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# PRECLINICAL AND CLINICAL EVIDENCE OF EXERCISE TRAINING AS A COMPLEMENTARY CHAGAS DISEASE TREATMENT

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# PRECLINICAL AND CLINICAL EVIDENCE OF EXERCISE TRAINING AS A COMPLEMENTARY CHAGAS DISEASE TREATMENT

Dissertação apresentada como parte dos requisitos para obtenção do título de Mestre em Ciências Biológicas pela Universidade Federal de Alfenas. Área de concentração: Interação patógeno-hospedeiro.

Orientador: Prof. Dr. Rômulo Dias Novaes.

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A Banca examinadora abaixo-assinada aprova a Dissertação/Tese apresentada como parte dos requisitos para a obtenção do título de Mestre em Ciências Biológicas pela Universidade Federal de Alfenas. Área de concentração: Interação Patógeno-Hospedeiro.

Aprovada em: 28 de julho de 2022.

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**RESUMO**

***Contexto:*** A doença de Chagas (DC) é uma condição negligenciada correlacionada com a pobreza e a principal causa de cardiomiopatia infecciosa em todo o mundo. Embora contraindicado no passado, o treinamento de exercícios (TE) foi recentemente sugerido como um tratamento complementar para a DC. No entanto, a base mecanicista que apoia essa recomendação ainda não está clara. ***Objetivo:*** Utilizamos uma estrutura de revisão sistemática para investigar as evidências pré-clínicas e clínicas sobre a relevância do TE para o tratamento da DC. ***Métodos:*** Uma pesquisa estruturada em dois níveis baseada na estratégia PRISMA foi aplicada aos bancos de dados PubMed/Medline, Web of Science, Scopus e Embase. Foram investigados desfechos parasitológicos, bioquímicos, imunológicos e cardiorrespiratórios. ***Resultados:*** Evidências pré-clínicas indicam que o treinamento físico pré-infecção é seguro e aumenta a resistência do hospedeiro à infecção por *T. cruzi*, regulando positivamente a função contrátil do coração e dos cardiomiócitos, os desfechos imunológicos (ex: INF-γ, TNF-α, NO) e antioxidantes (ex: CAT, GR, GPx, SOD); atenuando a parasitemia, carga parasitária, biossíntese de espécies reativas de oxigênio (EROS) e nitrogênio (ERN), dano molecular e microestrutural dos músculos cardíaco e esquelético. Por outro lado, o treinamento de exercícios concomitantemente administrado à infecção aguda pode exacerbar o parasitismo celular, as respostas pró-inflamatórias e pró-oxidantes (por exemplo, oxidação de lipídios e proteínas); potencializando a lesão de órgãos-alvo. Além disso, evidências clínicas indicam que o treinamento aeróbico com dosimetria ajustada por testes de exercício limitados por sintomas é igualmente seguro para melhorar a tolerância ao exercício, a função cardiorrespiratória e a qualidade de vida em pacientes com DC crônica. ***Conclusão:*** Evidências pré-clínicas e clínicas sustentam o TE como estratégia complementar para o tratamento da DC, indicando que a dosimetria de treinamento e o estágio da infecção devem ser rigorosamente controlados para garantir benefícios cardiovasculares, imunológicos e parasitológicos livres de eventos adversos.

***Palavras-chave***: Treinamento com exercícios; Parasitologia; Protozooses sistêmicas; *Trypanosoma cruzi*.

## ABSTRACT

***Background*:** Chagas disease (ChD) is a neglected condition closely correlated to poverty and the leading cause of infectious cardiomyopathy worldwide. Although contraindicated in the past, exercise training (ET) was recently suggested as a complementary treatment for ChD. However, the mechanistic basis that supports this recommendation is still unclear. ***Aim*:** We used a systematic review framework to investigate the preclinical and clinical evidence on the relevance of ET for ChD management. ***Methods*:** A two-level PRISMA-based search was applied to PubMed/Medline, Web of Science, Scopus, and Embase databases. Parasitological, biochemical, immunological and cardiorespiratory outcomes were investigated. ***Results:*** Preclinical evidence indicates that preinfection exercise training is safe and increases host resistance to *T. cruzi* infection by upregulating heart and cardiomyocytes contractile function, immune (e.g., INF-γ, TNF-α, NO) and antioxidants (e.g. CAT, GR, GPx, SOD) defenses; attenuating parasitemia, parasite load, reactive oxygen (ROS) and nitrogen (RNS) species biosynthesis, cardiac and skeletal muscle molecular and microstructural damage. Conversely, concomitant infection-exercise training can exacerbate cell parasitism, proinflammatory and prooxidant responses (e.g., lipid and protein oxidation); potentiating target organs damage. Additionally, clinical evidence indicates that dosimetry-adjusted (e.g., symptom-limited exercise tests) aerobic training is equally safe to improve exercise tolerance, cardiorespiratory function and quality of life in patients with chronic ChD. ***Conclusion:*** Preclinical and clinical evidence support ET as a complementary strategy for ChD treatment, indicating that training dosimetry and infection stage must be strictly controlled to ensure cardiovascular, immunological and parasitological benefits free from adverse events.



***Keywords***: Exercise training; Parasitology; Systemic protozooses; *Trypanosoma cruzi*.

## FIGURES LIST

## LIST OF ABBREVIATIONS

| | |
|---|---|
| 6MWT | Six-minute walk test |
| CAT | Catalase |
| CCC | Chronic Chagas Cardimyopathy |
| ChD | Chagas Disease |
| CKMB | Creatine kinase isoenzyme MB |
| DNA | Deoxyribonucleic acid |
| ELISA | Enzyme-linked immunosorbent assay |
| GR | Glutathione reductase |
| GST | Glutathione S-transferase |
| H2O2 | Hydrogen peroxide |
| IFN-γ | Interferon gamma |
| IL | Interleukin |
| MCP-1 | Monocyte chemoattractant Protein-1 |
| MyHC | Myosin heavy chain |
| NO | Nitric oxide |
| NPA | Non-protein antioxidante |
| NYHA | New York Heart Association |
| PICO | Problem, Intervention, Comparison and Outcome |
| PRISMA | Preferred Reporting Items for Systematic Reviews and Meta-analyses |
| PROSPERO | International Prospective Register of Systematic Reviews |
| SOD | Superoxide dismutase |
| TGF-β | Transforming growth factor β |
| Th1 | T-helper one |
| Th2 | T-helper two |
| TNF-α | Tumor necrosis factor-alpha |
| $VO_2$ | Oxygen uptak |

**SUMMARY**

# ARTICLE: PRECLINICAL AND CLINICAL EVIDENCE OF EXERCISE TRAINING AS A COMPLEMENTARY CHAGAS DISEASE TREATMENT

## INTRODUCTION

Chagas disease (ChD) is an anthropozoonosis caused by the protozoan parasite *Trypanosoma cruzi*. This pathogen infects about 6-8 million people worldwide, mainly in endemic countries in Latin America (WHO, 2022; DNDi, 2022). However, this disease is on the rise in non-endemic countries in North America, Europe and Oceania, especially due to cases of infections associated with the donation of contaminated blood and organs, vertical transmission (hand to fetus), and laboratory accidents (Lidani et al., 2019; Guhl and Ramírez, 2021). Chagas disease is a neglected infection, which has not aroused the interest of the pharmaceutical industry for investment in the development of new and more effective antiparasitic treatments (DNDi, 2022). This disease is highly disabling and potentially fatal, manifesting progressive deterioration of the heart structure and function in about 30% of infected individuals, a condition known as chronic Chagas cardiomyopathy (CCC) (Nunes et al., 2018; WHO, 2022). Accordingly, *T. cruzi* infection is the leading cause of infectious cardiomyopathy worldwide, and the third condition associated to heart transplantation in Latin America (Nogueira et al., 2018).

The specific treatment for ChD is based on benznidazole and nifurtimox, which are associated to marked systemic toxicity, serious side effects (e.g., blood marrow depression, peripheral neuropathy and dermatitis) and low cure rates in chronic infections (10-20%) (Sales Junior et al., 2017; Caldas et al., 2019). Thus, pharmacological (e.g., anti-inflammatory and antioxidant agents) and non-pharmacological (e.g., exercise training) complementary strategies have been proposed for ChD management (Lima et al., 2010; De Souza et al., 2020). Physical training was considered an absolute contraindication for chagasic patients until the 1990s, as it was believed that cardiovascular overload could worsen cardiac injuries

and accelerate the evolution of CCC (Gallo et al., 1975; Dias and Coura, 1997). However, this indication has been revised in the last decades, and this paradigm shift was supported by the therapeutic success of exercise training in cardiomyopathies with non-infectious etiology. In these cases, chronic exercise provided marked improvements in the immune response (Gevaert et al., 2020), antioxidant defenses (Sties et al., 2018; Tofas et al., 2019) and cardiovascular function in heart disease patients (Morris and Chen, 2019), events that were later recognized to offer cardioprotection and improve quality of life in CCC patients (Lima et al., 2010; Lucchetti et al., 2017). In addition, physical training also appears to be relevant in increasing host resistance to *T. cruzi* infection, especially by attenuating parasitemia, cell parasitism, myocarditis (Novaes et al., 2016, 2017; Santos et al., 2019), oxidative stress (Mendonça et al., 2019; Santos et al., 2019), disautonomia (Nascimento et al., 2014), as well as improving cardiomyocytes contractility (Novaes et al., 2016) and cardiovascular function (Lucchetti et al., 2017; Sarmento et al., 2021) in ChD.

Currently, evidence on the impact of exercise training on *T. cruzi*-infected hosts remains fragmented, making it difficult to establish a clear relationship between the therapeutic benefits associated with different exercise training protocols. Accordingly, mapping the evidence on specific exercise modalities and dosimetry parameters (e.g., modality, time, intensity, frequency and progression) is essential to clarify the best training protocols, contributing to prescribing exercises in a more objective and rational way in ChD. Thus, this study was designed to investigate the preclinical and clinical evidence on the applicability and relevance of exercise training for ChD treatment. In addition to mapping exercise protocols adopted, its impact on redox metabolism, parasitological and immunological parameters were related to the morphophysiology of target organs in *T. cruzi*-infected animals and humans. Considering the refinement

of research relating exercise training and ChD, the methodological quality of all reviewed studies was evaluated, pointing out the main limitations/sources of bias in the accumulated evidence that must be overcome in further investigations.

## METHODOLOGY

### Research question and protocol registration

Our guiding question was based on the PICO (P= Problem, I= Intervention, C= Comparison and O= outcome) strategy (Eriksen et al., 2018). Thus, the guiding question for this review was: Animals and humans infected by *T. cruzi* and treated with exercise training exhibits improved parasitological, immunological, oxidative and morphofunctional outcomes of target organs compared to *T. cruzi*-infected and untreated hosts? The methodological protocol used in this systematic review was registered in PROSPERO (International Prospective Register of Systematic Reviews) database, receiving the register number CRD420191500275.

### Search strategy

This revision was developed according to standardized guideline PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analyses) (Page et al., 2020). A two-level search was designed to identify relevant studies investigating the impact of exercise training on *T. cruzi*-infected animals and humans. The primary (direct) search was based on study retrieval in four comprehensive electronic databases: PubMed/Medline, Web of Science, Scopus (Felizardo et al., 2018), and Embase (Bramer et al., 2017). The secondary (indirect) search was carried out by the screening of the reference lists of all relevant studies identified in the primary search (Pereira et al., 2017). For all electronic databases, indexed records were retrieved

using advanced searches based on structured strategies, which were developed by combining search filters organized into two categories: (i) Disease (Chagas disease or American trypanosomiases) and (ii) Intervention (exercise training). Population data (animals and human) and research outcomes were intentionally omitted from our search filters to enhance the search sensitivity rather than specificity (Jenkins, 2004).

**Structured search filters**

The search filters were initially developed from standardized descriptors extracted from the PubMed thesaurus (MeSH). The commands [MeSH Terms] and [TIAB] were combined to ensure the retrieval of indexed studies and those in indexing process. All descriptors were combined using Boolean operators (AND/OR) (Pereira et al., 2017; Souza-Silva et al., 2019). The same descriptors and keywords used in the PubMed/Medline database were adapted for Scopus, Web of Science and Embase. Thus, specific search algorithms were used to establish the standardized syntax required in each database (Pereira et al., 2017). The complete search strategy for each database is detailed in the supplementary files (Table S1). No chronological or language limits were applied in our search strategy. Thus, all studies identified and published up to December 2021 were included in this systematic review.

**PRISMA workflow and records screening**

The PRISMA strategy (Page et al., 2020) was used to identify and select all relevant studies. All steps of the PRISMA flow diagram were executed by two independent reviewers (M.A.S. and R.R.F.), and disagreements were resolved from arbitration by expert researcher (R.D.N). All duplicate records retrieved in the primary search were removed. For this, overlapping records between Medline and Scopus

were automatically excluded using the AND NOT INDEX (Medline) algorithm. Duplicates between Medline and Embase were automatically excluded from the Venn diagram tool (Sources tab), selecting the field of studies exclusively indexed in Embase. Additional duplicates were removed using the Mendeley reference manager software (McKeown and Mir, 2021) and by comparing indexing metadata of all identified studies. After this step, the abstracts of all research records were analyzed and irrelevant studies (not related to the investigated subject) were excluded. The remaining studies were collected in full text and evaluated for eligibility according to well-defined inclusion and exclusion criteria.

**Eligibility criteria and inter-rater agreement**

Original and indexed studies investigating the impact of exercise training on the cardiovascular and musculoskeletal systems in *T. cruzi*-infected animals and humans were retrieved. The following exclusion criteria adopted were: (i) full text unavailable, (ii) secondary studies (literature reviews, comments, letters to the editor and editorials), (iii) gray literature (studies not indexed and not submitted to a formal process peer review), (iv) studies with multiple interventions where the effect of exercise cannot be isolated, (v) studies without a control group (infected untreated), and (vi) studies unrelated to cardiorespiratory and musculoskeletal functions. The reference list of all relevant studies retrieved from electronic databases were manually searched for additional articles (Marques et al., 2018; Pereira et al., 2017), which were submitted to the same inclusion and exclusion criteria. The result obtained from the complete search strategy applied by the independent researchers was compared using the Cohen's kappa coefficient (κ), which was calculated to establish the inter-rater agreement (Marcelino et al., 2022).

**Studies categorization and data extraction**

According to the methodological requirements of each research design, data extraction masks were built specifically for pre-clinical and clinical studies (Souza-Silva et al., 2019). Characteristics such as authors and year of publication were drawn from all studies. Additional information from preclinical studies were extracted, such as: (i) animal model: species, lineage, sex and age; (ii) disease model: *T. cruzi* strain, inoculum size, route of administration and time of infection; (iii) intervention: type, dosimetry of exercise training (session duration, intensity, frequency and training period); (iv) primary outcomes: parasitemia, parasite load and mortality; and (v) secondary outcomes: immunological, biochemical, microstructural and functional cardiovascular and musculoskeletal findings. Additional information were also extracted from randomized clinical studies, such as: (i) patients: age and sex; (ii) disease: diagnostic methods and stage; (iii) primary outcomes: parasitemia, parasite load, cardiorespiratory function and mortality; (iv) secondary outcomes: immunological markers (cytokines and antibodies).

**Research bias in preclinical and clinical studies**

The SYRCLE's RoB tool was used to assess potential sources of bias in preclinical animal studies (Hooijmans et al., 2014). This tool is based on the Cochrane Risk of Bias (RoB) tool and was originally tailored for specific aspects of bias that have a relevant impact on animal intervention studies. The SYRCLE's tool is structured into ten topics, which are related to multiple potential sources of bias, such as: (i) selection, (ii) performance, (iii) detection, (iv) friction, (v) reporting and (vi) sources of bias addition not covered by other domains. The detailed analysis of bias for each animal study reviewed is presented in the supplementary files (Tables S4).

The Downs and Black checklist was used to assess the methodological quality and potential sources of bias in randomized clinical trials (Downs and Black, 1998; Nogueira et al, 2018). This tool is based on a scale constructed with 27 questions structured into five categories: (i) reporting quality, (ii) external validity, (iii) bias, (iv) confounding, and (v) statistical power. This scale presented high test-retest reliability (r = 0.88) and internal consistency (KR20 formula = 0.89). Considering previous recommendations, the statistical power (question 27) was omitted due to the high ambiguity and risk of inaccurate inferences (Nogueira et al., 2018).

## RESULTS

### Studies recovered and inter-rater agreement

Six hundred and ninety-six research records were found in the electronic databases, and 3 citations were identified in the reference lists of studies selected in the primary search. Duplicates were removed (n= 406) and 34 studies were submitted to eligibility analysis. Finely, 11 preclinical studies and 4 randomized controlled clinical trials were found from our primary and secondary searches (Fig. 1). Our combined search strategy indicated a substantial inter-rater reliability, as revealed by the Cohen's kappa coefficient (κ= 0.728). This coefficient and the list of all studies included in the systematic review are shown in Table S2. Most preclinical studies were developed in Brazil (81.82%, n= 10) between 1965 and 2019. All clinical studies (100%, n= 4) were conducted in Brazil between 2010 and 2021.

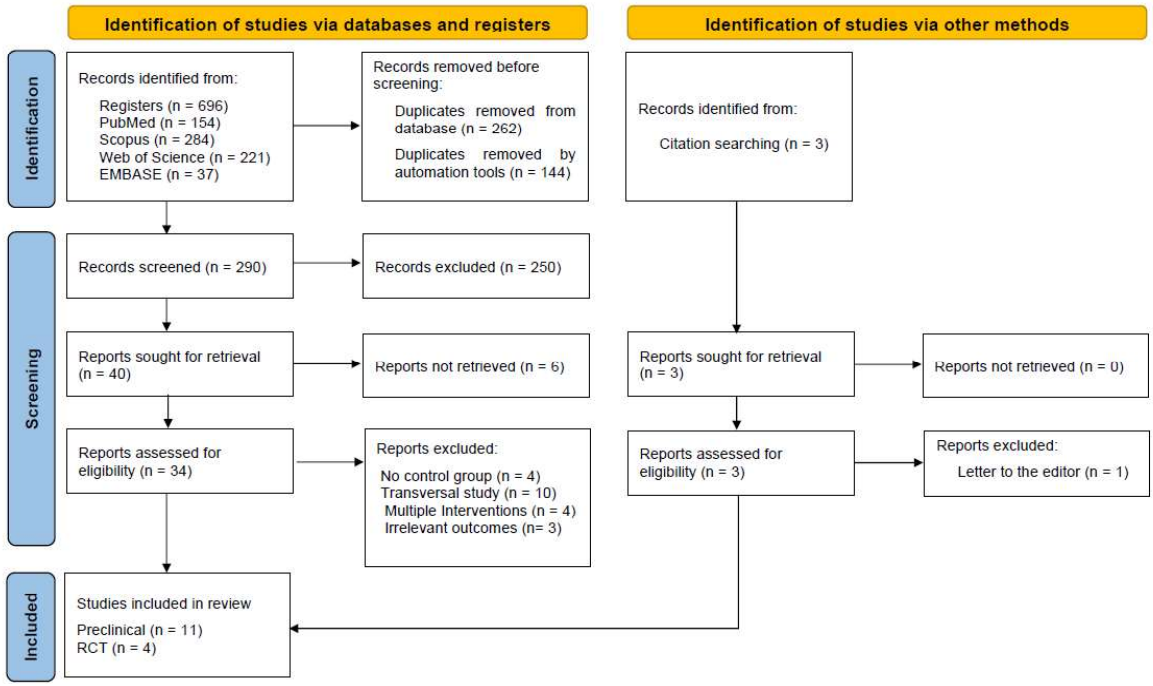


**Fig. 1.** Flowchart detailing selection of studies included in the systematic review. Based on the PRISMA statement "Preferred Reporting Items for Systematic Reviews and Meta-Analyses". Available at: www.prisma-statement.org

## Preclinical studies of Chagas disease

### *Characteristics of preclinical models*

As shown in Table S3, most studies used male mice as animal model (63.64%, n= 7), followed by Wistar rats (36.36%, n= 4). Isogenic mice (BALB/c and C557BL/6) were more frequent (45.46%, n= 5), followed by heterogenic *Swiss* mice or both (9.09%, n= 1 each). All studies investigated young adult animals, ranging from 1 to 16 weeks. Male mice were most frequently used (54.55%, n= 6), followed by female (27.27%, n= 3) and a combination of male and female animals (9.09%, n= 1). This data was not reported in 1 study (9.09%). *T. cruzi* Y strain was used to induce Chagas disease in all studies (100%, n= 11). Inoculum size ranged from 500 to 9000 parasites in mice and 150,000 to 600,000 trypomastigotes / 100g body mass in rats. Infection time ranged from 11 to 180 days in mice and 30 to 63 days in rats.

*Characteristics of exercise protocols*

As shown in Table S4, all preclinical studies adopted aerobic exercise training based on treadmill running (90.91%, n= 10) or swimming training (9.09%, n= 1). All studies with running training used well-delimited intensity progression in structured protocols. Intensity progression was not adopted for swimming training, which used fixed exercise time (30 or 60 min.). Five weekly exercise sessions were reported in most studies (90.91%, n= 10). Only one study used a protocol with 7 days exercise per week (9.09%, n= 1). Exercise training was administered for 4 to 12 weeks. Exercise training before infection was administered in 7 studies (63.64%), while exercise concomitant with infection was reported in 4 studies (36.36%). Both exercise protocols (before and during infection) were included in 1 study (9.09%).

*Parasitological and immunological outcomes*

As indicated in Table S5, parasitological parameters were reported in most studies (81.82%, n= 9). Mean parasitemia and peak of parasitemia were significantly reduced in 8 studies (73.73%) with preinfection exercise training. Conversely, these parameters were increased in 1 study (9.1%) with animals receiving concomitant infection-exercise training. Cytokines were quantified in 9 studies (81.82% %). In general, increased TNF-α, IL-6 (Novaes et al., 2016), IL-10 (Novaes et al., 2017a; Santos et al., 2019), IL-4 and IFN-γ (Santos et al., 2019) levels were reported, while reduced TNF-α (Lucchetti et al., 2017; Novaes et al., 2017a), MCP-1 (Santos et al., 2019), IL-10 and IFN-γ (Lucchetti et al., 2017) levels were also indicated. Conversely, similar TNF-α (Schebeleski-Soares et al., 2009, 2010; Santos et al., 2019), TGF-β (Novaes et al., 2016), IFN-γ (Novaes et al., 2016, 2017), IL-6 (Novaes et al., 2017a), IL-17 (Santos et al., 2019) levels were identified in studies with preinfection exercise training. In addition, TNF-α (Schebeleski-Soares et al., 2010), IL-10 and IL-4 levels

were unchanged, while TNF-α, IFN-γ, IL-17, and MCP-1 levels were increased by concomitant acute infection-exercise training (Mendonça et al., 2019). Finally, IL-6, IL-10, IL-12, TNF-α, IFN-γ, and MCP-1 were unchanged in animals chronically infected and trained (Pedra-Rezende et al., 2020).

*Biochemical, microstructural and functional outcomes*

*Trypanosoma cruzi* infection was often associated with marked oxidative stress (Table S6). In general, preinfection exercise training reduced nitric oxide (NO) production (Santos et al., 2019), lipid and protein oxidation (Novaes et al., 2017a; Santos et al., 2019) and upregulated CAT, SOD (Novaes et al., 2017a; Santos et al., 2019), NPA and GST (Santos et al., 2019) activities in the heart. Preinfection training also increased exercise tolerance (Novaes et al., 2017a; Santos et al., 2019), cardiomyocytes contractile properties (e.g., shortening rate, contraction and relaxation velocities) (Novaes et al., 2016), resting heart rate (Lucchetti et al., 2017), MyHC I expression and myocytes distribution in skeletal muscle (Novaes et al., 2017a). In addition, CKMB (Soares et al., 2021), cardiac damage, inflammatory infiltrate (Novaes et al., 2016, 2017; Lucchetti et al., 2017; Santos et al., 2019), and cardiac parasitism (Lucchetti et al., 2017) were attenuated in trained animals. In turn, NO, hydrogen peroxide ($H_2O_2$), lipid and protein oxidation, CAT, GR, inflammatory infiltrate were increased, and cardiomyocytes distribution was reduced by concomitant acute infection-exercise training (Mendonça et al., 2019). However, increased capillaries distribution (Preto et al., 2015), exercise tolerance, cardiac functional area (Aves et al., 2019), reduced collagen content, cardiomyocytes hypertrophy (Preto et al., 2015) were also associated with concomitant infection-exercise training.

## Clinical trials on Chagas disease

*Patient characteristics*

As indicated in Table S7, 134 volunteers with chronic Chagas disease were investigated in randomized controlled studies. The patients were aged between 30 and 71 years. Immunodiagnostic methods were based on serology for anti-*T. cruzi* antibodies detection. Immunofluorescence and/or enzyme-linked immunosorbent assay (ELISA) were used to confirm Chagas disease in all studies (n= 4, 100%). Heart function was evaluated from echocardiography and electrocardiography in all studies. Heart function received specific classifications in 3 studies (75.0%). Heart function classified according the NYHA criteria indicated patients with scores I and II. Heart function classified according the Goldman criteria indicated patients with scores I, II or III. Maximal oxygen consumption rate was similar at baseline, ranging from 15.4 ± 6.3 to 31.4 ± 7.2 mL/kg/min in the untrained group and 17.6 ± 4.7 to 27.6 ± 5.9 mL/kg/min in trained participants.

*Characteristics of exercise training protocols*

As shown in Table S8, all studies adopted aerobic exercise training based on monitored walk (50%, n= 2) or a multimodal training based on treadmill or ergometer exercises combined with stretching and strengthening exercises (50%, n= 2). All studies used well-delimited exercise intensity, which was based on symptom-limited exercise tests and structured in exercise protocols including warm-up, aerobic training and cooling-down phases. All training protocols were based on 60-minute exercise sessions 3 days a week, ranging from 12 to 16 weeks. Intensity progression was not objectively reported in all studies.

*Cardiorespiratory outcomes*

As indicated in Table S9, all clinical studies indicated cardiorespiratory benefits of exercise training in patients with chronic ChD. In general, exercise training increased $VO_2$ max and exercise tolerance measured from ergometric test or six-minute walk test (6MWT) (Lima et al., 2010; Nascimento et al., 2014; De Souza et al., 2020; Sarmento et al., 2021). In addition, peak heart rate (Lima et al., 2010), muscle sympathetic nerve activity, systolic blood pressure variability (Sarmento et al., 2021), functional aerobic impairment (De Souza et al., 2020) were reduced, while Goldman index (Nascimento et al., 2014), muscle blood flow, hear rate variability and cardiac barorreflex sensitivity were increased (Sarmento et al., 2021) in trained participants. Conversely, brain natriuretic peptide levels (Lima et al., 2010), heart rate at rest (Lima et al., 2010; Sarmento et al., 2021), maximum inspiratory and expiratory pressures (De Souza et al., 2020), mean blood pressure, systolic blood pressure, left ventricle ejection fraction, end-diastolic volume, and end-systolic volume were unchanged in trained patients (De Souza et al., 2020; Sarmento et al., 2021).

**Risk of bias in preclinical and clinical studies**

No preclinical study met all the methodological criteria analyzed in the SYRCLE's tool. The highest support to the quality criteria evaluated was obtained by Novaes et al. 2016, 2017 and Santos et al. 2019 (60%). The highest risk of bias was identified in the studies developed by Schebeleski-Soares et al. 2010; Soares et al. 2012 and Lucchetti et al. 2017 (20%), followed by Schebeleski-Soares et al. 2009, Preto et al. 2015, Pedra-Rezende et al., 2021 (30%), and Alves et al. 2019 (40%). Allocation sequence (54.55%), baseline characteristics (72.73%), absence of selective outcomes (81.82%) and complete report of the exercise protocols (72.73%) were the main criteria met. Conversely, aspects such as blinding of caregivers and evaluators, random

assessment of animals and outcomes were neglected in all preclinical research reports (Table S10).

Similarly, no clinical study met all methodological criteria analyzed from the Downs and Black Quality checklist (Table S11). However, a moderate quality score was obtained in all studies, ranging from 65.39% (Lima et al., 201) to 76.92% (Nascimento et al., 2014). Reports related to sample representativeness for the recruited population, experimental blinding, randomization of patients and intervention groups, and adjustment for confounding variables were neglected in all clinical trials. Except for the adverse events (25%) and attempts to blind the evaluators (75%), the remaining domains were fully met (100%).

## DISCUSSION

### General preclinical and clinical findings

From a comprehensive search strategy, the relevance and safety of exercise training during ChD infection was investigated. Currently, the evidence available is mainly based on preclinical studies, while randomized controlled trials investigating patients with Chagas disease are still scarce. Interestingly, preclinical and clinical studies converge in indicating beneficial effects and the absence of adverse events of preinfection exercise training in animal models of *T. cruzi* infection, as well as aerobic training in subjects with chronic ChD. In both conditions, exercise training was able to induce favorable cardiometabolic and immunological adaptations to the host, which are schematically summarized in Fig. 2. In preclinical studies, this protective response was closely correlated to the upregulation of antioxidant defenses (Novaes et al., 2017a; Santos et al., 2019) and anti-*T. cruzi* proinflammatory effectors (Novaes et al., 2016; Santos et al., 2019), resulting in a more efficient parasitological control

(Schebeleski-Soares et al., 2009; Soares et al. 2012; Novaes et al., 2016, 2017; Lucchetti et al., 2017; Santos et al., 2019), attenuation of tissue damage (Preto et al., 2015; Novaes et al., 2016, 2017; Lucchetti et al., 2017; Santos et al., 2019) and mortality rates (Lucchetti et al., 2017; Pedra-Rezende et al., 2021). In clinical trials, exercise tolerance, cardiorespiratory function (Lima et al., 2010; Nascimento et al., 2014; De Souza et al., 2020; Sarmento et al., 2021) and quality of life (Lima et al., 2010) were improved in trained patients with chronic ChD, which did not manifested adverse effects. Potential deleterious effects of exercise training during acute infection was reported in a single preclinical study (Mendonça et al., 2019). In this case, exercise training was proposed as an additional cardiometabolic overload in cases of patent parasitemia. Accordingly, was suggested that the overlap of exercise and acute infection can overwhelm the host's defenses, shifting the redox and immune balance in favor of the parasite, exacerbating target organs damage and the risk of host mortality.

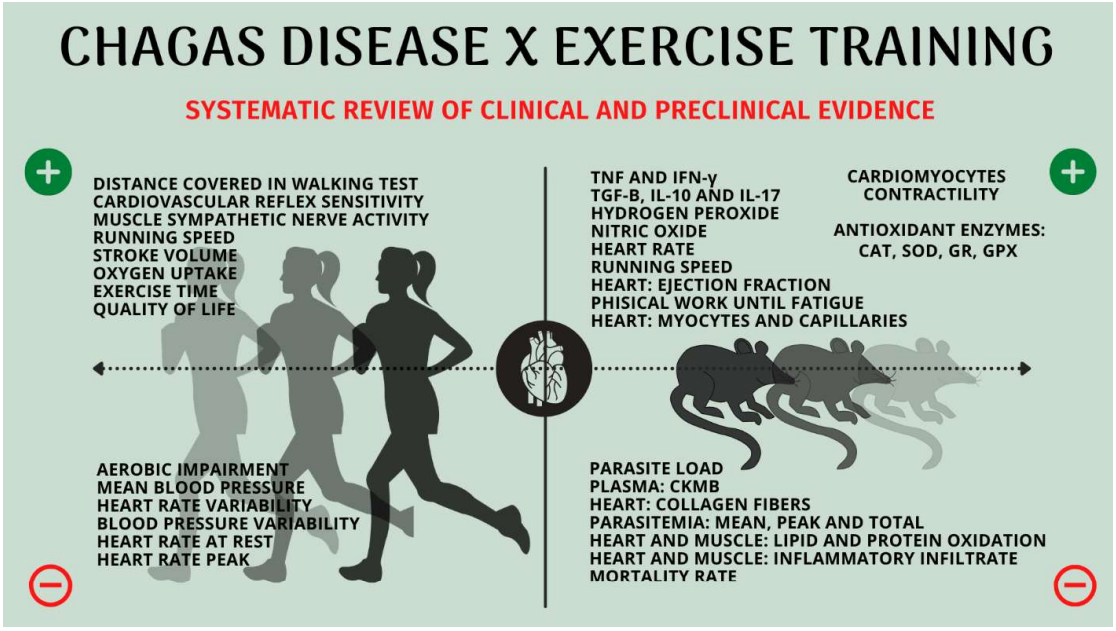


**Fig. 2.** Main outcomes obtained from exercise training in animals and humans with Chagas disease. Symbols: Events upregulated ⊕ and downregulated ⊖ by exercise training in humans and animals with Chagas disease. Left side: Human adaptations. Right side: Non-human animal adaptations. CAT: catalase, SOD: superoxide dismutase, GR: glutathione reductase, GPX: glutathione peroxidase.

**Preclinical models of Chagas disease**

As expected, most studies identified were developed in endemic countries. Despite the positive effects reported in these studies, current evidence does not support the replacement of antiparasitic chemotherapy with exercise training. Thus, there seems to be a consensus to admit exercise training as a complementary strategy for ChD management. From a methodological perspective, the pre-clinical evidence was integrally based on different murine models. Thus, species-specific variability in the profile of resistance and susceptibility to *T. cruzi* infection must be considered to interpret the validity and relevance of ChD models in simulating human disease (Chatelain and Konar, 2015; Lana, 2017). Fortunately, Wistar rats were used few studies (Novaes et al., 2016, 2017; Mendonça et al., 2019; Santos et al., 2019), since these animals show marked resistance to *T. cruzi* infection (Novaes et al., 2011; Lana, 2017). Accordingly, even with parasite inoculations about 150 times greater than in mice, rats often manifest low parasitemia, rapid parasite clearance, subtle tissue damage, and no mortality (Novaes et al., 2011; Santos et al., 2019). This response is known to be linked to an intense and highly polarized Th1 phenotype, ensuring rapid and effective natural infection control in rats (Mendonça et al., 2019; Santos et al., 2019). Thus, animals highly resistant to *T. cruzi* are not ideal ChD preclinical models, since exacerbated antiparasitic responses make it difficult to isolate research outcomes related to treatment or the host's natural resistance (Lana, 2017). Conversely, mice are susceptible to *T. cruzi* infection, manifesting high parasitemia, parasite load, extensive tissue damage and high mortality even in animals challenged with low inoculum (e.g., 1000-2000 parasites) (Felizardo et al., 2018; Chatelain and Konar, 2020; Pereira-Santos et al., 2022). Despite rapid disease evolution, these animals develop prominent biochemical, immunological and microstructural changes

in target organs, which provide a better estimate of the effects of pharmacological and non-pharmacological therapy (Souza-Silva et al., 2019; Pereira-Santos et al., 2022). In addition, isogenic mice (e.g., C57BL/6 and BALB/c) contribute to obtain more homogeneous responses, which are favorable to investigate the mechanistic basis associated with the treatments administered (Pereira et al., 2017; Ferreira et al., 2018). However, the high genetic and phenotypic variability observed in heterogeneous animals (e.g., *Swiss*) may be more relevant in studies on ChD treatment, since they are more realistic considering the human infection (Campbell et al., 2004; Lana et al., 2017). Despite the variability in animal models, infection was consistently induced using *T. cruzi* Y strain in all studies. Interestingly, this strain determines high infectivity, cardiotropism and pathogenicity (Rodriguez et al., 2014; Santos et al., 2015; Souza-Silva et al., 2019), being relevant and in line with the models of acute ChD investigated in the studies reviewed.

**Preclinical exercise protocols**

In addition to the ChD model, training characteristics have a direct influence on therapeutic outcomes. Despite different dosimetry, most preclinical and clinical studies reviewed adopted aerobic training based on well-structured protocols with progressive intensity. This finding is not surprising, since cardiovascular changes are the main causes of morbidity and mortality associated with ChD (Nogueira et al., 2018; Lidani et al., 2019). In general, these alterations result from direct organs damage caused by cellular parasitism (Pérez-Molina and Molina; 2018; Lidani et al., 2019), as well as secondary immune-mediated damage (e.g., cytotoxic cellular response, autoimmunity, and inflammatory-based redox imbalance) (Bonney et al., 2019). Thus, the protective cardiometabolic and immunological adaptations induced by chronic exercise provide a

rationale for the selection of aerobic training in all studies reviewed. Interestingly, most preclinical studies reviewed adopted preinfection exercise training. The motivation for this type of training was not always explicit. However, it may be related to the misperception of exercise training as an absolute contraindication in cases of ChD. Although this concept lasted up to 2 decades ago (Gallo et al., 1975; Bocchi, 2010) and may be rational in more severe cases of CCC, recent preclinical (Lucchetti et al., 2017; Santos et al., 2019; Mendonça et al., 2019; Pedra-Rezende et al., 2021) and clinical (Lima et al., 2010; Nascimento et al., 2014; De Souza et al., 2020; Sarmento et al., 2021) studies indicate that aerobic training can contribute to ChD management.

**Preclinical immunological outcomes**

In the preclinical context, the studies reviewed proved the effective role of preinfection exercise training in improving physical performance (e.g., exercise tolerance, physical work, and lactate threshold) in animal models (Novaes et al., 2016, 2017; Lucchetti et al., 2017; Alves et al., 2029). Along with this effect, animals trained before infection and challenged with *T. cruzi* developed cardiometabolic protective adaptations, especially increased production of proinflammatory antiparasitic effectors (e.g., IFN-γ, TNF, and NO) (Novaes et al., 2016; Santos et al., 2019), enzymatic (e.g., CAT, GST, SOD) and non-enzymatic antioxidant defenses (Novaes et al., 2017a; Santos et al., 2019). Thus, trained hosts developed greater resistance to *T. cruzi* infection, which was evidenced by better parasitological control (e.g., attenuated parasitemia and parasite load) (Schebeleski-Soares et al., 2009; Soares et al., 2012; Novaes et al., 2016, 2017; Lucchetti et al., 2017; Santos et al., 2019), attenuated oxidative stress (e.g., lipid and protein oxidation) (Novaes et al., 2016, 2017; Santos et al., 2019) and microstructural damage to target organs (e.g., myocarditis and/or

skeletal myositis) (Novaes et al., 2016, 2017; Lucchetti et al., 2017; Santos et al., 2019), improved cardiac function (e.g., heart rate and cardiomyocyte contractility) (Novaes et al., 2016; Lucchetti et al., 2017) and lower mortality rates (Lucchetti et al., 2017; Pedra-Rezende et al., 2021). Overall, the preclinical evidence clearly demonstrates an interesting alignment between immunological and parasitological findings. Accordingly, by upregulating IFN-γ, TNF-α and NO production, exercise training was efficient in stimulating the Th1 immune response, which is the main defense barrier against *T. cruzi* that restricts parasitemia and parasite load on target organs (Rodrigues et al., 2017; Felizardo et al., 2018). In addition to these effectors, training demonstrated potential immunomodulatory relevance by stimulating IL-17 and IL-10 production (Novaes et al., 2017a; Santos et al., 2019; Mendonça et al., 2019). Although these molecules are antagonistic to the Th1 phenotype (Lana et al., 2017; Rodrigues et al., 2017), IL-17 and IL-10 act in a counterregulatory manner to balance the inflammatory response, preventing exacerbated reactions often associated with the worsening of the microstructural damage in parasitized organs, especially the heart (Felizardo et al., 2018; Pereira-Santos et al., 2022). Thus, there is evidence that IFN-γ, TNF-α, IL-10 (Golgher and Gazzinelli, 2004) and IL-17 (Miyazaki et al., 2010) knockout animals challenged with *T. cruzi* develop more severe infections. In these cases, animals evolve with higher parasitemia and parasite load, severe myocarditis, and high mortality rates compared to wild-type animals, reinforcing the proposition that antagonistic immunological responses are simultaneously activated to modulates the balance between host resistance and susceptibility in Chagas disease (Rodrigues et al., 2017; Pereira-Santos et al., 2022).

**Preclinical immunological and metabolic outcomes**

To achieve better parasitological control, some preclinical studies indicated that the Th1 antiparasitic response in trained animals was associated with redox metabolism upregulation (Novaes et al., 2016, 2017; Santos et al., 2019). This finding was expected, since the respiratory burst in activated leukocytes and M1 macrophages polarization induced by IFN-γ and TNF-α trigger reactive species production, especially $H_2O_2$ and NO (Rodrigues et al., 2017; Chistiakov et al., 2018). Although these molecules are toxic to the parasite, they also cause lipids, proteins and DNA oxidation in host cells, which can induce cell death (Bonney et al., 2019; Maldonado et al., 2020). In fact, reviewed studies that evaluated reactive stress markers identified increased $H_2O_2$, NO, oxidized lipids and oxidized proteins levels in *T. cruzi*-infected animals (Novaes et al., 2016, 2017; Lucchetti et al., 2017; Santos et al., 2019). However, these markers were attenuated in trained animals; an effect potentially mediated by the upregulation of enzymatic and non-enzymatic antioxidant effectors, which are potent scavengers of radical and non-radical reactive species (Novaes et al., 2016, 2017; Santos et al., 2019). Although the improvement in oxidative metabolism is a classic adaptation of aerobic training (Sties et al., 2018; Tofas et al., 2019), this is not a trivial response in ChD. There is consistent evidence that CCC progression is linked to *T. cruzi*-induced redox imbalance (Wen et al., 2004; Maldonado et al., 2020). Accordingly, increased reactive species production and antioxidant defenses attenuation are important predictors of microstructural organs damage (Wen et al., 2004; Felizardo et al., 2018; Novaes et al., 2017b) and cardiac electromechanical disturbances (Maçao et al., 2007; Barbosa et al., 2017) in ChD. Thus, by improving the redox balance, exercise training has a potentially beneficial effect on mechanisms centrally involved in CCC pathogenesis. Apparently, this protective effect is not restricted to the heart,

indicating that exercise training can also reinforce antioxidant defenses and attenuate skeletal myositis in *T. cruzi*-infected animals, contributing to an improved physical performance in trained animals (Novaes et al., 2017a).

**Preclinical microstructural and functional outcomes**

As expected, most of the studies reviewed indicated that better parasitological control and modulation of the immune and redox metabolism by exercise training were also associated with marked attenuation of pathological cardiac and skeletal muscle remodeling. Microstructural alterations of muscle tissues are evident in the acute phase of *T. cruzi* infection; especially diffuse inflammatory infiltrate, cardiomyocytolysis, myonecrosis, connective stromal expansion, vascular collapse and microembolic processes (Lana et al., 2017; Pérez-Molina JA, Molina, 2018; Bonney et al., 2019). Although these changes are classically identified by histopathological methods, biochemical markers such as CKMB (Cortes-Serra et al., 2020) and myosin heavy chain I (MyHC I) (Giordanengo et al., 2000) are useful in the diagnosis of microstructural cell damage in acute ChD. Although these markers have been marginally investigated in the studies reviewed, microstructural protection was consistent with CKMB attenuation (Soares et al., 2012) and MyHC I levels (Novaes et al., 2017a) in trained animals. There is sufficient evidence that parasitological and inflammatory control are essential to attenuate the progression of experimental and human ChD (Novaes et al., 2017a; Pérez-Molina et al., 2020). Thus, it has been systematically proved that antiparasitic and anti-inflammatory drugs are efficient to attenuate the severity of tissue damage and the mortality in *T. cruzi*-infected hosts (Novaes et al., 2017b; Mendonça et al., 2020). However, attenuating oxidative stress through antioxidant drugs does not seem sufficient to prevent pathological cardiac

remodeling, indicating that consistent parasitological and inflammatory control are essential to ensure more favorable pathological outcomes (Marim et al., 2012; Novaes et al., 2017b), which are partially achieved from exercise training.

Unfortunately, the effect of preinfection exercise training on cardiac function in infected animals was poorly investigated in the reviewed studies. However, preinfection training also exerted a protective cardiovascular effect, improving resting heart rate (Lucchetti et al., 2017), heart functional area, ejection fraction (Alves et al., 2019) and cardiomyocytes contractility (Novaes et al., 2016). Apparently, these effects were related to better parasitological, immunological and oxidative control. Previous studies indicated that cell parasitism trigger intense pathological heart remodeling, which deteriorates cardiac electromechanical function in response to parenchymal destruction, tissue fibrosis, microvascular hypoperfusion, and blockages of the heart's excitation and conduction system (Pérez-Molina et al., 2018; Bonney et al., 2019). In addition, exacerbated cytokine levels (e.g., TNF-α and IFN-γ) and reactive species (e.g., NO and $H_2O_2$) are able to attenuating cardiomyocytes contractility and cardiac function in ChD (Novaes et al., 2016; Cruz et al., 2017; Roman-Campos et al., 2019). Thus, exercise training seems to modulate a complex network of parasitological, immunological and oxidative interactions to improve cardiac function in ChD, a remarkable effect considering that CCC is the most severe and deadly manifestation of this disease (Nogueira et al., 2018; Bonney et al., 2019).

**Controversial preclinical outcomes**

It is essential to consider that exercise training benefits do not seem unequivocal in relation to the stage of *T. cruzi* infection. Thus, no parasitological benefit (Alves et al., 2019; Pedra-Rezende et al., 2021) or even parasitemia, systemic inflammation,

oxidative stress and myocarditis aggravation were reported when training was administered in the patent period of acute infection (Mendonça et al., 2019). The limited number of studies investigating concomitant infection-exercise training precludes a conclusive understanding of this interaction. However, it is well known that exercise training and patent infection are independent sources of cardiometabolic stress (Gallo et al., 1975; Bocchi et al., 2010; Mendonça et al., 2019). This overload is more relevant in the initial period of exposure to exercises, since the cardiovascular, antioxidant and immunological benefits are late adaptations to training protocols (Sties et al., 2018; Morris and Chen, 2019; Tofas et al., 2019). Thus, it is rational to assume that the metabolic demands of active infections and the initial phase of exercise act synergistically or additively, determining metabolic overload capable of overcome the host's protective counterregulatory mechanisms. This proposition is reinforced considering that animals trained during acute infection manifested higher reactive species (e.g., NO and $H_2O_2$) levels and intense molecular oxidative damage (e.g., increased malondialdehyde and protein carbonyl) compared to untrained infected animals (Mendonça et al., 2019). Despite upregulation of antioxidant enzyme activity (e.g., CAT and SOD), this response was not sufficient to prevent oxidative stress in trained animals, corroborating evidence that additional exercise training overload can be harmful in the presence of patent parasitemia and active myocarditis (Mendonça et al., 2019). Apparently, this concern extends to patients with ChD (Bocchi et al., 2010). Thus, the moment when training is administered has a potential impact on pathological outcomes in ChD, which must be considered to design efficient and safe training programs.

**Patients, diagnostic tools and exercise dosimetry in clinical studies**

From an extensive literature search, we identified that current clinical evidence is still scarce and based on a limited number of patients included in randomized controlled trials (Lima et al., 2010; Nascimento et al., 2014; De Souza et al., 2020; Sarmento et al., 2021). However, these studies show remarkable methodological control and used robust methods for infection diagnosis (e.g., ELISA and immunofluorescence) and cardiac function assessment (e.g., electrocardiography and echocardiography). The combination of parasitological and cardiovascular diagnostic tools are essential to assess ChD progression (Dias et al., 1997; Nogueira et al., 2018; Pérez-Molina and Molina, 2018). These methods make it possible to assess the disease stage, since cardiac abnormalities are pathognomonic of the determined chronic phase, since they are absent in the indeterminate chronic phase despite positive serology (Dias et al., 1997; Pérez-Molina and Molina, 2018). Thus, the current evidence is mainly based on adult subjects with specific ChD, without limitation or with mild or moderate limitation to physical exercise. Currently, exercise recommendation during acute infections is still controversial and often contraindicated (Bocchi et al., 2010; Mendonça et al., 2019). In this case, the etiological treatment based on the drugs benznidazole (first-line chemotherapy) or nifurtimox (second choice) remains the antiparasitic therapy of reference (Sales Junior et al., 2017; Caldas et al., 2019; Pérez-Molina et al., 2020). However, the clinical studies available are consistent with the reality of late ChD diagnosis, which is mainly discovered in the chronic phase (Pérez-Molina and Molina, 2018; Caldas et al., 2019), when the indication of exercise training can be better defined based on a careful cardiovascular assessment. As a central element for adequate exercise prescription, cardiovascular safety in all investigated studies also took into account the intensity of training effort (Lima et al., 2010; Nascimento et al., 2014; De Souza et al., 2020; Sarmento et al., 2021). Thus, the effort

dosimetry was based on adjusted $VO_2$ max rate calculated from symptom-limited exercise tests for each patient, reinforcing the individualized characteristic and the reproducibility of the training protocols used. In addition, baseline $VO_2$ max was similar in trained and control groups, while the more intense exercise stages were based on 30-40 min walking or treadmill running 3 time/week for 12-16 weeks were adopted in all studies, favoring the comparison of clinical outcomes.

**Cardiorespiratory clinical outcomes**

As expected, mechanistic aspects were not investigated in clinical trial due to evident ethical limitations involving human research. However, exercise training was associated with marked cardiovascular benefits in all studies. Accordingly, significant improvements in physical performance and exercise tolerance (e.g., $VO_2$ max, time and distance covered in 6MWT) (Lima et al., 2010; Nascimento et al., 2014; De Souza et al., 2020; Sarmento et al., 2021), heart rate of rest (Lima et al., 2010), muscle sympathetic nerve activity, systolic blood pressure variability, cardiac baroreflex sensitivity and Goldman risk index (Sarmento et al., 2021). Taken together, these findings indicate a remarkable therapeutic potential of exercise training in patients with ChD. Interestingly, these benefits were associated with important functional aspects limited by CCC, which often worsen as the disease progresses over decades (Dias and Coura, 1997; Nunes et al., 2018; Pérez-Molina and Molina, 2018). Although exercise-training purpose is not to achieve parasitological cure, mitigating CCC sequelae are equally relevant endpoints in ChD management (Gonçalves and Novaes et al., 2018). Improving physical performance can prolong the autonomy of ChD patients to perform work and daily life activities (Lima et al., 2010; Fialho et al., 2012). In addition, there is a direct relationship between physical performance and psychological well-being (Lima et al., 2010, Almeida et al., 2022), which may partially

explain the improvements in multiple quality of life domains (e.g., vitality, emotional aspects, and mental health) in trained ChD patients (Lima et al., 2010). Thus, by exerting a multidimensional impact, exercise training also offers a relevant opportunity to complement the clinical ChD management.

**Risk of bias in preclinical and clinical studies**

From a critical interpretation of the evidence guided by standardized tools (Fig. 3), methodological quality assessment in all preclinical studies indicated that 39.29 ± 15.42% criteria evaluated were achieved, with an adherence ranging from 20 to 60% criteria met (high to moderate risk of bias). Conversely, a high average methodological score (70.19 ± 4.84%) indicated and a reduced risk of bias in randomized controlled clinical trials, ranging from 65.39% to 76.92% criteria met (De Souza et al., 2020). Considering that preclinical and clinical studies can be similarly designed, as well as the greater possibility of experimental control in preclinical studies, the risk of bias indicated that reporting quality is an important limitation of the preclinical evidence. Conversely, the high methodological rigor in human research provides more robust clinical evidence. Contrary to expectations, the quality index did not show a clear time-dependent behavior (influence of the publication year), indicating that the variability detected in the quality scores may be linked to the systematic replication of confounding factors (sources of bias) despite the advances applied to the design, operationalization and monitoring of preclinical or clinical studies, as well as greater availability of sensitive and specific analytical tools.

In cases of partial methodological adherence, the least met criteria were associated to limited information on random allocation of animals to intervention groups, blinding of evaluators, random data collection, and incomplete reporting of

incomplete outcome data. In addition, the main sources of bias in clinical studies were related to incomplete reporting on sample representativeness in relation to the entire population, blinding of patients and evaluators, randomization of patients into treatment groups, and outcome adjustments for confounding variables. Admittedly, these methodological limitations undermine the reproducibility, internal and external validity of the reviewed studies, limiting evidence reliability (Downs and Black, 1998; Torrico et al., 2018; 2021). However, it is important to consider that these quality scores do not indicate flaws in the experimental protocols, since they exclusively point out limitations in the research report.

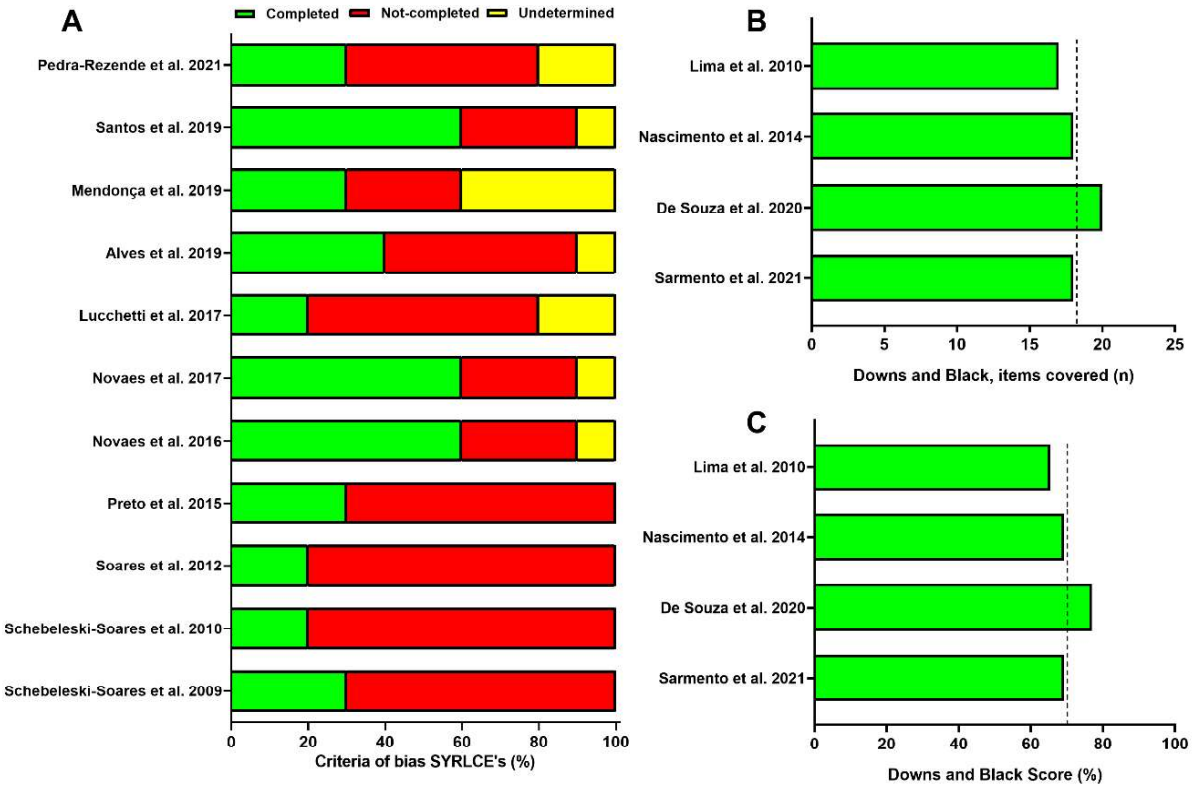


**Fig. 3.** Risk of bias in preclinical (A) and clinical studies (B and C). (A) Items completed, not-completed and undetermined in preclinical studies analyzed from the SYRCLE's risk of bias toll. (B) Number (n) and (C) percentage (%) of items meet in randomized controlled trials analyzed from the Downs & Black checklist for quality assessment.

## CONCLUSION

Preclinical and clinical evidence are consistent in supporting aerobic exercise training as a complementary strategy for ChD treatment, indicating that training dosimetry and infection stage must be strictly controlled to ensure cardiovascular, immunological, antioxidant and parasitological benefits free from adverse events. Currently, the main benefits associated with exercise training are due to improvement in exercise tolerance, cardiorespiratory function, increased production of antiparasitic immunological effectors, attenuation of oxidative and nitrosative stress, parasitemia, tissue parasitism, as well as *T. cruzi*-induced cardiac and muscle microstructural damage. Although exercise training administered before infection (preinfection) and during the chronic disease is safe, concomitant acute infection-exercise training are potentially harmful to the host. Thus, current evidence suggests that training overload by can aggravate *T. cruzi* infection by adding to the metabolic overload imposed by the intense parasitism and cellular destruction that occurs in the acute disease. Despite exercise training-induced positive effects, preclinical and clinical evidence exhibited variable methodological quality. Thus, by mapping potential bias sources in all investigated studies, this review provides objective support to delimit further investigations with greater methodological rigor, providing unequivocal evidence on the efficacy of exercise training for ChD management.

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**Conflict of interest**

None to declare.

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**Table S1:** Complete search strategy with search filters and number of studies recovered in databases PubMed-Medline, Embase, Scopus, and Web of Science.

| ***PubMed-MEDLINE – Search filters*** |
|---|
| **#1 Disease:** ("Chagas disease"[MeSH Terms] OR "*Trypanosoma cruzi*"[MeSH Terms] OR "Chagas disease"[TIAB] OR "American trypanosomiasis"[TIAB] OR "*Trypanosoma cruzi*"[TIAB]) |
| **#2 Intervention (exercise):** ("Exercise"[MeSH Terms] OR "Exercise"[TIAB] OR "physical exercise"[TIAB] OR "physical activity"[TIAB] OR "physical training"[TIAB] OR "strength training"[TIAB] OR "resistance training"[TIAB] OR "aerobic training"[TIAB] OR "swimming training"[TIAB] OR "exercise therapy"[TIAB] OR "exercise test"[TIAB] OR "acute exercise"[TIAB] OR "chronic exercise"[TIAB] OR "exercise training"[TIAB]) |
| **#3 Combined search: (**#1 AND #2) |

**Table S1 (*continuation*).** Complete search strategy with search filters and number of studies recovered in databases PubMed-Medline, Embase, Scopus, and Web of Science.

| ***Embase – Search filters*** |
|---|
| **#1 Disease:** ('Chagas disease':de,ab,ti OR 'American trypanosomiasis':de,ab,ti OR 'Trypanosoma cruzi':de,ab,ti) |
| **#2 Intervention (exercise):** (Exercise:de,ab,ti OR physical exercise:de,ab,ti OR physical activity:de,ab,ti OR physical training:de,ab,ti OR strength training:de,ab,ti OR resistance training:de,ab,ti OR aerobic training:de,ab,ti OR swimming training:de,ab,ti OR exercise therapy:de,ab,ti OR exercise test:de,ab,ti OR acute exercise:de,ab,ti OR chronic exercise:de,ab,ti OR exercise training:de,ab,ti) |
| **#3 Combined search:** #1 AND #2 |
| **#4 Search limit (Sources):** Embase |

**Table S1 (*continuation*).** Complete search strategy with search filters and number of studies recovered in databases PubMed-Medline, Embase, Scopus, and Web of Science.

| **SCOPUS – *Search filters*** |
|---|
| **#1 Disease:** (TITLE-ABS-KEY("Chagas disease") OR TITLE-ABS-KEY("American trypanosomiasis") OR TITLE-ABS-KEY("Trypanosoma cruzi")) |
| **#2 Intervention (exercise):** (TITLE-ABS-KEY("Exercise") OR TITLE-ABS-KEY("physical exercise") OR TITLE-ABS-KEY("physical activity") OR TITLE-ABS-KEY("physical training") OR TITLE-ABS-KEY("strength training") OR TITLE-ABS-KEY("resistance training") OR TITLE-ABS-KEY("aerobic training") OR TITLE-ABS-KEY("swimming training") OR TITLE-ABS-KEY("exercise therapy") OR TITLE-ABS-KEY("exercise test") OR TITLE-ABS-KEY("acute exercise") OR TITLE-ABS-KEY("chronic exercise") OR TITLE-ABS-KEY("exercise training")) |
| **#3 Search limit:** NOT INDEX (medline) |
| **#4 Combined search:** #1 AND #2 AND #3 |

**Table S1 (*continuation*).** Complete search strategy with search filters and number of studies recovered in databases PubMed-Medline, Embase, Scopus, and Web of Science.

| **Web of Science – *Search filters*** |
| --- |
| **#1 Disease:** TS=Chagas disease OR TS=American trypanosomiasis OR TS=Trypanosoma cruzi<br><br>**#2 Intervention (exercise):** TS=Exercise OR TS= physical exercise OR TS= physical activity OR TS= physical training OR TS= strength training OR TS= resistance training OR TS= aerobic training OR TS= swimming training OR TS= exercise therapy OR TS= exercise test OR TS= acute exercise OR TS= chronic exercise OR TS= exercise training<br><br>**#3 Combined search:** #1 AND #2 |

**Table S2**. Cohen's kappa coefficient (κ) calculated to measure inter-rater reliability from the full search strategy applied by two independent researchers.

| | *Kappa calculation** | **Researcher 1** | |
|---|---|---|---|
| | | **Included study** | **Excluded study** |
| **Researcher 2** | **Included study** | **10** | **4** |
| | **Excluded study** | **3** | **277** |

*Statistical calculator: https://www.graphpad.com/quickcalcs/kappa1/

**Statistical results:**

Number of observed agreements: 287 (97.62% of the observations)

Number of agreements expected by chance: 268.2 ( 91.24% of the observations)

Kappa= 0.728

SE of kappa = 0.098

95% confidence interval: From 0.536 to 0.921

***One way to interpret kappa is with this scale:***

Kappa < 0: No agreement

Kappa between 0.00 and 0.20: Slight agreement

Kappa between 0.21 and 0.40: Fair agreement

Kappa between 0.41 and 0.60: Moderate agreement

Kappa between 0.61 and 0.80: Substantial agreement

Kappa between 0.81 and 1.00: Almost perfect agreement.

**Complete list of papers selected and included in the systematic review.**

- ***Preclinical studies***

1. Alves RL, Cardoso BRL, Ramos IPR, Oliveira BDS, Dos Santos ML, de Miranda AS, de Almeida TCS, Vieira MAR, Machado FS, Ferreira AJ, de Avelar GF. Physical training improves exercise tolerance, cardiac function and promotes changes in neurotrophins levels in chagasic mice. Life Sci. 2019;232:116629. doi: 10.1016/j.lfs.2019.116629.
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9. Schebeleski-Soares, C., Occhi-Soares, R. C., Franzói-de-Moraes, S. M., de Oliveira Dalálio, M. M., Almeida, F. N., de Ornelas Toledo, M. J., & de Araújo, S. M. (2009). Preinfection aerobic treadmill training improves resistance against *Trypanosoma cruzi* infection in mice. *Applied Physiology, Nutrition and Metabolism*, *34*(4), 659–665. https://doi.org/10.1139/H09-053.
10. Schebeleski-Soares, C., Occhi, R. C., De Carvalho, L. G. L., Franzói-de-Moraes, S. M., Dalálio, M. M. D. O., & De Araújo, S. M. (2010). Production of tumoral necrosis factor-alpha and hydrogen peroxide during *Trypanosoma cruzi* infection in mice submitted to exercise. *Acta Scientiarum - Health Sciences*, *32*(1), 57–60. https://doi.org/10.4025/actascihealthsci.v32i1.6757.
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- ***Clinical studies***

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**Table S3.** General characteristics of *in vivo* preclinical studies investigating the impact of exercise training in *Trypanosoma cruzi*-infected animals.

| Studies | Animal lineage | Number | Age | Sex | *T. cruzi* strain | Inoculum size (parasite number) | Administration route | Infection time |
|---|---|---|---|---|---|---|---|---|
| ***Preinfection Exercise Training*** | | | | | | | | |
| Schebeleski-Soares *et al*., 2009 | BALB/c mice | (-) | 4 w | Female | Y | 1400 | Intraperitoneal | 13 d |
| Schebeleski-Soares *et al*., 2010 | BALB/c mice | 63 | 1 w | Female | Y | 1400 | Intraperitoneal | 13 d |
| Soares *et al*., 2012 | Swiss and BALB/c mice | 356 | 4 w | Male and Female | Y | 1400 | Intraperitoneal | 11 d |
| Novaes *et al*., 2016 | Wistar rats | (-) | 16 w | Male | Y | 600000/100g body mass | Intraperitoneal | 63 d |
| Novaes *et al*., 2017 | Wistar rats | (-) | 16 w | Male | Y | 150000/100g body mass | Intraperitoneal | 63 d |
| Lucchetti *et al*., 2017 | Swiss mice | 120 | (-) | (-) | Y | 5000 | Intraperitoneal | 60 d |
| Santos *et al*., 2019 | Wistar rats | (-) | 7 w | Male | Y | 150000/100g body mass | Intraperitoneal | 30 d |

(d) Days. (w) Weeks. (-) Data not reported.

**Table S3 *(continuation)*:** General characteristics of *in vivo* preclinical studies investigating the impact of exercise training in *Trypanosoma cruzi*-infected animals.

| Studies | Animal lineage | Number | Age | Sex | *T. cruzi* strain | Inoculum size (parasite number) | Administration route | Infection time |
|---|---|---|---|---|---|---|---|---|
| ***Concomitant Infection-Exercise Training*** | | | | | | | | |
| Pedra-rezende *et al*., 2021 | BALB/c | 56 | 4-8 w | Female | Y | 500 | Intraperitoneal | 180 d |
| Schebeleski-Soares *et al*., 2010* | BALB/c | 63 | 1 w | Female | Y | 1400 | Intraperitoneal | 13 d |
| Preto *et al*., 2015 | BALB/c mice | 20 | 5-7 w | Male | Y | 1000 | (-) | 40 d |
| Alves *et al*., 2019 | C557BL/6 mice | 28 | 7-8 w | Male | Y | 1000 | Intraperitoneal | 45 d |
| Mendonça *et al*., 2019 | Wistar rats | 45 | 8 w | Male | Y | 600,000/100g body mass | Intraperitoneal | 30 d |

(d) Days. (w) Weeks. (-) Data not reported. * Study cited twice once that evaluated training before and during infection

**Table S4.** Characteristics of exercise protocols adopted in *in vivo* preclinical studies investigating the impact of exercise training in *Trypanosoma cruzi*-infected animals.

| Studies | Exercise modality | Exercise dosimetry | Training frequency | Training period |
|---|---|---|---|---|
| ***Preinfection Exercise Training*** | | | | |
| Schebeleski-Soares *et al*., 2009 | Aerobic/treadmill running | **Week 1**: 30–45 min at a speed of 6–14 m/min<br>**Week 2**: 45–60 min at 8–16 m/min<br>**Weeks 3-8**: 60 min at 10–20 m/min | 5 days/week | 8 weeks |
| Schebeleski-Soares *et al*., 2010 | Aerobic/treadmill running | **Week 1**: 30–45 min<br>**Week 2**: 45–60 min<br>**Weeks 3-8**: 60 min<br>**Weeks 1-4:** 14 m/min<br>**Weeks 5-8:** 18 m/min | 5 days/week | 8 weeks |
| Soares *et al*., 2012 | Aerobic/treadmill running | **Week 1**: 30–45 min at 6–14 m/min;<br>**Week 2**: 45–60 min at 8–16 m/min;<br>**Weeks 3-8**: 60 min at 10–20 m/min | 5 days/week | 8 weeks |
| Novaes *et al*., 2016 | Aerobic/treadmill running | **Weeks 1-2:** 15-60 min at 17 m/min.<br>**Weeks 3-4:** 60 min at 17 m/min<br>**Week 5:** 8 min at 17 m/min (warm-up)<br>45 min at 20 m/min<br>5 min at 17 m/min (warm-down)<br>**Weeks 6, 7, 8 and 9:**<br>8 min at 20 m/min (warm-up)<br>45 min at 23 m/min<br>5 min at 18 m/min (warm-down) | 5 days/week | 9 weeks |

**Table S4 *(continuation)*:** Characteristics of exercise protocols adopted in *in vivo* preclinical studies investigating the impact of exercise training in *Trypanosoma cruzi*-infected animals.

| Studies | Exercise modality | Exercise dosimetry | Training frequency | Training period |
|---|---|---|---|---|
| ***Preinfection Exercise Training*** | | | | |
| Lucchetti *et al*., 2017 | Aerobic/treadmill running | **Weeks 1-2:** 10 min at 8 m/min<br>**Weeks 6-9:** 3 m/min every 3 minutes (6-33 m/min) | 5 days/week | 9 weeks |
| Novaes *et al*., 2017 | Aerobic/treadmill running | **Weeks 1-2**: 15-60 min at 17 m/min.<br>**Weeks 3-4**: 60 min at 17 m/min<br>**Week 5:** 8 min at 17m/min (warm-up) 45 min at 20 m/min 5 min at 17 m/min (warm-down)<br>**Weeks 6, 7, 8 and 9:** 8 min at 20 m/min (warm-up) 45 min at 23 m/min 5 min at 18 m/min (warm-down). | 5 days/week | 9 weeks |
| Santos *et al*., 2019 | Aerobic/treadmill running | **Weeks 1-2:** 15- 60 min at 17 m/min<br>**Weeks 3-4:** 60 min at 17 m/min<br>**Week 5:** 8 min at 17 m/min (warm-up), 45 min at 20 m/min 5 min at 17 m/min (warm-down).<br>**Weeks 6, 7, 8 and 9**: 8 min at 20 m/min (warm-up) 45 min at 23 m/min 5 min at 18 m/min (warm-down) | 5 days/week | 9 weeks |

**Table S4 *(continuation)*:** Characteristics of exercise protocols adopted in *in vivo* preclinical studies investigating the impact of exercise training in *Trypanosoma cruzi*-infected animals.

| Studies | Exercise modality | Exercise dosimetry | Training frequency | Training period |
|---|---|---|---|---|
| ***Concomitant Infection-Exercise Training*** | | | | |
| Schebeleski-Soares *et al*., 2010* | Aerobic/treadmill running | **1-2 days:** 30 min at 6-8 m/min<br>**3-5 days:** 45 min at 8-16 m/min<br>**6-7 days:** 55 min at 10-16 m/min | 7 days/week | 8 weeks |
| Preto *et al*., 2015 | Aerobic/ swimming | 30 min/day: Free swimming with no extra charge | 5 days/week | 8 weeks |
| Alves *et al*., 2019 | Aerobic/treadmill running | 60 min/day at 70% of maximum speed to fatigue estimated from incremental running tests | 5 days/week | 12 weeks |
| Mendonça *et al*., 2019 | Aerobic/treadmill running | 40 min/day at 80% of the lactate threshold estimated from incremental running tests | 5 days/week | 4 weeks |
| Pedra-Rezende *et al*., 2021 | Aerobic/treadmill running | **Adaptation:** 15 min at 4-6-m/min<br>**Week 1:** 30 min 6-14 m/min<br>**Week 2:** 45 min 8-16 m/min<br>**Week 3:** 60 min 10-18 m/min<br>**Week 4:** 60 min 20 m/min | Adaptation: 3 days/week<br>Training: 5 days/week | Adaptation 1 week<br>Training 4 weeks |

* Study cited twice once that evaluated training before and during infection.

**Table S5.** Parasitological and immunological outcomes identified in preclinical studies investigating the effect of exercise training in *Trypanosoma cruzi*-infected animals.

| Author | Parasitemia / Parasite load | Cytokines |
|---|---|---|
| ***Preinfection Exercise Training*** | | |
| Schebeleski-Soares *et al*., 2009 | **Parasites/ml × $10^6$**<br>**NT+I PA**$^b$: 1.2 ± 2.3<br>**T+I PA**$^b$: 1.1 ± 1.7<br>**NT+I pPA**$^b$: 7.2 ± 0.7<br>**T+I pPA**$^b$: 4.9 ± 0.6* | **(pg/ml)**<br>**NT+I TNF-α**$^b$: 164.8 ± 14.8<br>**T+I TNF-α**$^b$: 190.5 ± 16.8 |
| Schebeleski-Soares *et al*., 2010 | (-) | **(pg/ml)**<br>**NT+I TNF-α**$^b$: 166.7 ± 12.8<br>**T+I TNF-α**$^b$: 189.4 ± 19.9 |
| Soares *et al*., 2012 | **Parasites/ml × $10^3$**<br>**Male BALB/c mice**<br>**NT+I PA**$^a$: 9.9 ± 7.3<br>**T+I PA**$^a$: 9.2 ± 1.5<br>**Female BALB/c mice**<br>**NT+I PA**$^a$**:** 11.7 ± 3.9<br>**T+I PA**$^a$**:** 10.4 ± 5.8<br>**Male Swiss mice**<br>**NT+I PA**$^a$: 16.9 ± 9.6<br>**T+I PA**$^a$: 9.4 ± 4.6*<br>**Female Swiss mice**<br>**NT+I PA**$^a$: 19.7 ± 6.1<br>**T+I PA**$^a$: 6.0 ± 5.5* | (-) |
| Novaes *et al*., 2016 | **Parasites/ml × $10^3$**<br>**NT + I PA**$^a$**:** 14.4 ± 6.1<br>**T+I PA**$^a$**:** 8.4 ± 4.8*<br>**NT + I pPA**$^a$**:** 24.5 ± 34.6<br>**T+I pPA**$^a$**:** 21.9 ± 17.7 | **(pg/ml)**<br>**NT+I TNF-α**$^c$**:** 210.5<br>**T+I TNF-α**$^c$**:** 295.11*<br>**NT+I INF-γ**$^c$**:** 62.3<br>**T+I INF-γ**$^c$**:** 67.9<br>**NT+I IL-6**$^c$**:** 59.5<br>**T+I IL-6**$^c$**:** 96.7* |

Groups: T+I: trained infected. NT+I: non-trained infected. (*a*) Mean ± standard deviation. (*b*) Mean ± Standard Error. *Statistical difference ($p<0.05$). (-) Data underreported or not analyzed. PA: mean parasitemia. pPA: peak of parasitemia. tPA: total mean parasitemia. TNF-α: tumor necrosis factor alpha. INF-γ: Interferon-gamma. MCP-1 monocyte chemoattractant protein-1. TGF-β: Transforming growth factor beta.

**Table S5** ***(continuation)*****:** General measure outcome extracted from preclinical studies included in the systematic review.

| Author | Parasitemia / Parasitic load | Cytokines |
|---|---|---|
| ***Preinfection Exercise Training*** | | |
| Novaes *et al*., 2017 | **Parasites/ml × $10^3$**<br>**NT+I PA**$^a$**:** 3.8 ± 1.5<br>**T+I PA**$^a$**:** 1.9 ± 0.6*<br>**NT+I pPA**$^a$: 21.6 ± 2.8 ×$10^3$<br>**T+I pPA**$^a$: 20.6 ± 2.6 × $10^3$ | **(pg/mg protein)**<br>**NT+I TNF-α**$^a$: 59.8 ± 11.9<br>**T+I TNF- α** $^a$: 40.0 ± 8.7*<br>**NT+I IFN-γ**$^a$: 79.4 ± 12.2<br>**T+I IFN-γ**$^a$: 72.3 ± 13.6<br>**NT+I IL-10**$^a$: 12.7 ± 2.3<br>**T+I IL-10**$^a$: 17.6 ± 1.5*<br>**NT+I IL-6**$^a$: 19.4 ± 3.9<br>**T+I IL-6**$^a$: 17.4 ± 3.4 |
| Lucchetti *et al*., 2017 | **Parasites/ml × $10^6$**<br>**NT+I PA**$^b$: 0.7 ± 5.9<br>**T+I PA**$^b$: 0.5 ± 5.9<br>**NT+I pPA**$^b$: 3.32 ± 0.95<br>**T+I pPA**$^b$: 1.17 ± 0.28* | **(ng/ml)**<br>**NT+I TNF-α**$^b$: 77.2 ± 21.1<br>**T+I TNF-α**$^b$: 16.8 ± 7.2*<br>**NT+I IL-10**$^b$: 1508.4 ± 240.1<br>**T+I IL-10**$^b$: 1120.5 ± 190.8*<br>**NT+I INF-γ**$^b$: 1649.3 ± 204.0<br>**T+I INF-γ**$^b$: 663.4 ± 204.0* |
| Santos *et al*., 2019 | **Parasites/0.1ml × $10^3$**<br>**NT+I pPA**$^a$**:** 39.5 ± 3.9<br>**T+I pPA**$^a$**:** 19.7 ± 1.5*<br>**NT+I pPA**$^a$**:** 6.3 ± 1.2<br>**T+I pPA**$^a$**:** 3.9 ± 1.0* | **(pg/mg protein)**<br>**NT+I TNF- α**$^a$: 60.6 ± 17.1<br>**T+I TNF-α**$^a$: 59.0 ± 18.7<br>**NT+I IL-10**$^a$: 16.2 ± 2.5<br>**T+I IL-10**$^a$: 23.4 ± 8.7*<br>**NT+I IL-4**$^a$: 10.6 ± 4.6<br>**T+I IL-4**$^a$:16.7 ± 5.0*<br>**NT+I IL-17**$^a$: 15.3 ± 4.9<br>**T+I IL-17**$^a$: 16.5 ± 6.1<br>**NT+I INF-γ**$^a$:54.4 ± 13.2<br>**T+I INF-γ** $^a$: 91.2 ± 10.3*<br>**NT+I MCP-1**$^a$: 28.8 ± 5.5<br>**T+I MCP-1**$^a$: 17.8 ± 6.2* |

Groups: T+I: trained infected. NT+I: non-trained infected. (*a*) Mean ± standard deviation. (*b*) Mean ± Standard Error. (*c*) Median. *Statistical difference ($p<0.05$). PA: mean parasitemia. pPA: peak of parasitemia. tPA: total mean parasitemia. IL: Interleukin. TNF-α: tumor necrosis factor alpha. TGF-β: Transforming growth factor beta. MCP-1 monocyte chemoattractant protein-1.

**Table S5** ***(continuation)*****:** General measure outcome extracted from preclinical studies included in the systematic review.

| Author | Parasitemia / Parasitic load | Cytokines |
|---|---|---|
| ***Concomitant Infection-Exercise Training*** | | |
| Schebeleski-Soares *et al*., 2010 † | (-) | **(pg/ml)**<br>**NT+I TNF-α**[b]: 272.3 ± 63.1<br>**T+I TNF- α**[b]: 246.1 ± 14.2 |
| Alves *et al*., 2019 | **Parasites/0.05 ml**<br>**T+I PA**[b]**:** 47.3 ± 62.2<br>**NT+I PA**[b]**:** 63.6 ± 105.7<br>**NT+I pPA**[b]**:** 252.2 ± 62.5<br>**T+I pPA**[b]**:** 156.8 ± 19.1 | (-) |
| Mendonça *et al*., 2019 | **Parasites/0.1 ml × 10³**<br>**NT+I PA:** 3.1 ± 0.7<br>**T+I PA:** 8.3 ± 2.4*<br>**NT+I pPA**[b]**:** 9.2 ± 3.7<br>**T+I pPA**[b]**:** 17.6 ± 5.3* | **(pg/mg protein)**<br>**NT+I IFN-ɤ**[a] **:** 66.1 ± 15.4<br>**T+I IFN-ɤ**[a]**:** 103.5 ± 19.3*<br>**NT+I TNF-α**[a]**:** 49.3 ± 7.2<br>**T+I TNF-α**[a]**:** 74.0 ± 11.4*<br>**NT+I IL-10**[a]**:** 17.5 ± 5.0<br>**T+I IL-10**[a]**:** 19.8 ± 3.9<br>**NT+I IL-4**[a]**:** 12.9 ± 3.9<br>**T+I IL-4**[a]**:** 14.7 ± 3.1<br>**NT+I IL-17**[a]**:** 12.43 ± 2.4<br>**T+I IL-17**[a]**:** 18.33 ± 3.4*<br>**NT+I MPC-1**[a]**:** 15.7 ± 3.8<br>**T+I MPC-1**[a]**:** 23.7 ± 3.3* |
| Pedra-Rezende *et al*., 2021 | **NT+I PL**[b]**:** 0.17 ± 0.04<br>**T+I PL**[b]**:** 0.21 ± 0.07 | **NT+I IFN-ɤ**[b] **:** 2.51 ± 0.37<br>**T+I IFN-ɤ**[b]**:** 2.71 ± 0.89<br>**NT+I TNF-α**[b]**:** 13.66 ± 4.66<br>**T+I TNF-α**[b]**:** 13.27 ± 4.59<br>**NT+I IL-10**[b]**:** 5.23 ± 2.69<br>**T+I IL-10**[b]**:** 20.03 ± 8.13<br>**NT+I IL-6**[b]**:** 1.33 ± 0.60<br>**T+I IL-6**[b]**:** 2.94 ± 0.80<br>**NT+I IL-12**[b]**:** 32.7 ± 13.2<br>**T+I IL-12**[b]**:** 53.3 ± 23.0<br>**NT+I MPC-1**[b]**:** 19.77 ± 6.66<br>**T+I MPC-1**[b]**:** 24.96 ± 7.23 |

Groups: T+I: trained infected. NT+I: non-trained infected. (a) Mean ± standard deviation. (b) Mean ± standard error. *Statistical difference ($p<0.05$). PA: mean parasitemia. pPA: peak of parasitemia. PL: Parasite load. TNF- α: tumor necrosis factor alpha. IL-12: interleukin 12. IL-10: interleukin 10. IL-4: interleukin 4. IL-17: interleukin 17. INF-γ: Interferon-gamma. MCP-1 monocyte chemoattractant protein-1. PL: Cardiac parasite load. † Study cited twice once that evaluated training before and during infection.

**Table S6.** Biochemical and microstructural outcomes identified in preclinical studies investigating the effect of exercise training in *Trypanosoma cruzi*-infected animals.

| Author | Oxidation markers | Antioxidant markers |
|---|---|---|
| ***Preinfection Exercise Training*** | | |
| Schebeleski-Soares *et al*., 2009 | **NT+I Hydrogen peroxide (nmol)[b]:** 2.4 ± 0.03<br>**T+I Hydrogen peroxide (nmol)[b]:** 2.5 ± 0.2 | (-) |
| Schebeleski-Soares *et al*., 2010 | **NT+I Hydrogen peroxide [b]:** 2.35 ± 0.07<br>**T+I Hydrogen peroxide [b]:** 2.44 ± 0.26 | (-) |
| Novaes *et al*., 2017 | **TBARS (nmol/ mg protein) [a]**<br>**NT+I:** 0.037 ± 0.006<br>**T+I:** 0.021 ± 0.004*<br>**Protein carbonyl (nmol/ml) [a]**<br>**NT+I:** 17.54 ± 3.22<br>**T+I:** 11.08 ± 2.01* | **Catalase (U/mg protein) [a]**<br>**NT+I:** 0.81 ± 0.18<br>**T+I:** 1.28 ± 0.25*<br>**SOD (µmol/min/g × $10^2$) [a]**<br>**NT+I:** 1.74 ± 0.30<br>**T+I:** 2.30 ± 0.23* |
| Lucchetti *et al*., 2017 | **Nitric oxide (µm) [# b]**<br>**NT+I plasma:** 97.3 ± 82.4<br>**T+I plasma:** 82.4 ± 11.3*<br>**NT+I Heart:** 9.9 ± 4.3<br>**T+I Heart:** 1.9 ± 0.6 | (-) |
| Santos *et al*., 2019 | **NT+I Hydrogen peroxide (ng/ml)[a]**: 68.3 ± 7.9<br>**T+I Hydrogen peroxide (ng/ml)[a]**: 53.2 ± 5.9*<br>**NT+I Nitric oxide (µmol)[a]**: 53.1 ± 13.3<br>**T+I Nitric oxide (µmol)[a]**: 40.4 ± 6.6*<br>**NT+I Malondialdehyde (nmol/mg)[a]**: 0.1 ± 0.02<br>**T+I Malondialdehyde (nmol/mg)[a]**: 0.04 ± 0.01*<br>**NT-I Protein carbonyl (nmol/ml)[a]**: 22.6 ± 11.4<br>**T+I Protein carbonyl (nmol/ml)[a]**: 20.4 ± 12.3 | **NT+I NPA (nmol)[a]**: 3.9 ± 3.2<br>**T+I NPA (nmol)[a]**: 10.14 ± 4.9*<br>**NT+I Catalase (U/mg)[a]**: 1.9 ± 0.5<br>**T+I Catalase (U/mg)[a]**: 2.1 ± 0.5*<br>**NT+I GST (nmol)[a]**: 3.0 ± 1.1<br>**T+I GST (nmol)[a]**: 4.2 ± 1.1*<br>**NT+I SOD (U/mg)[a]**: 3.7 ± 1.0<br>**T+I SOD (U/mg)[a]**: 3.4 ± 1.1 |

Groups: T+I: trained infected. NT+I: non-trained infected. (a) Mean ± standard deviation. (b) Mean ± Standard error. *Statistical difference compared to non-trained infected ($p<0.05$). TBARS: Thiobarbituric acid reactive substances. NPA: non-protein antioxidants. GST: Glutathione S-transferase. SOD: superoxide dismutase. (-) Data not analyzed. #20 days post-infection.

**Table S6.** Biochemical and microstructural outcomes identified in preclinical studies investigating the effect of exercise training in *Trypanosoma cruzi*-infected animals.

| Author | Oxidation markers | Antioxidant markers |
|---|---|---|
| ***Concomitant Infection-Exercise Training*** | | |
| Schebeleski-Soares *et al*., 2010 | **Hydrogen peroxide in macrophages (nmol)**[b]<br>**NT+I:** 1.1 ± 0.2<br>**T+I:** 1.5 ± 0.2 | (-) |
| Mendonça *et al*., 2019 | **Heart nitric oxide (µM)**[a]<br>**NT+I:** 44.6 ± 8.5<br>**T+I:** 64.4 ± 8.3*<br>**Heart hydrogen peroxide (M)** [a]<br>**NT+I:** 5.4 ± 0.7<br>**T+I:** 7.7 ± 0.9*<br>**Heart malondialdehyde (nmol/mg protein)** [a]<br>**NT+I:** 0.1 ± 0.11<br>**T+I:** 0.1 ± 0.02*<br>**Heart protein carbonyl (nmol/mL)** [a]<br>**NT+I:** 14.9 ± 2.6<br>**T+I:** 21.9 ± 3.5* | **Heart antioxidant effectors**<br>**Catalase (U/mg protein)** [a]<br>**NT+I:** 1.6 ± 0.3<br>**T+I:** 2.3 ± 0.4*<br>**Glutathione reductase (umol/min/g × 10²)** [a]<br>**NT+I:** 34.9 ± 8.3<br>**T+I:** 39.3 ± 6.8<br>**Superoxide dismutase (U/mg protein)** [a]<br>**NT+I:** 2.9 ± 0.6<br>**T+I:** 4.3 ± 0.4*<br>**Non-enzymatic antioxidants (nmol)** [a]<br>**NT+I:** 7.11 ± 2.21<br>**T+I:** 6.47 ± 2.21 |

Groups: T+I: trained infected. NT+I: non-trained infected. (a) Mean ± standard deviation. (b) Mean ± Standard Error. FU, fluorescence intensity. *Statistical difference compared to control animals ($p<0.05$).

**Table S6.** Biochemical and microstructural outcomes identified in preclinical studies investigating the effect of exercise training in *Trypanosoma cruzi*-infected animals.

| Author | Oxidation markers | Antioxidant markers |
|---|---|---|
| ***Concomitant Infection-Exercise Training*** | | |
| Pedra-Rezende *et al*., 2021 | **Cardiac reactive species (FU)** [b]<br>**NT+I:** 64.21 ± 4.37<br>**T+I:** 51.7 ± 2.2 | **Catalase (nmols/min/mg)** [b]<br>**NT+I:** 5.29 ± 0.51<br>**T+I:** 3.92 ± 0.63<br>**Glutathione peroxidade (nmols/min/mg)** [b]<br>**NT+I:** 2.41 ± 0.39<br>**T+I:** 1.91 ± 0.11<br>**Superoxide dismutase (U/mg)** [b]<br>**NT+I:** 5.32 ± 0.19<br>**T+I:** 5.57 ± 0.06 |

Groups: T+I: trained infected. NT+I: non-trained infected. (a) Mean ± standard error. *Statistical difference compared to control animals ($p<0.05$).

**Table S6.** Biochemical and microstructural outcomes identified in preclinical studies investigating the effect of exercise training in *Trypanosoma cruzi*-infected animals.

| Author | Biochemical findings | Microstructural findings | Functional findings |
|---|---|---|---|
| ***Preinfection Exercise Training*** | | | |
| Soares *et al*., 2012 | **BALB/c mice CKMB (U/l)**[a]<br>**NT+I** male: 73.3 ± 65.9<br>**T+I** male: 0.0 ± 0.0*<br>**NT+I** female: 38.8 ± 38.3<br>**T+I** female: 23.0 ± 27.9*<br>**Swiss mice CKMB (U/l)** [a]<br>**NT+I** male: 32.4 ± 28.5<br>**T+I** male: 1.3 ± 0.8*<br>**NT+I** female: 0.8 ± 0.4<br>**T+I** female: 1.1 ± 0.5* | (-) | (-) |

Groups: T+I: trained infected. NT+I: non-trained infected. (a) Mean ± standard deviation. *Statistical difference compared to control animals ($p<0.05$). CKMB: Creatine kinase-MB. (-) Data underreported or not analyzed.

**Table S6 *(continuation)*:** Biochemical and histopathological outcomes identified in preclinical studies investigating the effect of exercise training in *Trypanosoma cruzi*-infected animals.

| Author | Biochemical findings | Microstructural findings | Functional findings |
|---|---|---|---|
| Novaes *et al*., 2016 | **Collagen (µg/mg protein)**[a]<br><br>**Right atrium:**<br>**NT+I:** 28.6 ± 1.6<br>**T+I:** 23.7 ± 1.1*<br><br>**Left Ventricle:**<br>**NT+I:** 30.9 ± 1.9<br>**T+I:** 25.3 ± 1.2* | **Myocardial cellularity (cells/170 × $10^3$ µm2)**[a]<br>**Right atrium**<br>**NT+I:** 356.4 ± 14.0<br>**T+I:** 179.6 ± 16.6*<br>**Left Ventricle**<br>**NT+I:** 625.6 ± 84.6<br>**T+I:** 25.3 ± 1.2*<br>**Cardiomyocytes volume (pl)**[a]<br>**Left Ventricle:**<br>**NT+I:** 21.2 ± 1.8<br>**T+I:** 26.5 ± 1.3 | **Cardiomyocytes contractility (%)**[b]<br>**Left Ventricle**<br>**Shortening:**<br>**NT+I:** 6.30 ± 0.2<br>**T+I:** 7.79 ± 0.2*<br>**Maximal rate of contraction (µm/s):**<br>**NT+I:** 56.4 ± 1.2<br>**T+I:** 68.3 ± 1.7*<br>**Maximal rate of relaxation (µm/s):**<br>**NT+I:** 56.0 ± 1.7<br>**T+I:** 69.3 ± 1.7* |
| Novaes *et al*., 2017 | **MyHC I (%)**[a]<br><br>**NT+I:** 21.81 ± 4.84<br>**T+I:** 43.63 ± 4.24*<br><br>**Lactate threshold (mmol/L)**[a]<br><br>**NT+I:** 2.61 ± 0.50<br>**T+I:** 2.43 ± 0.44 | **Vv myocytes (%)**[a]<br>**NT+I:** 55.7 ± 7.1<br>**T+I:** 72.3 ± 6.2*<br>**Vv necrosis (%)**[a]<br>**NT+I:** 9.65 ± 3.2<br>**T+I:** 4.28 ± 1.1<br>**$QA_{IC}$ (cells/$mm^2$)**<br>**NT+I:** 2951.8 ± 617.3<br>**T+I:** 1808.1 ± 415.7*<br>**$QA_{TC}$ (nests/$mm^2$)**<br>**NT+I:** 11.4 ± 5.1<br>**T+I:** 20.3 ± 7.4 | **Time to lactate threshold (min)**<br><br>**NT+I:** 16 ± 2<br>**T+I:** 27 ± 3*<br><br>**Time to fatigue (min)**[a]<br><br>**NT+I:** 24 ± 2<br>**T+I:** 33 ±3* |

Groups: T+I: trained infected. NT+I: non-trained infected. (a) Mean ± standard deviation. (b) Mean ± standard error. Vv: Volume density. $QA_{IC}$: Number density of interstitial/inflammatory cells. $QA_{TC}$: Number density of *Trypanosoma cruzi* nests. *Statistical difference compared to control animals ($p<0.05$).

**Table S6 *(continuation)*:** Biochemical and histopathological outcomes identified in preclinical studies investigating the effect of exercise training in *Trypanosoma cruzi*-infected animals.

| Author | Microstructural findings | Functional findings |
|---|---|---|
| Lucchetti *et al*., 2017 | **Amastigote nests 20 dpi / 10 mm²**<br>**NT+I**[b]: 8.55 ± 1.88<br>**T+I**[b]: 0.22 ± 0.11*<br>**Heart cellularity 20 dpi / 1mm²**<br>**NT+I**[b]: 5219.6 ± 356.2<br>**T+I**[b]: 3481.6 ± 168.7* | **Mean arterial pressure (mmHg)** [# a]<br>**NT+I**[b]: 80.4 ± 4.4<br>**T+I**[b]: 77.2 ± 5.6<br>**Heart rate (bpm)** [# a]<br>**NT+I** [b] : 699.7 ± 42.4<br>**T+I**[b] : 742.1 ± 25.4* |
| Santos *et al*., 2019 | **Mononuclear cells (n/mm² × 10³)**[c]<br>**NT+I**: 1.65<br>**T+I**: 1.32*<br>**Polymorphonuclear cells (n/mm² × 10³)**[c]<br>**NT+I**: 0.42<br>**T+I**: 0.23*<br>**Vv cardiomyocytes (%)**[c]<br>**NT+I**: 75.2<br>**T+I**: 76.8<br>**Vv connective tissue (%)**[c]<br>**NT+I**: 14.8<br>**T+I**: 13.2 | **Lactate (mmol/l)**[a]<br>**NT+I**: 1.5 ± 0.4<br>**T+I**: 1.6 ± 0.6<br>**Time to fatigue (min)** [a]<br>**NT+I**: 15 minutes<br>**T+I**: 24 minutes*<br>**Total physical work (kgm)**[a]<br>**NT+I**: 8.3 ± 2.4<br>**T+I**: 30.4 ± 5.1* |

Groups: T+I: trained infected. NT+I: non-trained infected. (a) Mean ± standard deviation. (b) Mean ± standard error. (c) Mean ± interquartile interval. *Statistical difference compared to non-trained infected ($p<0.05$). Vv: Volume density. dpi: Days post-infection. # Nine weeks exercise training.

**Table S6 *(continuation)*:** Biochemical and histopathological outcomes identified in preclinical studies investigating the effect of exercise training in *Trypanosoma cruzi*-infected animals.

| Author | Microstructural findings | Microstructural findings (*continuation*) |
|---|---|---|
| ***Concomitant Infection-Exercise Training*** | | |
| Preto *et al*., 2015 | **Left ventricle Vv [myocytes]%**[b]<br>**NT+I:** 88.4 ± 0.6<br>**T+I:** 89.7 ± 1.2<br>**Left ventricle Vv [collagen]%**[b]<br>**NT+I:** 3.05 ± 0.06<br>**T+I:** 1.3 ± 0.05*<br>**Left ventricle Vv [capillaries]%**[b]<br>**NT+I:** 1.3 ± 0.1<br>**T+I:** 4.3 ± 0.7*<br>**Left ventricle Vv [ct]%**[b]<br>**NT+I:** 10.3 ± 0.6<br>**T+I:** 6.00 ± 0.8*<br>**Left ventricle CSA (µm)**[b]<br>**NT+I:** 232.5 ± 11.2<br>**T+I:** 181.6 ± 11.9*<br>**Right ventricle Vv [myocytes]%**[b]<br>**NT+I:** 94.1 ± 0.4<br>**T+I:** 90.6 ± 0.5*<br>**Right ventricle Vv [collagen]%**[b]<br>**NT+I:** 2.9 ± 0.3<br>**T+I:** 2.2 ± 0.2* | **Right ventricle Vv [capillaries]%**[b]<br>**NT+I:** 0.9 ± 0.2<br>**T+I:** 1.3 ± 0.2<br><br>**Right ventricle CSA (µm)**[b]<br>**NT+I:** 184.6 ± 19.6<br>**T+I:** 233.5 ± 5.0* |

Groups: T+I: trained infected. NT+I: non-trained infected. (a) Mean ± standard deviation. (b) Mean ± Standard Error. *Statistical difference compared to control non-trained infected animals ($p<0.05$). Vv, volume density. Ct: connective tissue. cap, capillaries of heart. CSA, myocyte mean cross-sectional area.

**Table S6 *(continuation)*:** Biochemical and histopathological outcomes identified in preclinical studies investigating the effect of exercise training in *Trypanosoma cruzi*-infected animals.

| Author | Cardiotrophic effectors | Microstructural findings | Functional findings |
|---|---|---|---|
| ***Concomitant Infection-Exercise Training*** | | | |
| Alves *et al*., 2019 | **NGF (pg/100mg heart)**[b]<br>**NT+I:** 1135.4 ± 69.9<br>**T+I:** 1484.8 ± 104.8<br>**NGF (pg/100mg spleen)**<br>**NT+I:** 301.3 ± 26.2<br>**T+I:** 296.9 ± 21.8<br>**BDNF (pg/100mg heart)**[b]<br>**NT+I:** 4368.3 ± 141.3<br>**T+I:** 4715.2 ± 334.3<br>**BDNF (pg/100mg spleen)**<br>**NT+I:** 2788.0 ± 231.3<br>**T+I:** 2646.7 ± 128.5<br>**BDNF (pg/50uL serum)**[b]<br>**NT+I:** 85.5 ± 9.4<br>**T+I:** 67.1 ± 5.5<br>**GDNF (pg/100mg heart)**[b]<br>**NT+I:** 1243.9 ± 105.0<br>**T+I:** 1050.3 ± 45.9<br>**GDNF (pg/100mg spleen)**<br>**NT+I:** 357.8 ± 19.7<br>**T+I:** 259.4 ± 23.0* | **Left ventricle cardiomyocyte volume nuclei ($mm^3$)**[b]<br>**NT+I:** 944.8 ± 144.8<br>**T+I:** 744.8 ± 172.4<br>**Left ventricle cardiomyocyte / Fibroblast ratio (%)**[b]<br>**NT+I:** 1.6 ± 0.8<br>**T+I:** 1.3 ± 0.6 | **Physical work in the end 12th week (J)**[b]<br>**NT+I**: 0.61 ± 0.11<br>**T+I**: 3.20 ± 0.36*<br>**End-diastolic volume (µl)**[b]<br>**NT+I:** 76.0 ± 6.3<br>**T+I:** 77.6 ± 4.4<br>**End-systolic volume (µl)**[b]<br>**NT+I:** 42.5 ± 3.8<br>**T+I:** 35.0 ± 2.6<br>**Fractional area change (%)**[b]<br>**NT+I:** 31.9 ± 4.9<br>**T+I:** 46.1 ± 2.9*<br>**Ejection fraction (%)**[b]<br>**NT+I:** 43.4 ± 1.3<br>**T+I:** 53.5 ± 0.7*<br>**Stroke volume (µl)**[b]<br>**NT+I:** 33.0 ± 2.7<br>**T+I:** 41.5 ± 2.8<br>**Cardiac output (ml/min)**[b]<br>**NT+I:** 12.4 ± 1.2<br>**T+I:** 15.8 ± 1.6 |

Groups: T+I: trained infected. NT+I: non-trained infected. (a) Mean ± standard deviation. (b) Mean ± Standard Error. *Statistical difference compared to control non-trained infected animals ($p<0.05$). NGF: Nerve growth factor. GDNF: Glial cell line-derived neurotrophic factor. BDNF: Brain derived neurotrophic factor.

**Table S6 *(continuation)*:** Biochemical and histopathological outcomes identified in preclinical studies investigating the effect of exercise training in *Trypanosoma cruzi*-infected animals.

| Author | Biochemical findings | Microstructural findings | Functional findings |
|---|---|---|---|
| ***Concomitant Infection-Exercise Training*** | | | |
| Mendonça *et al*., 2019 | (-) | **Heart inflammatory infiltrate**<br>**IC (cells/mm² × $10^4$)**[a]<br>**NT+I:** 2.1 ± 0.3<br>**T+I:** 3.2 ± 0.5*<br>**Vv (cmy) %**[a]<br>**NT+I:** 42.6 ± 7.5<br>**T+I:** 17.9 ± 7.5*<br>**Vv (cnt) %**[a]<br>**NT+I:** 17.6 ± 2.3<br>**T+I:** 24.9 ± 2.6*<br>**Vv (bvs) %**[a]<br>**NT+I:** 1.8 ± 0.6<br>**T+I:** 1.9 ± 0.7<br>**SPR (cnt/cmy) %**[a]<br>**NT+I:** 0.2 ± 0.04<br>**T+I:** 0.3 ± 0.04*<br>**McvR (bvs/cmy)**[a]<br>**NT+I:** 0.02 ± 0.01<br>**T+I:** 0.03 ± 0.01 | (-) |

Groups: T+I: trained infected. NT+I: non-trained infected. (a) Mean ± standard deviation. (b) Mean ± Standard Error. *Statistical difference compared to control non-trained infected animals ($p<0.05$). (-) Data underreported or not analyzed. Vv, volume density. IC: Interstitial/Inflammatory cells. Cmy: Cardiomyocytes. Cnt: Connective tissue. Bvs: Blood vessels. SPR: Stroma/parenchyma ratio. McvR: micro-vascularization ratio.

**Table S6 *(continuation)*:** Biochemical and histopathological outcomes identified in preclinical studies investigating the effect of exercise training in *Trypanosoma cruzi*-infected animals.

| Author | Biochemical findings | Microstructural findings | Functional findings |
|---|---|---|---|
| ***Concomitant Infection-Exercise Training*** | | | |
| Pedra-Rezende *et al*., 2021 | **CKMB (OD 340 nm)** [b]<br>**NT+I:** 0.23 ± 0.82<br>**T+I:** 0.33 ± 1.15 | **Heart inflammatory cells (100 fields)** [b]<br>**NT+I:** 213.27 ± 12.38<br>**T+I:** 157.19 ± 5.15<br>**Heart collagen (%)** [b]<br>**NT+I:** 17.6 ± 3.4<br>**T+I:** 12.2 ± 1.3* | **Heart rate (BPM)** [b]<br>**NT+I:** 517.1 ± 10.0<br>**T+I:** 518.4 ± 3.8<br>**P wave interval (ms)** [b]<br>**NT+I:** 16.7 ± 1.1<br>**T+I:** 37.1 ± 0.6<br>**PR interval (ms)** [b]<br>**NT+I:** 40.2 ± 1.1<br>**T+I:** 37.1 ± 0.7<br>**QRS interval (ms)** [b]<br>**NT+I:** 15.3 ± 1.7<br>**T+I:** 12.1 ± 0.7<br>**LV ejection fraction (%)** [b]<br>**NT+I:** 47.8 ± 8.1<br>**T+I:** 51.8 ± 6.3<br>**Stroke volume (µl)** [b]<br>**NT+I:** 29.2 ± 1.6<br>**T+I:** 28.6 ± 1.8 |

Groups: T+I: trained infected. NT+I: non-trained infected. OD: Optical density. LV: Left ventricle. (b) Mean ± Standard Error. *Statistical difference compared to control non-trained infected animals ($p<0.05$). (-) Data underreported or not analyzed.

**Table S7.** General characteristics of patients with chronic Chagas disease submitted to exercise training in randomized trials.

| Study | Country | Patients number | Age (years) | Sex | Diagnostic methods | Cardiac functional classes† | $VO_2$ Baseline [a] |
|---|---|---|---|---|---|---|---|
| Lima *et al*., 2010 | Brazil | 40 | 30-65 (49.5 ± 7.8)[a] | ChNT: 63% M - 36% F ChT: 52% M - 48% F | Serology for anti-*T. cruzi* antibodies, electrocardiography and echocardiography | NYHA 62.5% I 37.5 % II Goldman 65% I 35% II and III | ChNT: 31.4 ± 7.2 ChT: 27.3 ± 5.7 |
| Nascimento *et al*., 2014 | Brazil | 40 | 49.5 ± 8[a] | M | Serology for anti-*T. cruzi* antibodies and echocardiography | NYHA 64.9 % I 35.1% II Goldman 59.5 % I 40.5 % II and III | ChNT: 31.3 ± 7.2 ChT: 27.6 ± 5.9 |

(a) Mean ± standard deviation. M: Male. F: Female. (-): Data not reported or not evaluated. †Classification established according to New York Heart Association (NYHA) and Goldman cardiac risk index. ChNT: Chagasic non-trained. ChT: Chagasic trained. $VO_2$: maximal oxygen uptake during exercise.

**Table S7 (continuation).** General characteristics of patients with chronic Chagas disease submitted to exercise training in randomized trials.

| Study | Country | Patients number | Age (years) | Sex | Diagnostic methods | Cardiac functional classes† | $VO_2$ Baseline [a] |
|---|---|---|---|---|---|---|---|
| De Souza *et al*., 2020 | Brazil | 30 | ChT: 57.8 ± 9.4[a]<br>ChNT: 60.7 ± 10.7[a] | ChT: 73.3% M - 26.7% F<br>ChNT: 60% M - 40% F | ELISA, immunofluorescence, electrocardiography and echocardiography | NYHA I or II | ChNT: 15.4 ± 6.3<br>ChT: 17.6 ± 4.7 |
| Sarmento *et al*., 2021 | Brazil | 24 | 30-60<br>ChT: 47.8 ± 2.4[a]<br>ChNT: 51.0 ±1.9[a] | ChT: 24% M - 76% F<br>ChNT: 50% M/F | ELISA, immunofluorescence, and echocardiography | (-) | ChNT: 25.2 ± 1.8<br>ChT: 24.3 ± 2.4 |

(a) Mean ± standard deviation. M: Male. F: Female. (-): Data not reported or not evaluated. †Classification established according to New York Heart Association (NYHA) and Goldman cardiac risk index. ChNT: Chagasic non-trained. ChT: Chagasic trained. $VO_2$: maximal oxygen uptake during exercise.

**Table S8.** Characteristics of specific exercise protocols applied in the treatment of patients with chronic Chagas disease.

| Study | Exercise modality | Exercise dosimetry | Time of training session | Training frequency | Training period |
|---|---|---|---|---|---|
| Lima *et al*., 2010 | Aerobic: Monitored walk | **Weeks 1 and 2**: 55% to 65% HR reached in the symptom-limited exercise test<br>**Weeks 3-12**: 50-70% HR peak calculated using the Karvonen formula<br>**15 min:** Warm-up<br>**30 min:** Walking<br>**15 min:** Cooling-down | 60 minutes | 3 times/week | 12 weeks |
| Nascimento *et al*., 2014 | Aerobic: Monitored walk | 50-70% HR peak reached in the symptom-limited exercise test and calculated using the Karvonen formula<br>**15 min:** Warm-up<br>**30 min:** Walking<br>**15 min:** Cooling-down | 60 minutes | 3 times/week | 12 weeks |

HR: Heart Rate. AT: anaerobic threshold.

**Table S8** ***(continuation).*** Characteristics of specific exercise protocols applied in the treatment of patients with chronic Chagas disease.

| Study | Exercise modality | Exercise dosimetry | Time of training session | Training frequency | Training period |
|---|---|---|---|---|---|
| De Souza *et al*., 2020 | Aerobic: Treadmill walking,<br>Strengthening and stretching | HR peak reached in the symptom-limited exercise test: Month 1: 90% - 100% HR AT<br>Months 2-6: 100% to 110% HR AT<br>OR HR calculated using the Hellerstein formula<br>Month 1: 70% predicted HR<br>Months 2-6: 85% predicted HR<br>**30 min:** Treadmill walking<br>**20 min:** Strength exercises<br>**10 min:** Stretching exercises | 60 minutes | 3 times/week | 24 weeks |
| Sarmento *et al*., 2021 | Aerobic: Monitored treadmill running or ergometer,<br>Strengthening and stretching | HR peak and BP reached at AT in the symptom-limited exercise test<br>**5 min:** Stretching exercises<br>**40 min:** Treadmill running/ergometer<br>**10 min:** Strengthening exercises<br>**5 min:** Cooling-down: Stretching | 60 minutes | 3 times/week | 16 eeks |

HR: Heart Rate. BP: Blood Pressure. AT: anaerobic threshold.

**Table S9.** Cardiorespiratory outcomes obtained in patients with Chagas disease treated with different exercise training protocols.

| Lima *et al*., 2010 | Nascimento *et al*., 2014 | De Souza *et al*., 2020 |
|---|---|---|
| **$VO_2$ (ml/kg/min)**[c] | **EgT: $VO_2$ (ml/kg/min) post-training**[a] | **$VO_2$ (ml/kg/min)**[a] |
| **NT+I**: 33.3 (29.3-38.8) | **NT+I:** 33.5 ± 6.8 | **NT+I**: 13.0 ± 4.5 |
| **T+I**: 33.6 (31.0-36.3)* | **T+I:** 34.3 ± 4.9 | **T+I**: 19.4 ± 5.5* |
| **Exercise time (min)**[c] | **EgT: $VO_2$ (ml/kg/min) Δ pre-post training**[a] | **Maximum speed (km/h)**[a] |
| **NT+I**: 10.1 (8.4-12.4) | **NT+I:** 2.2 ± 4.8 | **NT+I**: 4.4 ± 1.6 |
| **T+I**: 10.0 (9.2-11.4)* | **T+I:** 7.0 ± 3.6* | **T+I**: 5.8 ± 1.0* |
| **6MWT distance (m)**[c] | **EgT time (min)**[a] | **FAI (%)**[a] |
| **NT+I**: 541.0 (485.0-579.0) | **NT+I:** 10.2 ± 3.0 | **NT+I**: 54.7 ± 14.4 |
| **T+I**: 574.0 (560.2-664.5)* | **T+I:** 10.6 ± 2.2 | **T+I**: 35.3 ± 21.3* |
| **HRr (bpm)**[a] | **EgT time (min) Δ pre-post training** | **Maximum SBP (mm Hg)**[a] |
| **NT+I**: 66.0 ± 9.6 | **NT+I:** 0.8 ± 2.0 | **NT+I**: 118.1 ± 36.4 |
| **T+I**: 58.4 ± 6.0 | **T+I:** 3.0 ± 1.6* | **T+I**: 117.3 ± 21.0 |
| **HRp (bpm)**[c] | **6MWT distance (m)**[a] | **Maximum HR (bpm)**[a] |
| **NT+I**: 142.0 (120.0–158.0) | **NT+I:** 530.3 ± 69.1 | **NT+I**: 106.6 ± 22.9 |
| **T+I**: 136.0 (123.8–146.5)* | **T+I:** 593.3 ± 78.5* | **T+I**: 113.3 ± 21.7 |
| **BNP (pg/ml)**[c] | **Goldman index improvement** | **OUES**[a] |
| **NT+I**: 87.4 (29.8–259.0) | **NT+I:** 1 (5.3%) | **NT+I**: 1041.0 ± 542.2 |
| **T+I**: 95.3 (43.3–147.0) | **T+I:** 8 (44.4%)* | **T+I**: 1404.3 ± 411.6 |

Groups: NT+I: non-trained infected. T+I: trained infected. [a] Mean ± standard deviation. [b] Mean ± standard error. [c] Median ± interquartile range. $VO_2$: maximum oxygen volume. EgT: ergometric test. 6MWT: six-minute walk test. BNP: Brain natriuretic peptide. ET: ergonomic test. HR: Heart rate (beats per minute). HRp: Heart rate peak (bpm – beats per minute). HRr: Heart rate at rest (bpm – beats per minute). MSNA: Muscle sympathetic nerve activity. HRV: Heart rate variability. LV: Left ventricle. CBS: Cardiac baroreflex sensitivity. SBP: Systolic blood pressure. FAI: Functional aerobic impairment. OUES = oxygen uptake efficiency slope. *Statistical difference.

**Table S9** ***(continuation)*****:** Cardiorespiratory outcomes obtained in patients with Chagas disease treated with different exercise training protocols.

| De Souza *et al*., 2020[2] | Sarmento *et al*., 2021 | Sarmento *et al*., 2021[2] |
|---|---|---|
| **Ejection fraction (%)**[a]<br>**NT+I:** 33.9 ± 7.0<br>**T+I:** 32.3 ± 8.7 | **ET: $VO_2$ (ml/kg/min)**[b]<br>**NT+I:** 24.7 ± 2.1<br>**T+I:** 28.1 ± 1.7* | **MSNA (bursts/min)**[b]<br>**NT+I:** 32.9 ± 3.0<br>**T+I:** 22.8 ± 2.6* |
| **LV ESV (ml)**[a]<br>**NT+I:** 99.4 ± 35.0<br>**T+I:** 116.3 ± 40.2 | **HRr (bpm)**[b]<br>**NT+I**: 61.9 ± 1.15<br>**T+I:** 59.4 ± 3.3 | **Muscle blood flow (ml/min)**[b]<br>**NT+I:** 1.5 ± 0.1<br>**NT+I:** 1.7 ± 0.1* |
| **LV EDV (ml)**[a]<br>**NT+I:** 147.0 ± 47.8<br>**T+I: 171.1** ± 47.8 | **Mean blood pressure (mmHg)**[b]<br>**NT+I:** 93.2 ± 4.0<br>**T+I:** 86.7 ± 3.6 | **HRV low frequency (nu)**[b]<br>**NT+I:** 66 ± 2<br>**T+I:** 60 ± 4* |
| **SRV' (cm/s)**[a]<br>**NT+I:** 8.6 ± 2.8<br>**T+I:** 10.3 ± 1.9 | **Systolic blood pressure (mmHg)**[b]<br>**NT+I:** 123.8 ± 5.0<br>**T+I:** 124.4 ± 5.0 | **HRV high frequency (nu)**<br>**NT+I:** 34 ± 2<br>**T+I:** 40 ± 4* |
| **E/e'Ratio**[a]<br>**NT+I:** 11.8 ± 3.7<br>**T+I:** 14.7 ± 4.6 | **LV ejection fraction (%)**[b]<br>**NT+I:** 61.8 ± 1.2<br>**T+I:** 60.1 ± 0.6 | **SBP variability**<br>**Low frequency (nu)**[b]<br>**NT+I:** 48 ± 2<br>**T+I:** 37 ± 3* |
| **MIP (cm $H_2O$)**[a]<br>**NT+I:** 74.2 ± 33.6<br>**T+I:** 81.1 ± 22.9 | **End-diastolic volume (ml)**[b]<br>**NT+I:** 100.6 ± 3.7<br>**T+I:** 108.4 ± 3.2 | **CBS increase SBP**[b]<br>**NT+I:** -0.24 ± 0.1<br>**T+I:** 0.46 ± 0.1* |
| **MEP (cm $H_2O$)**[a]<br>**NT+I:** 101.8 ± 33.5<br>**T+I:** 119.1 ± 23.8 | **End-systolic volume (ml)**[b]<br>**NT+I:** 38.1 ± 1.2<br>**T+I:** 43.4 ± 1.8 | **CBS decrease SBP**[b]<br>**NT+I:** -0.52 ± 0.1<br>**T+I:** 1.0 ± 0.3* |

Groups: NT+I: non-trained infected. T+I: trained infected. [b] Mean ± standard error. ESV: End-systolic volume. EDV: End-diastolic volume. SRV': Right ventricular peak myocardial systolic velocity. E/e': ratio between early mitral inflow velocity and mitral annular early diastolic velocity. MIP: maximum inspiratory pressure. MEP: maximum expiratory pressure. $VO_2$: maximum oxygen volume. EgT: ergometric test. ET: ergonomic test. HRr: Heart rate at rest (bpm – beats per minute). MSNA: Muscle sympathetic nerve activity. HRV: Heart rate variability. CBS: Cardiac baroreflex sensitivity. SBP: Systolic blood pressure. [2] Repeated study due to large data amount. *Statistical difference.

**Table S10.** Bias analysis in all preclinical studies included in the review (Syrcle's Quality Index[1]).

| Studies | Was the allocation sequence adequately generated and applied? | Were the groups similar at baseline or were they adjusted for confounders in the analysis? | Was the allocation to the different groups adequately concealed during? | Were the animals randomly housed during the experiment? | Were the caregivers and/or investigators blinded from knowledge which intervention each animal received during the experiment? | Were animals selected at random for outcome assessment? | Was the outcome assessor blinded? | Were incomplete outcome data adequately addressed? | Are reports of the study free of selective outcome reporting? | Did the study present a full report of the exercise training protocol? | **Quality score (%)** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Schebeleski-Soares *et al*., 2009 | No | Yes | No | No | No | U | No | No | Yes | Yes | 30 |
| Schebeleski-Soares *et al*., 2010 | No | No | No | No | No | U | No | No | Yes | Yes | 20 |
| Soares *et al*., 2012 | No | No | No | No | No | U | No | No | Yes | Yes | 20 |
| Preto *et al*., 2015 | Yes | Yes | No | No | No | U | No | No | Yes | U | 30 |
| Novaes *et al*., 2016 | Yes | Yes | No | Yes | No | U | No | Yes | Yes | Yes | 60 |
| Novaes *et al*., 2017 | Yes | Yes | No | Yes | No | U | No | Yes | Yes | Yes | 60 |
| Lucchetti *et al*., 2017 | No | Yes | U | No | No | U | No | No | No | Yes | 20 |
| Santos *et al*., 2019 | Yes | Yes | No | Yes | No | U | No | Yes | Yes | Yes | 60 |
| Alves *et al*., 2019 | Yes | Yes | No | No | No | U | No | No | Yes | Yes | 40 |
| Mendonça *et al*., 2019 | Yes | Yes | No | U | No | U | No | U | Yes | U | 30 |
| Pedra-Rezende *et al*., 2021 | U | U | No | U | No | U | U | Yes | Yes | Yes | 30 |
| **Quality score / items-Yes (n)** | 6 | 8 | 0 | 3 | 0 | 0 | 0 | 3 | 9 | 8 | |
| **Quality score / items (%)** | 54.55 | 72.73 | 0 | 27.27 | 0 | 0 | 0 | 27.27 | 81.82 | 72.73 | |

Yes: Criteria attended. No: Criteria not completed. U: Criteria undefined / insufficient information. [1]Hooijmans, C. R. *et al*. (2014). BMC Med. Res. Methodol. 14:43.

**Table S11.** Bias analysis in all clinical studies included in the systematic review (Downs and Black Quality Index[1]).

| Studies | Is the hypothesis/aim/objective of the study clearly described? | Main outcomes were clearly described in the Introduction or Methods? | Are the characteristics of the patients included in the study clearly described? | Interventions were clearly described? | Distributions of confounders in each group compared was clearly described? | Are the main findings of the study clearly described? | Provide estimates of the random variability in the data for the main outcomes? | Adverse events as consequence of the intervention were reported? | Have the characteristics of patients lost to follow-up been described? | Probability reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability is less than 0.001? | The subjects were representative of the entire population from which they were recruited? | Were the subjects prepared to participate representative of the entire population from which they were recruited? | Were the staff, places, and facilities, representative of the treatment the majority of patients receive? | Was an attempt made to blind study subjects to the intervention? |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lima *et al*., 2010 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 |
| Nascimento *et al*., 2014 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 |
| De Souza *et al*., 2020 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 |
| Sarmento *et al*., 2021 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 |
| **Partial score / items (n)** | 4 | 4 | 4 | 4 | 4 | 4 | 0 | 1 | 4 | 4 | 0 | 0 | 4 | 0 |
| **Partial score / items (%)** | 100 | 100 | 100 | 100 | 100 | 100 | 0 | 25 | 100 | 100 | 0 | 0 | 100 | 0 |

[1]Downs and Black N. (1998). J. Epidemiol. Commun Health. 52(6), 377–384.

**Table S11** ***(continuation)*.** Bias analysis in all clinical studies included in the systematic review (Downs and Black Quality Index[1]).

| Studies | Was an attempt made to blind those measuring the main outcomes of the intervention? | Results based on " data dredging", were clearly reported? | Do the analyses adjust for different lengths (follow-up or period between the intervention and outcome) ? | Were the statistical tests used to assess the main outcomes appropriate? | Was compliance with intervention/s reliable? | Were the main outcome measures used accurate (valid and reliable)? | Were the patients in different groups or were the cases and controls recruited from the same population? | Were study subjects in different groups or were the cases and controls recruited over the same period of time? | Were study subjects randomized in groups? | Randomized intervention assignment concealed from patients and health care staff until recruitment was complete? | Adequate adjustment for confounding from which the main findings were drawn? | Losses of patients were considered? | **Downs and Black Quality Index[1] N / %** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lima *et al*., 2010 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 17 / 65.39 |
| Nascimento *et al*., 2014 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 18 / 69.23 |
| De Souza *et al*., 2020 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 20 / 76.92 |
| Sarmento *et al*., 2021 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 18 / 69.23 |
| **Quality score / items (n)** | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 1 | 0 | 0 | 4 | |
| **Quality score / items (%)** | 75 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 25 | 0 | 0 | 100 | |

[1]Downs and Black N. (1998). J. Epidemiol. Commun Health. 52(6), 377–384.