
Lee, 2020[43]	Antioxidants (eg, SOD, catalase), autophagy/mitophagy (eg, AMPK, mTOR), apoptosis (Bax, BCL2)	Yes	A postconditioning PE program improved basal autophagy and mitophagy and counteracted doxorubicin-induced oxidative stress compared with nonexercised controls
Lien, 2015[44]	SERCA2a	Yes	Short-term forced and voluntary exercise interventions prevented doxorubicin-induced LV dysfunction in vivo and ex vivo compared with nonexercised controls. Both programs preserved SER2CA expression, yet the FTM intervention appeared to be more effective in the higher doxorubicin dose
Mackay, 2019[45]	Markers of oxidative stress (malondialdehyde, glutathione), iron regulation	No	Doxorubicin treatment significantly altered myocardial iron regulation, which was not prevented by a PE program nor metformin treatment before doxorubicin administration
Marques-Aleixo, 2015[46]	Markers of oxidative stress (malondialdehyde, aconitase), antioxidants (SOD), mitochondrial biogensis (PGC-1α receptor), mitochondrial functioning	Yes	Both FTM and voluntary wheel running interventions prevented doxorubicin-induced increase in oxidative stress and preserved mitochondrial functioning. Cardiac ultrastructure alterations (eg, percentage of abnormal mitochondria) were counteracted by the 2 PE programs. No major differences between the 2 PE programs were found
Marques-Aleixo, 2018[47]	mPTP, autophagy/mitophagy (eg, Beclin2, Pink, Parkin, P62) apoptosis (caspases, Bax, Bcl2)	Yes	Compared with nonexercised controls, forced and voluntary PE programs during doxorubicin treatment mitigated doxorubicin-induced mPTP susceptibility, and increased autophagic and apoptotic signaling, without substantial differences between the two exercise modalities
Morton, 2019[48]	mPTP, doxorubicin accumulation, reactive oxygen species emission, ABC-transporter expression	Yes	A short-term preconditioning PE program prevented doxorubicin-induced LV dysfunction on echocardiography and mitigated alteration in mPTP susceptibility compared with nonexercised controls. In addition, less mitochondrial doxorubicin accumulation and increased expression of ABC transporters were found

(*Continued*)

Table 2. Continued

Reference of animal study	Pathway(s)	Effect of PE intervention	Summary of main results
Parry, 2015[49]	Doxorubicin accumulation, multi-drug resistance protein expression	Yes	In tumor-inoculated rats, a voluntary wheel running program before doxorubicin treatment preserved cardiac function in vivo as well as ex vivo and reduced doxorubicin accumulation in cardiac tissue compared with nonexercised controls. The exercise program did not interfere with doxorubicin's therapeutic efficacy
Pfannenstiel, 2018[50]	Marker of oxidative stress (malondialdehyde), MHC distribution isoforms	Yes	A resistance training protocol before doxorubicin treatment preserved cardiac function in doxorubicin-treated animals and protected against MHC isoform distribution changes compared with nonexercised controls. No effect was found in the exercise program with lipid peroxidation
Phungphong, 2020[51]	Markers of oxidative stress (LDH, lipid peroxidation), inflammatory markers (IL-6), calcium homeostasis, MHC distribution isoforms	Moderately	In ovariectomized rats, preconditioning exercise program attenuated doxorubicin-induced oxidative stress and cardiac inflammation compared with nonexercised controls. No protective effect on cardiac function following exercise was found
Shirinbayan, 2012[52]	Markers of oxidative stress (malondialdehyde, creatine kinase, creatine phosphokinase-myocardial band), antioxidants (SOD), HSP (HSP70)	Yes	A preconditioning PE program significantly increased HSP70 and SOD and decreased malondialdehyde as opposed to nonexercised controls, regardless of differences in doxorubicin doses (10 or 20 mg/kg)
Smuder, 2013[53]	Autophagy (mRNA and protein synthesis)	Yes	Compared with nonexercised controls, a preconditioning treadmill program prevented doxorubicin-induced increase in autophagic signaling
Sturgeon, 2014[54]	MHC distribution isoforms	No	In a murine model with melanoma, a PE program before doxorubicin treatment did not mitigate doxorubicin-induced LV dysfunction on echocardiography nor changes in MHC isoform distribution but improved doxorubicin's antitumor efficacy compared with nonexercised controls
Werner, 2008[55]	Apoptosis (telomere-regulating proteins, TUNEL, p53)	Yes	A preconditioning voluntary wheel running program reduced doxorubicin-induced p53 expression and might prevent cardiomyocyte apoptosis. In animals not treated with doxorubicin, the exercise program upregulated telomere stabilizing proteins compared with nonexercised controls
Wonders,2008[56]	Marker of oxidative stress (malondialdehyde)	Yes	An exercise bout before doxorubicin treatment mitigated doxorubicin-induced LV dysfunction on isolated heart perfusion and attenuated an increase in oxidative stress compared with nonexercised controls
Yang, 2020[57]	Inflammation markers (AKT, COX-2), fibrotic markers (TGF-β)	Yes	A PE program during doxorubicin treatment ameliorated doxorubicin-induced expression of fibrosis factors and reduced cardiac fibrosis on histopathology compared with nonexercised controls. On echocardiography, LV function was preserved in the exercise group

AMPK indicates 5'-adenosine monophosphate-activated protein kinase; COX-2, cyclooxygenase-2; CRP, C-reactive protein; eNOS, endothelial nitric oxide synthase; FTM, forced treadmill; HNE, 4-hydroxy-2-nonenal protein; HSP, heat shock protein; IL-6, interleukin 6; IL-8, interleukin 8; IL-10, interleukin 10; LDH, lactate dehydrogenase; LV, left ventricular; MHC, myosin heavy chain; mPTP, mitochondrial permeability transition pore; NO, nitric oxide; PE, physical exercise; PGC-1α, peroxisome proliferator-activated receptor-γ coactivator 1α; SERCA2a, sarcoendoplasmatic reticulum calcium ATPase 2a; SOD, superoxide dismutase; TERT, telomerase reverse transcriptase; TGF-β, transforming growth factor β; TNF-α, tumor necrosis factor α; and TUNEL, terminal deoxynucleotidal transferase–mediated biotin–deoxyuridine triphosphate nick-end labeling.

doxorubicin dosage. Both studies reported simultaneous preservation of systolic function. However, 1 study in which doxorubicin administration significantly lowered SERCA2a by ≈80% found no effect of a 10-week preconditioning PE intervention.[37]

Induction and opening of the mitochondrial permeability transition pore (mPTP) could result from doxorubicin-induced oxidative stress and associated deregulation in calcium homeostasis.[66] The mPTP is a channel in the mitochondrial membrane susceptible to calcium loading conditions. Opening of this channel enhances membrane permeability, which can lead to mitochondrial dysfunction via cessation of ATP synthesis and apoptosis.[66] Four studies investigated the effect of PE on mPTP susceptibility, all of which found that a forced preconditioning PE program, varying from a single bout to an endurance protocol of 14 weeks, had a positive effect on doxorubicin-induced increased susceptibility to mPTP opening.[25,26,47,48] These findings were corroborated by simultaneous reduction in DIC on echocardiography[48] and histopathology.[26]

Another pathway related to oxidative metabolism is the peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α). The PGC-1α plays a pivotal

Table 3. Overview of Available Evidence Per Pathway

Pathway	No.
Antioxidants	18
HSPs	10
Cardiac inflammation	2
Calcium homeostasis	3
mPTP	4
PGC-1α	2
MHC isoform distribution	6
Autophagy	3
Apoptosis	10
Doxorubicin accumulation	4

HSP indicates heat shock protein; MHC, myosin heavy chain; mPTP, mitochondrial permeability transition pore; and PGC-1α, peroxisome proliferator-activated receptor-γ coactivator 1α.

role in mitochondrial biogenesis as well as lipid and carbohydrate metabolism,[67] and is believed to be cardioprotective, since knockout of PGC-1α in mice leads to cardiomyopathy.[68] One study found that a short-term PE program induced the expression of PGC-1α protein in cardiac (and skeletal muscle) tissue by ≈50%.[18] The authors proposed that this could prevent downstream doxorubicin-induced cardiac and skeletal muscle wasting.[18] Another study found a trend for preservation of PGC-1α expression by both forced and voluntary PE interventions during doxorubicin administration, which corresponded with fewer cardiotoxic changes on histopathology.[46]

The distribution of myosin heavy chain (MHC) isoforms, ie, the ratio of fast α-MHC to slower yet more metabolically efficient β-MHC is of importance for cardiac contractility. Upregulation of β-MHC at a cost of α-MHC is reported after exposure to doxorubicin[69] and has been attributed, in part, to doxorubicin-induced oxidative stress.[38] Six studies found that the distribution of MHC isoforms was unfavorably affected by doxorubicin.[35–38,50,51] Studies by Hydock et al[35,37,38] using both forced and voluntary PE programs found a consistent trend towards lower expression of β-MHC (≈5%–15% reduction) following exercise. These results were corroborated by studies by Pfannenstiel et al[50] and Phungphong et al[51] using a resistance and FTM PE program, respectively. However, 2 other studies found no significant effect of PE on MHC isoform distribution by doxorubicin administration alone or in combination with an FTM intervention.[30,54]

The process of autophagy is important for cellular survival by regulation of energy sources, removing damaged organelles (eg, mitochondria) or intracellular pathogens.[70] Dysregulation of autophagy could occur via doxorubicin-induced oxidative stress and could eventually lead to nonapoptotic cell death.[71] Two studies showed that both a 12-day and a 14-week PE intervention during treatment can effectively prevent a doxorubicin-mediated increase in autophagy.[47,53] However, using a postconditioning protocol, Lee et al[43] found no attenuation of autophagy in nonexercised animals, while autophagy was significantly promoted in the exercised animals.

Apoptosis is an important and final step in the development of DIC, which can be promoted through oxidative stress (among others via increased mPTP opening) and the formation of topoisomerase IIβ-doxorubicin-DNA complexes.[5,72] The latter is believed to be a key mechanism underlying DIC, since depletion of topoisomerase IIβ can prevent cardiac toxicity caused by doxorubicin.[72] Attenuation of apoptotic signaling by exercise was investigated in 10 studies,[¶] of which the majority measured caspase 3 or Bcl2 family proteins (proapoptotic and antiapoptotic protein Bax and Bcl2, respectively). All of the studies of caspase 3 activity reported that various PE programs counteracted doxorubicin-induced increase in caspase 3 activity.[25,26,30,42,43,47,51] Results from studies of the Bcl2 proteins are less consistent. Alihemmati et al[23] found that a 6-week preconditioning high-intensity interval training program significantly counteracted doxorubicin-induced upregulation of Bax from ≈50% to ≈10% change and downregulation of Bcl2 from ≈80% to ≈55% change. Two other studies using a 14-week preconditioning[26] or concomitant[47] PE protocol also found relatively lower expression of Bax in the exercise groups but no significant changes or trend in Bcl2.[26,47] No attenuation of either Bax or Bcl2 was found by the other studies that investigated these proteins.[25,43] Werner et al[55] demonstrated that a 3-week voluntary running protocol mitigated expression of proapoptotic proteins, including p53, compared with nonexercised controls. However, another study using levels of cleaved poly(ADP-ribose) polymerase as a marker for apoptosis found no effect of either a 2-week FTM intervention or doxorubicin administration alone on apoptotic signaling.[54]

Doxorubicin Accumulation

Compared with other cytosolic compartments of a cell, doxorubicin predominantly localizes in mitochondria.[48] Four studies using FTM[39,48] or VWR[34,39,49] interventions investigated whether a PE program can counteract doxorubicin accumulation. Three of these studies found significantly lower concentrations of doxorubicin, varying from ≈25% to 40%, in the left ventricles of exercised animals compared with nonexercised animals within 2 days after injection.[39,48,49]

[¶]References 23,25,26,30,42,43,47,51,54,55

A study by Hall et al[34] reported a nonsignificant trend of a 38% reduction of doxorubicin accumulation in the left ventricle, favoring the 4-month preconditioning group. Parry et al,[49] using tumor-inoculated animals, reported no differences between the study groups in changes in doxorubicin accumulation within the tumor, indicating that the PE program did not interfere with doxorubicin's therapeutic efficacy. All 4 studies described preservation of myocardial function (on echocardiography or isolated heart perfusion) or histology.

DISCUSSION

The aim of this meta-analysis and systematic review was to generate an estimate of the effect of PE on DIC and to systematically evaluate mechanisms underlying exercise-induced cardioprotection. The included clinical studies reported favorable results for some but not all outcomes related to cardiac function and the available data are therefore not sufficient to demonstrate a protective effect of PE in humans. For animal studies, our meta-analysis indicated that both forced and voluntary PE interventions significantly improve in vivo (echocardiography) and ex vivo (isolated heart perfusion) cardiac parameters compared with nonexercised doxorubicin-treated animals. We identified oxidative stress and related mechanisms, and less doxorubicin accumulation in cardiac tissue, as pathways through which exercise could exert a protective effect on DIC.

Clinical Studies

In the 2 reports by Kirkham et al,[19,20] no conclusive evidence was provided that treadmill interventions are effective in preserving cardiac function following treatment with doxorubicin. However, the sample size was small (n=24) and imaging was limited to 2-dimensional echocardiography. Given the limitations of this modality in terms of temporal variability in serial LV assessment,[73,74] subtle between- and within-group changes in this small sample could remain unrecognized. Although the study by Ma was rated as having relatively low methodological quality and high risk of bias, their results were sufficiently promising to warrant replication in a larger sample of patients with cancer, with adequate follow-up. Currently, several clinical initiatives are ongoing. An example is the EXACT2 (Exercise to Prevent Anthracycline-based CardioToxicity 2.0) study,[75,76] which is investigating the effect of a 12-week supervised PE program during chemotherapy. The primary outcome of this randomized controlled trial (estimated study sample of n=100) is change in LV ejection fraction on 2-dimensional echocardiography from baseline to post-treatment (13 weeks) and 6 months after baseline. Another example is the ongoing Pact-Paces-Heart (Evaluation of Heart Function After Physical Activity During Adjuvant Chemotherapy in Breast Cancer Patients) study,[77] which evaluates the effect a moderate- to-high-intensity supervised PE program during breast cancer treatment on cardiotoxicity after a long period of follow-up (≈8 years after treatment). This study has a relatively large sample size (n≈180) and an extensive cardiac assessment, including cardiac magnetic resonance imaging and 3-dimensional echocardiography. Results of these studies are expected to provide new insights into the effect of PE on cardiotoxicity in humans.

Meta-Analysis in Animal Studies on the Effect of Exercise on Cardiotoxicity

The animal studies in our quantitative analysis yielded evidence that PE interventions that vary in terms of type, duration, timing, and intensity can provide protection against DIC. The effects of forced exercise interventions appeared slightly stronger than those of voluntary interventions. Also, contrary to other studies included in our review, the study by Sturgeon et al,[54] which used an exercise program with a lower intensity compared with other studies, found no protective effect of PE on cardiotoxicity. This could suggest that a certain threshold of exercise intensity is needed in order to achieve cardioprotection. Similarly, both exercise programs starting before as well as during doxorubicin administration appeared to be cardioprotective, although effects of the former were somewhat stronger. It seems intuitive that exercise interventions during doxorubicin administration would be (more) effective, given that our qualitative analysis on underlying pathways identified accelerated doxorubicin clearance as an important mechanism underlying exercise-mediated cardioprotection. Nonetheless, initiating a PE program before the start of treatment is likely to have added value because of preconditioning of the cardiomyocytes via, among other pathways, upregulation of α-MHC expression or ABC transporters (as discussed in a later section). These hypotheses, however, need to be investigated in future studies.

The reported effect sizes of our meta-analysis correspond with absolute changes in percentage points in FS for FTM and VWR of 8.5% and 5.8%, respectively. In rodents, echocardiography is the modality of choice for evaluating cardiac function, since it is noninvasive, versatile, cheap, and reproducible. Recently, reference values for FS for adult rats and mice were published, which ranged from 41% to 48% and 31% to 43%, respectively.[78] This indicates that the observed effects are likely to be of clinical importance in rodents. In addition, for LVP, beneficial results were found for both FTM and VWR interventions as compared with control.

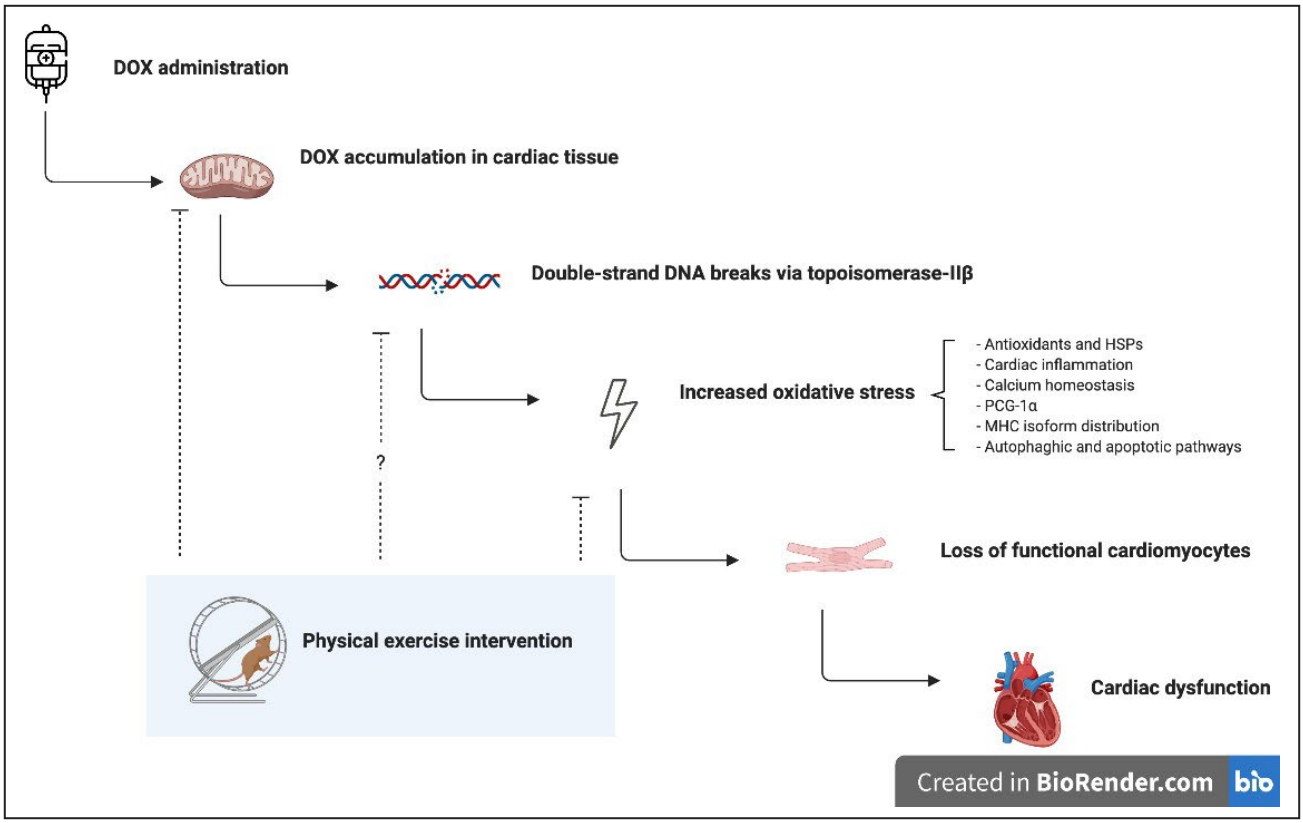


Figure 4. Suggested pathways underlying exercise-mediated protection against doxorubicin-induced cardiotoxicity (DIC) in rodents.
Exercise prevents accumulation of doxorubicin in cardiac tissue, thereby inhibiting downstream pathways, through which doxorubicin can induce cardiotoxicity. HSP indicates heat sock protein; MHC, myosin heavy chain; and PGC-1α, peroxisome proliferator-activated receptor-γ coactivator 1α.

Isolated heart perfusion has been proposed as a reliable model for the assessment of myocardial function, especially since it provides insight into cardiac performance in the absence of neurohumeral influences and variation in loading conditions.[79] Thus, these results, complemented by the in vivo–derived evidence, support the notion that exercise indeed has cardioprotective potential in rodents.

Mechanisms Underlying Exercise-Mediated Cardioprotection

In a previous study, reduction of doxorubicin-induced oxidative stress and less doxorubicin accumulation have been described as pathways underlying exercise-mediated cardioprotection.[10] Our results complement the results of that study by providing an update of the novel articles on these topics, as well as describing a number of additional underlying pathways.

Oxidative stress and related pathways are the most extensively studied mechanisms, and many studies found that PE can counterbalance doxorubicin-induced increased markers of oxidative stress.[#]

[#]References 22,25–27,29,31–33,42,43,45,46,51,52,56

Research on how exercise could alleviate doxorubicin-induced oxidative stress has yielded more ambiguous results. Most studies have focused on upregulation of antioxidants or HSPs. Although some of these studies yielded positive effects, others reported that attenuation of these proteins/enzymes is not necessary in order for exercise to exert its cardioprotective effect. This is supported by clinical studies in which no significant effect of antioxidants (eg, L-carnitine, coenzyme Q10) on the incidence or extent of cardiotoxicity was found.[80] Studies of the other proposed mechanisms are limited in number and have yielded varying results. For example, caspase 3, a marker for apoptotic signaling, has been investigated in 7 studies, all of which reported that PE mitigated a doxorubicin-induced increase in activity of the enzyme. Nevertheless, the only study that investigated whether this corresponded to less DIC in vivo reported no attenuation in LV function by PE.[51] This finding, as well as those investigating antioxidants, suggest that other, more upstream pathways are also involved. In this regard, it is conceivable that accumulation of doxorubicinin cardiac tissue acts as an overarching mechanism, since blocking this phenomenon effectively enables PE interventions to

tackle all downstream doxorubicin-induced effects (Figure 4). This is supported by the fact that all included studies demonstrated that lowered doxorubicin accumulation coincided with reduced DIC.[34,39,48,49] As for how PE mitigates doxorubicin accumulation, current evidence suggests that doxorubicin accumulation is influenced by exercise through upregulation of ABC transporters.[39,48,49] These transporters can export a wide range of substances, eg, doxorubicin, out of cells or cell organelles. Knockout mice, lacking these receptors, show prolonged presence of doxorubicinin cardiac tissue.[81] However, since causality between upregulation of ABC transporters and reduction of doxorubicin accumulation has not been established, lower doxorubicin accumulation might equally result from exercise-mediated alterations in doxorubicin uptake or metabolism. Further research is therefore needed to elucidate the exact underlying mechanisms of exercise-mediated reduction in doxorubicin accumulation.

The strengths of the study are the large number of studies included using various PE protocols and the systematic approach to evaluate the effect of PE on cardiotoxicity and underlying mechanisms. A limitation is that many components of the methodology of the included animal studies were not adequately reported, which could limit the internal validity of these studies, as well as the comparison of results among studies. For the current report, authors were contacted in case a component was scored as "unclear." This resulted in clarification of a substantial amount of risk-of-bias information, thereby improving the quality of the evidence. In addition, the wide variety of study protocols made it challenging to quantify the protective effects of specific forms of PE and to draw definitive conclusions regarding underlying mechanisms. Last, the majority of the preclinical studies used FS as a parameter for LV function. Since LV ejection fraction is currently recommended in clinical guidelines,[74] this hampers the generalizability to patients with cancer. Limitations of the clinical studies are the small sample sizes and the fact that imaging was limited to 2-dimensional echocardiography. In addition, follow-up time was too short to detect all relevant cases of cardiotoxicity, given that anthracycline-induced cardiotoxicity can manifest within a year after treatment (ie, not necessarily directly after completion of treatment). This limited our ability to draw any conclusions about the effects of PE on DIC in humans.

CONCLUSIONS

Our meta-analysis and systematic review indicate that PE is an effective intervention for reducing DIC in rodents. Less doxorubicin accumulation in cardiomyocytes could act as an overarching mechanism underlying the protective effects of exercise against DIC. While clinical studies in humans are limited, the observed effects are congruent with the hypothesis that PE yields cardioprotection. Larger, more sophisticated clinical studies with an adequate period of follow-up are needed in order to document the role of PE in preventing cardiotoxicity in patients with cancer.

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Disclosures

None.

Supplementary Material

Data S1–S5
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Supplemental Material

Data S1.

Supplemental Methods

Search strings

Pubmed

PubMed	
Chemotherapy	Anthracyclines[Mesh] OR Chemotherapy, Adjuvant[Majr:NoExp] OR Mitoxantrone[Mesh] OR doxorubicin[Title/Abstract] OR DOX[Title/Abstract] OR Adriamycin[Title/Abstract] OR Daunorubicin[Title/Abstract] OR Cerubidine[Title/Abstract] OR Idarubicin[Title/Abstract] OR Idamycin[Title/Abstract] OR Epirubicin[Title/Abstract] OR Ellence[Title/Abstract] OR Mitoxantrone[Title/Abstract] OR Novantrone[Title/Abstract]
Cardiotoxicity	Cardiotoxicity[MeSH Terms] OR Cardiovascular Diseases[Majr:NoExp] OR Heart Failure[Majr] OR cardiotoxicity[Title/Abstract] OR cardiomyopathy[Title/Abstract] OR cardiomyopathies[Title/Abstract] OR cardiotoxic[Title/Abstract] OR CTRCD[Title/Abstract] OR Cardiac Failure[Title/Abstract] OR Cardiac damage[Title/Abstract] OR Cardiac dysfunction[Title/Abstract] OR Cardiac myopathy[Title/Abstract] OR cardiac apoptosis[Title/Abstract] OR heart failure[Title/Abstract] OR heart toxicity[Title/Abstract] OR heart damage[Title/Abstract] OR heart dysfunction[Title/Abstract] OR myocardial failure[Title/Abstract] OR Myocardial toxicity[Title/Abstract] OR myocardial damage[Title/Abstract] OR myocardial dysfunction[Title/Abstract] OR "ventricular failure"[Title/Abstract] OR "ventricular toxicity"[Title/Abstract] OR "ventricular damage"[Title/Abstract] OR "ventricular dysfunction"[Title/Abstract] OR cardiomyocyte damage[Title/Abstract] OR cardiomyocyte toxicity[Title/Abstract] OR cardiomyocyte dysfunction[Title/Abstract] OR cardiomyocyte apoptosis[Title/Abstract] OR "cardiomyocytes damage"[Title/Abstract] OR "cardiomyocytes toxicity"[Title/Abstract] OR "cardiomyocytes apoptosis"[Title/Abstract] OR "cardiomyocytes dysfunction"[Title/Abstract] OR cardiac injury[Title/Abstract]

	OR heart failures[Title/Abstract] OR heart toxicities[Title/Abstract] OR "ventricular toxicities[Title/Abstract] OR myocardial oxidative damage[Title/Abstract] OR cardiac oxidative damage[Title/Abstract]
Exercise	Exercise[MeSH Terms] OR sports[MeSH Terms] OR Exercise Therapy[MeSH Terms] OR kinesiotherapy[Title/Abstract] OR walking[Title/Abstract] OR weight lifting[Title/Abstract] OR sport[Title/Abstract] OR sports[Title/Abstract] OR ((Physical[Title/Abstract] OR Aerobic[Title/Abstract] OR exercise[Title/Abstract] OR endurance[Title/Abstract] OR fitness[Title/Abstract] OR training[Title/Abstract]) AND (activity[Title/Abstract] OR exercise[Title/Abstract] OR therapy[Title/Abstract] OR program[Title/Abstract] OR training[Title/Abstract] OR conditioning[Title/Abstract] OR activities[Title/Abstract] OR exercises[Title/Abstract] OR therapies[Title/Abstract] OR programs[Title/Abstract] OR trainings[Title/Abstract])) OR ((Activity[Title/Abstract]) AND (program[Title/Abstract] OR conditioning[Title/Abstract])))

Embase

EmBase	
Chemotherapy	'anthracycline antibiotic agent'/exp OR 'cancer chemotherapy'/mj OR 'mitoxantrone'/de OR 'doxorubicin':ti,ab,kw OR 'DOX':ti,ab,kw OR 'Adriamycin':ti,ab,kw OR 'Daunorubicin':ti,ab,kw OR 'Cerubidine':ti,ab,kw OR 'Idarubicin':ti,ab,kw OR 'Idamycin':ti,ab,kw OR 'Epirubicin':ti,ab,kw OR 'Ellence':ti,ab,kw OR 'Mitoxantrone':ti,ab,kw OR 'Novantrone':ti,ab,kw
Cardiotoxicity	'Cardiotoxicity'/exp OR 'cardiovascular disease'/mj OR 'Heart Failure'/exp OR 'cardiotoxicity':ti,ab,kw OR 'cardiomyopathy':ti,ab,kw OR 'cardiomyopathies':ti,ab,kw OR 'cardiotoxic':ti,ab,kw OR 'CTRCD':ti,ab,kw OR 'Cardiac Failure':ti,ab,kw OR 'Cardiac damage':ti,ab,kw OR 'Cardiac dysfunction':ti,ab,kw OR 'Cardiac myopathy':ti,ab,kw OR 'cardiac apoptosis':ti,ab,kw OR 'heart failure':ti,ab,kw OR 'heart

	toxicity':ti,ab,kw OR 'heart damage':ti,ab,kw OR 'heart dysfunction':ti,ab,kw OR 'myocardial failure':ti,ab,kw OR 'Myocardial toxicity':ti,ab,kw OR 'myocardial damage':ti,ab,kw OR 'myocardial dysfunction':ti,ab,kw OR 'ventricular failure':ti,ab,kw OR 'ventricular toxicity':ti,ab,kw OR 'ventricular damage':ti,ab,kw OR 'ventricular dysfunction':ti,ab,kw OR 'cardiomyocyte damage':ti,ab,kw OR 'cardiomyocyte toxicity':ti,ab,kw OR 'cardiomyocyte dysfunction':ti,ab,kw OR 'cardiomyocyte apoptosis':ti,ab,kw OR 'cardiac injury':ti,ab,kw OR 'heart failures':ti,ab,kw OR 'heart toxicities':ti,ab,kw OR 'ventricular toxicities':ti,ab,kw OR 'myocardial oxidative damage':ti,ab,kw OR 'cardiac oxidative damage':ti,ab,kw
Exercise	'Exercise'/exp OR 'sport'/exp OR 'kinesiotherapy'/exp OR 'sport':ti,ab,kw OR 'sports':ti,ab,kw OR 'walking':ti,ab,kw OR 'weight lifting':ti,ab,kw OR (('Physical':ti,ab,kw OR 'Aerobic':ti,ab,kw OR 'exercise':ti,ab,kw OR 'endurance':ti,ab,kw OR 'fitness':ti,ab,kw OR 'training':ti,ab,kw) AND ('activity':ti,ab,kw OR 'exercise':ti,ab,kw OR 'therapy':ti,ab,kw OR 'program':ti,ab,kw OR 'training':ti,ab,kw OR 'conditioning':ti,ab,kw OR 'activities':ti,ab,kw OR 'exercises':ti,ab,kw OR 'therapies':ti,ab,kw OR 'programs':ti,ab,kw OR 'trainings':ti,ab,kw)) OR (('Activity':ti,ab,kw) AND ('program':ti,ab,kw OR 'conditioning':ti,ab,kw))

Cochrane

#1	MeSH descriptor: [Anthracyclines] this term only
#2	MeSh descriptor: [Chemotherapy, Adjuvant] this term only
#3	MeSh descriptor: [Mitoxantrone] this term only
#4	'doxorubicin' OR 'DOX' OR 'Adriamycin' OR 'Daunorubicin' OR 'Cerubidine' OR 'Idarubicin' OR 'Idamycin' OR 'Epirubicin' OR 'Ellence' OR 'Mitoxantrone' OR 'Novantrone'
#5	MeSh descriptor: [Cardiotoxicity] explode all trees
#6	MeSH descriptor: [Cardiovascular Diseases] this term only

#7	MeSH descriptor: [Heart Failure] explode all trees
#8	"cardiotoxicity" OR "cardiomyopathy" OR "cardiomyopathies" OR "cardiotoxic" OR "CTRCD" OR "Cardiac Failure" OR "Cardiac damage" OR "Cardiac dysfunction" OR "Cardiac myopathy" OR "cardiac apoptosis" OR "heart failure" OR "heart toxicity" OR "heart damage" OR "heart dysfunction" OR "myocardial failure" OR "Myocardial toxicity" OR "myocardial damage" OR "myocardial dysfunction" OR "ventricular failure" OR "ventricular toxicity" OR "ventricular damage" OR "ventricular dysfunction" OR "cardiomyocyte damage" OR "cardiomyocyte toxicity" OR "cardiomyocyte dysfunction" OR "cardiomyocyte apoptosis" OR "cardiomyocytes damage" OR "cardiomyocytes toxicity" OR "cardiomyocytes dysfunction" OR "cardiomyocytes apoptosis" OR "cardiac injury" OR "heart failures" OR "heart toxicities" OR "ventricular toxicities" OR "myocardial oxidative damage" OR "cardiac oxidative damage"
#9	MeSH descriptor: [Exercise] explode all trees
#10	MeSH descriptor: [Sports] explode all trees
#11	MeSH descriptor: [Exercise Therapy] explode all trees
#12	"sport" OR "sports" OR "walking" OR "weight lifting" OR "kinesiotherapy" OR (("Physical" OR "Aerobic" OR "exercise" OR "endurance" OR "fitness" OR "training") AND ("activity" OR "exercise" OR "therapy" OR "program" OR "training" OR "conditioning" OR "activities" OR "exercises" OR "therapies" OR "programs" OR "trainings")) OR (("Activity") AND ("program" OR "conditioning"))
Search	(#1 OR #2 OR #3 OR #4) AND (#5 OR #6 OR #7 OR #8) AND (#9 OR #10 OR #11 OR #12) in Trials

Data S2.

References of articles excluded on the basis of full-text screening

All conference abstracts are not shown.

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is this the explanation for the cytoprotection of the heart against oxidative stress-induced redox imbalance in comparison to skeletal muscle cells? *Cell Biochem Funct*. 2007;25:23–32.

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Data S3.

Risk of bias assessment

The risk of bias assessment for the human studies is presented in Figure 2a. The two studies by Kirkham et al.[19,20] were scored as one, since they made use of the same study population and largely the same methodology. These studies were scored as low risk of bias on all items. An exception was the item on performance bias which was scored as 'high', since blinding was not possible due to the nature of the intervention. For Ma, the items on selection bias were scored 'unclear', since no information on the randomization procedure was provided. Performance bias was scored as high risk of bias, similar to the reports by Kirkham et al. Since information on loss of participations was not adequately provided, the risk of attrition bias was rated as high. The item on selective reporting could not be assessed, as no pre-specified protocol was available.

The risk of bias assessment and reporting of quality indicators for the animal studies are presented in Figure 2b and Figure 2c, respectively. Many studies did not adequately report on important aspects of the methodology; hence most items were initially scored as 'unclear'. After contacting authors (response rate: 73%, n=29/40), 127 of the 'unclear' ratings were modified to either 'low' (*n*=109) or 'high' (*n*=18) risk of bias. Risk of selection bias, assessed via entries on random sequences generation, comparability of baseline characteristics and concealment of allocation, were scored low in approximately two-thirds of the studies, 'unclear' in about 25% and high in the remaining studies. Compared to other sources of bias, risk of performance bias comprising entries on random housing and blinding of the trial caregivers and researchers (when possible), was scored high in 14% and 35%, respectively. Risk of detection bias (random outcome assessment and blinding of outcome assessors) were scored relatively low. Drop-outs were inadequately reported in approximately one-fifth of the studies, leading to a high risk of attrition bias in these studies. The item on reporting bias, i.e. whether outcomes were selectively reported, was scored 'unclear' for all studies, since no-preregistered protocols were available.

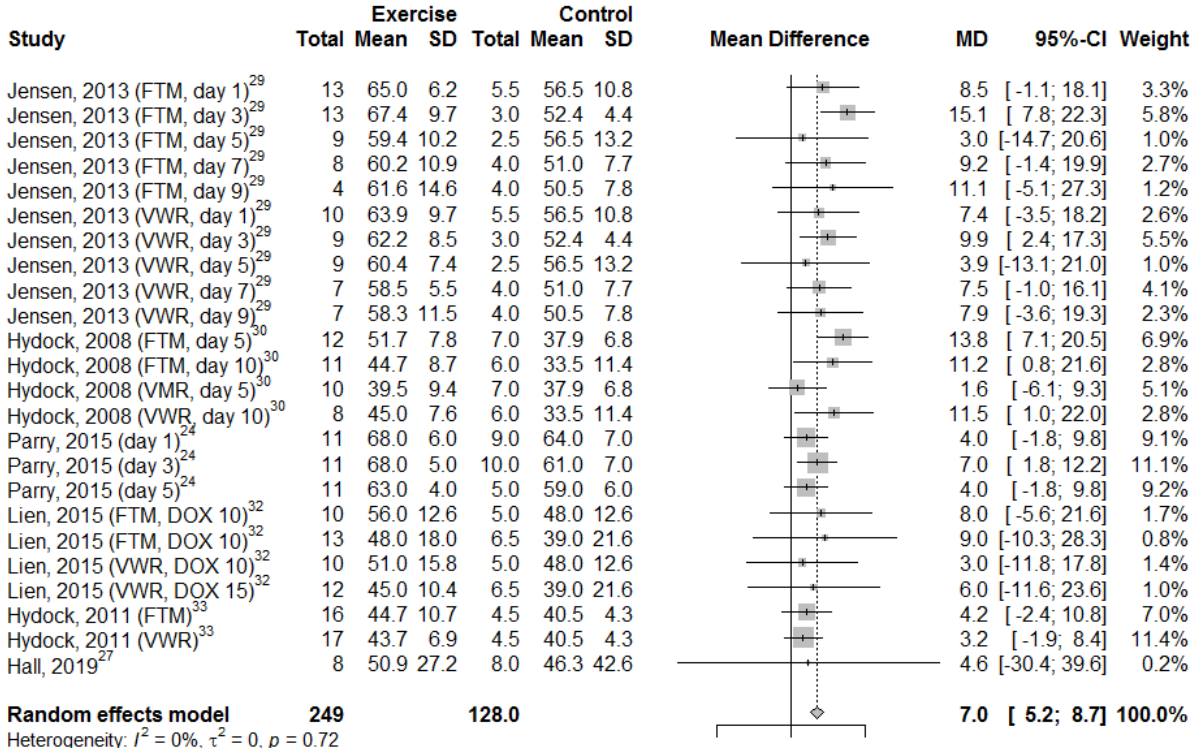
As for the reporting of methodology, none of the studies provided a sample size calculation, and many studies did not mention any form of blinding. Ethical approval was, however, reported in the vast majority of the studies.

Data S4.

Sub-analysis on the timing of the physical exercise intervention

Forest plot (Figure S1) present the results of the preconditioning physical exercise interventions (i.e. started before DOX treatment) and plot (Figure S2)) presents the results of those interventions given concomitant with DOX administration.

1



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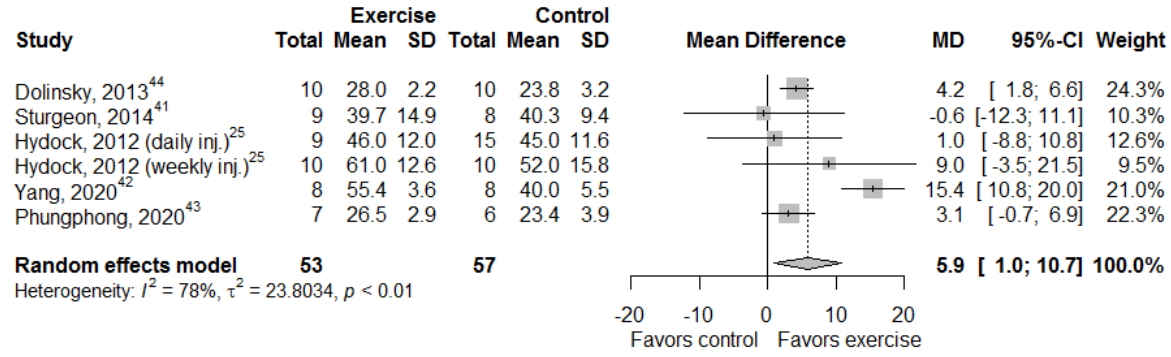


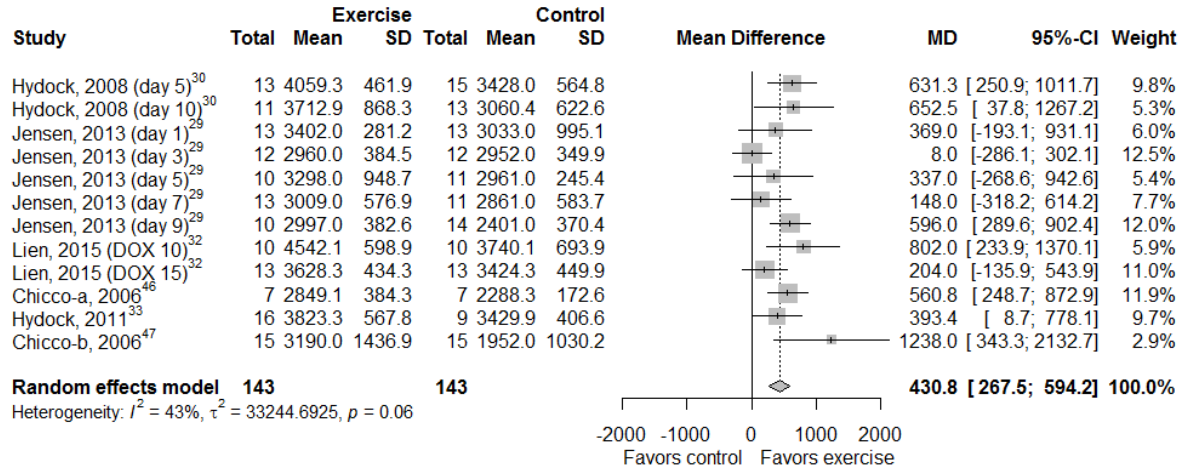
Data S5.

Results on +dP/dt and –dP/dt

For both +dP/dt and –dP/dt, results are favoring physical exercise groups (Figures S3-S6). In +dp/dt, a pooled analysis of studies using forced exercise interventions demonstrated a MD of 430.8 mm Hg (95CI%: 267.5; 594.1), T^2=21392.1. Results were comparable, yet slighter stronger in the analysis on voluntary exercise interventions; MD of 500.3 mm Hg (95CI%: 274.7; 725.9), T^2=138438.2.

In –dP/dt, a MD of -374.5 (95%CI:-508.9; -240.1), T^2=20895.3, and -407.5 (9%CI: -596.9; -218.1) mm Hg was found for respectively forced and voluntary exercise interventions compared to non-exercised rodents.

3



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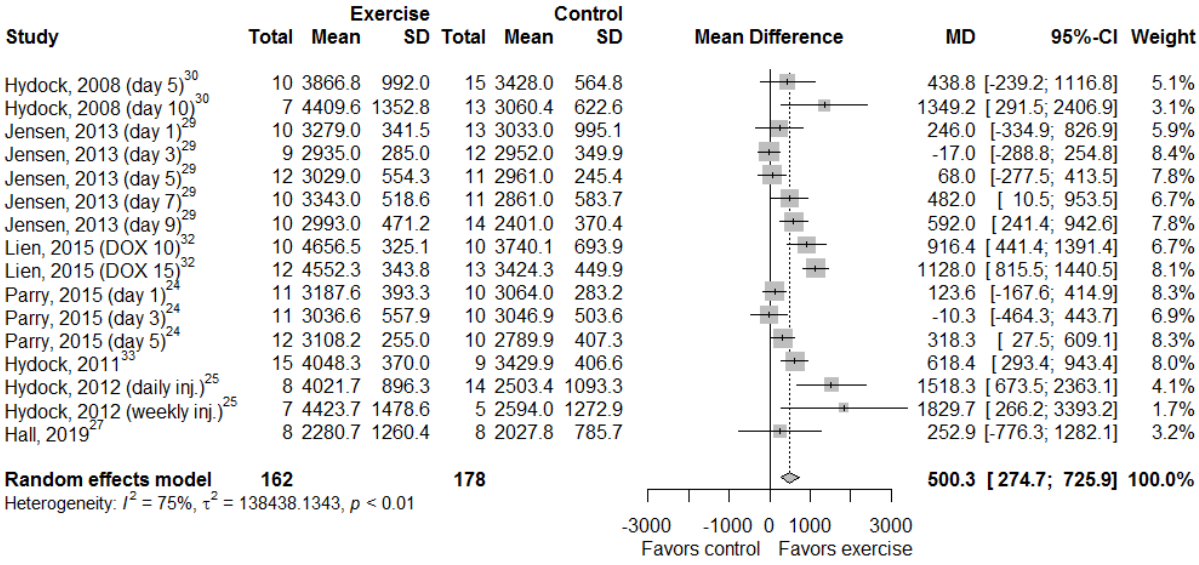
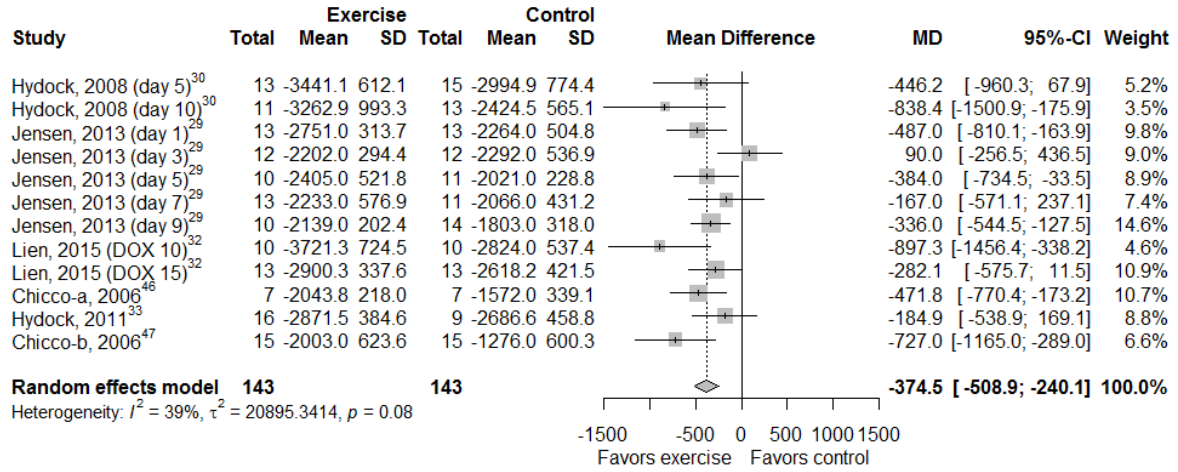


5



6