REVIEW ARTICLE

Detrimental effects of malaria, toxoplasmosis, leishmaniosis and Chagas disease on cardiac and skeletal muscles

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Abstract

The pathogenic mechanisms of several diseases triggered by protozoan parasites, such as the causative agents of Chagas disease, toxoplasmosis, leishmaniosis, and malaria, have demonstrated to cause direct detrimental effect on cardiac and skeletal muscle. These are amongst the most prevalent and epidemiologically relevant protozoan infections worldwide and infecting millions of people per year. As such, this review focuses on the current knowledge on the pathogenic mechanisms of these diseases on muscles. Case studies and original research addressing the mechanisms of action for direct and indirect damage to cardiac and skeletal muscle were analyzed and the main findings summarized. Importantly, all diseases reviewed here produce an intense inflammatory response, with the associated oxidative stress and pro-inflammatory cytokine production leading to or furthering these detrimental effects. Critically, the disruption of cardiac muscle function can lead to minor arrhythmias and even death, and skeletal muscle damage can result in homeostatic imbalances serving to further morbidity and mortality. Strategies for preventing complications and determining the effectiveness of interventions designed with antioxidant and anti-inflammatory molecules to minimize muscle injury and help the millions of people with these diseases are an urgent need.

Introduction

Mammalian muscle tissues are divided into three types according to their function and morphology: skeletal, cardiac, and smooth. Skeletal muscle is the most abundant of the three forms of muscle in the human body, consisting of long, striated, and multinucleated fibers (also called striated muscle myocytes) that align in a regular pattern of fine red and white lines. This appearance is distinct and conserved across mammalian species (1). Cardiac muscle is also composed by striated myocytes, but consisting of mononucleated fibers, found only in the heart, and responsible for the circulation of blood throughout the body. Although mononucleated, cardiac muscle cells can undergo hypertrophy with two or three nuclei. In cardiac muscle, two or more myocytes can attach laterally via desmosomes. The striated microscopic appearance in both skeletal and cardiac muscles is from the filaments of actin and myosin responsible for muscle contraction (2). Myopathies, a general term used to describe diseases of muscle tissues dating back to the mid 1800's, negatively affects muscle fiber function, leading to muscle pain, weakness, and fatigue (3, 4). Muscle myopathies are either acquired or inherited. Acquired myopathies sub-classify in inflammatory, toxic, infectious, and associated with systemic diseases. Inherited myopathies are: muscular dystrophies, congenital myopathies, metabolic myopathies, and mitochondrial myopathies. (5). Infectious diseases caused by microorganisms, such as viruses, bacteria, and parasites, may cause direct and/or indirect damage in cardiac and skeletal muscle resulting in myopathies (6). An important aspect of these infectious diseases is the infecting component, their systemic nature, pro-inflammatory properties, and the induction of metabolic changes. They possess the intrinsic destructive capacity to combine several aspects that together can be largely detrimental to the musculoskeletal (MSK) system. We also interpret the MSK as buffering sentinel for the organism; at the expense of the MSK other organs can be preserved.

Most of the pathogenesis caused by intestinal protozoans, such as *Giardia* spp., *Entamoeba hystolitica*, *Balantidium* spp., *Cryptosporidium* spp., *Isospora* spp., and *Cyclospora* spp., induce diarrheas or dysenteries leading to electrolyte imbalance and dehydration. Without proper treatment, severe electrolyte imbalances can cause loss of muscle mass with or without the loss of fat mass (7, 8). While some of this damage can be indirect, the generation of action potentials to induce muscle contractions is fully dependent on a tight electrolyte balance, and disturbances can severely affect muscle function. Muscle weakness can develop, as well as cramps and muscle contractures, which under certain conditions can lead to rhabdomyolysis, a severe muscle damage that can also damage the kidneys due to the release of myoglobin in the blood. In the same way, the pathogenic mechanisms of other protozoan parasites, such as Chagas disease, toxoplasmosis, leishmaniasis, and malaria, have been demonstrated to cause direct detrimental effect on the musculoskeletal and cardiovascular system (**Table 1).**

Due to the worldwide importance of these protozoan parasites, this review is focused on the detrimental effects on skeletal and cardiac muscle caused by the infection of the protozoan parasites that result in serious and highly prevalent diseases; *Trypanosoma cruzi* (Chagas diseases), *Leishmania spp.* (leishmaniasis), *Toxoplasma gondii* (toxoplasmosis), and *Plasmodium* spp. (malaria). All but *Plasmodium* spp. have been detected inside striated muscle fibers and directly affect striated muscle and cardiac cells (**Table 1**). Although

Plasmodium spp. has never been observed infecting muscle cells, numerous reports have demonstrated that malaria infection can seriously affect skeletal and cardiac cells (9-11).

Of the four protozoan diseases we focus on here, Chagas disease, leishmaniasis, and toxoplasmosis are zoonosis; naturally transmitted between animals and humans. Leishmaniasis has shown completely different pathologies in canines when compared to humans, and to Chagas disease and toxoplasmosis, therefore, the results from animal models or in naturally infected animals should be interpreted with caution. Despite that limitation, the discussed studies brought insights to the knowledge of the pathologies caused by these zoonotic parasites. Finally, *Plasmodium spp*. causes malaria, a species-specific disease, with murine malaria being the most used experimental model used to compare effects with human malaria.

Table 1. Main protozoan parasites that cause human diseases and relationship with damage effect in striated muscle fibers and other human organs or cells.

Phylum	Genera	Diseases	Detected inside striated muscle fibers	Damage effect on muscles	Infect other cells and organs
Sarcomastigophora	***Leishmania***	Cutaneous, mucocutaneous and Visceral leishmaniasis,	**Yes**	**Yes**	macrophages, skin, visceral organs
	Trypanosoma	Chagas' disease, sleeping sickness	**Yes**	**Yes**	macrophages, heart, esophagi, intestine
	Giardia	Diarrhea	NE	NDE	small intestine
	Trichomonas	Vaginitis	NE	NDE	women lower genital tract, inside of the penis
	Entamoeba	Dysentery, liver abscess	NE	NDE	intestine, liver, lung, brain
	Dientamoeba	Colitis	NE	NDE	large intestine
	Naegleria and Acanthamoeba	Encephalitis	NE	NDE	central nervous system and cornea
Apicomplexa	*Babesia*	Babesiosis	NE	NDE	red blood cells
	Plasmodium	Malaria	**No**	**Yes**	hepatocytes and red blood cells
	Isospora	Diarrhea	NE	NDE	intestine
	Cryptosporidium	Diarrhea	NE	NDE	small intestine, heart
	Toxoplasma	Toxoplasmosis	**Yes**	**Yes**	central nervous system
Ciliophora	*Balantidium*	Dysentery	NE	NDE	large intestine

NE: no evidence. NDE: no direct evidence.

1. Trypanosomatid parasites and their effect in cardiac and skeletal muscles

The three major human diseases caused by trypanosomatids are African trypanosomiasis or "sleeping sickness" (caused by *Trypanosoma brucei* and transmitted by tsetse flies), American trypanosomiasis or Chagas disease (caused by *Trypanosoma cruzi* and transmitted in endemic areas by triatomine bugs), and the three manifestations of leishmaniasis diseases; cutaneous, mucocutaneous, and

visceral leishmaniasis (caused by various species of *Leishmania* transmitted by sandflies) (12). These vector-borne diseases affect humans and other mammals and are responsible for significant morbidity and mortality worldwide. Other species of *Trypanosoma* and *Leishmania* infect a wide range of vertebrate hosts, all transmitted by arthropods (13). Their pathogenic processes can cause detrimental effects on skeletal and cardiac muscle in vertebrates (14-19).

1.1. *Trypanosoma cruzi* and the Chagas diseases

The prevalence of *Trypanosoma cruzi* is approximately 6-8 million persons worldwide, although many experts in the field believe this number is underestimated, and approximately 10,000 die each year from Chagas disease complications (20). Around 30% of people with Chagas disease develop serious complications (21).

The effect of *T. cruzi* parasites on skeletal and cardiac muscles has been investigated for decades (16, 18). The high genetic diversity of the Chagas disease parasite produces heterogeneous clinical manifestations (22). This has been reflected in the differences observed in organ damage, with evidence of organ or tissue tropism highly associated to the *T. cruzi* strain (23). The crucial importance of the autoimmune response in the pathogenesis of cardiac Chagas was demonstrated by Wesley et al., where the analysis of *T. cruzi* kDNA minicircle integration into the mammalian host genome was correlated to the levels of IgG antibodies reactive to host cardiac proteins and interferon production, both influencing tissue inflammation (24). High levels of pro-inflammatory cytokines is linked to cardiac damage (25). However, a direct correlation between parasite load and tissue damage is not entirely clear as there are descriptions of parasite-triggered autoimmunity that can indirectly damage organs even in the absence of significant parasitemia (26).

Myotropic or non-myotropic strains of *T. cruzi* may show different behavior when in contact with muscle cells (27). The interaction of *T .cruzi* trypomastigotes from amyotropic strain (CL and Colombiana strains) comparing to a macrophage tropic strain (Y strain) with mice myoblasts and myotubes, showed that parasites from the myotropic strains were more infective to myoblasts than those from the macrophagotropic strain (16). Interestingly, myoblasts infected with parasites from non-myotropic strains are able to fuse with other infected and uninfected cells to form myotubes. However, the process of fusion was inhibited when the myoblasts were infected with parasites from the myotropic strains. Normal myogenic processes in murine cardiac cells were disrupted by myotropic strains exclusively (18).

Previous work by Báez et al. has provided building evidence that functional and structural alterations to mitochondria are responsible for chagasic cardiomyopathy (28-30). More recent studies from the same group have extended this effect to murine skeletal muscle, observing disruptions in citrate synthase activity and cellular respiration malfunction. All samples from infected mice showed at least one structural abnormality related to mitochondrial enzyme malfunction, such as increased matrix formation or cristae disorganization (28).

Acute and chronic Chagas disease can induce severe acute myositis, rapidly leading to the degeneration and necrosis of muscle fibers (31, 32). This significantly reduces skeletal muscle contractility and, in the early stages can be reversed by myogenic stem cell activation (31, 33, 34).

Throughout the chronic phase, cardiac tissue abnormalities present a variety of symptoms, ranging from slight electrocardiogram (EKG) alterations to

sudden cardiac death (SCD). Congestive heart failure (CHF) can also develop, presenting a myriad of complex healthcare conditions (35). Novaes et al. demonstrated reductions in aerobic exercise tolerance in infected rats (36-38). Despite this limitation, comparisons between sedentary and aerobically active groups suggest that exercise may induce beneficial adaptations. Active rats saw decreases in pro-inflammatory cytokines: interferon gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), and IL-6. Parasitemia, tissue inflammation and fibrosis, and cell atrophy markedly decreased while serum antioxidant enzymes increased. This resulted in decreased cardiac oxidative damage, limiting contractile dysfunction(37, 38). Similar effects have been seen in humans, greatly increasing cardiac functionality (39).

The suppression of cytokine signaling 2 (SOCS2) can regulate immune responses and may play a role in the pathogenesis of *T. cruzi* infection. Reductions in parasitemia and the expression of IFN-γ, TNF-α, SOCS1, and SOCS3 were detected in SOCS2 knockout (KO) mouse cardiac muscle. An increase in the growth and formation of T regulatory (Treg) cells with a decrease in memory cells in infected SOCS2 KO mice with *T. cruzi* has been reported, indicating reductions in inflammation and parasitemia in infected SOCS2-KO mice may be secondary to the increases in Treg cells, suggesting another future therapeutic direction (40).

Surface carbohydrates have been associated with processes of cell recognition and surface markers also play a role in *T. cruzi* infection and in system response (41). Galectin-1 (Gal-1) is an endogenous surface glycoprotein expressed on the surface of human and murine cardiac cells that reduces both the likelihood of infection and apoptosis induced by *T. cruzi*. Infected humans and rodents increase Gal-1 expression, and Gal-1 knockout (KO) mice present higher parasitemia, reduced cardiac and skeletal muscle tissue inflammation, and lower survival rates compared to wild-type (WT) mice (42).

Emerging stem cell-based therapies provide an impactful advance in the treatment of chronic-degenerative diseases. Transplantation of bone marrow cells (BMCs) can regenerate heart and skeletal muscle lesions caused by *T. cruzi* infection in experimental models, potentially mitigating the damage caused by *T. cruzi*. This supports the potential benefits of stem cell-based therapy during the chronic phase of Chagas disease, drastically accelerating the natural regeneration process (43), although such a therapy modality is economically challenging for most of the countries impacted by these diseases.

1.2. Effects of Leishmaniasis infection in skeletal and cardiac muscles

The *Leishmania* genus represents more than 30 different species, some infecting humans, causing a wide spectrum of clinical manifestations classified into cutaneous (CL), mucosal (ML) and visceral leishmaniasis (VL). Estimates suggest that between 10 and 50 million people are infected with *Leishmania* spp. in 98 countries causing more than 1.5 million cases per year. VL is the most serious and deadly form of this disease. Leishmaniasis is a serious disease, killing an estimated 50,000 people in 2010. This burden is not spread equally, with only six countries reporting 90% of all VL cases, and ten countries reporting 75% of all CL cases. (44).

Leishmania species can infect different tissues and organs of the host (26). The infection is initiated in macrophages, but other cell types can be infected, including fibroblasts, dendritic cells, and muscle fibers. Leishmaniasis are among the main causes of muscle lesions and muscle atrophy in canines

(14), the main domestic reservoir of the disease (45). Canines infected with *Leishmania* spp. have shown cardiac effects as well (15, 17, 19). Alves et al. showed cardiac and pulmonary alterations in both symptomatic and asymptomatic canines with visceral leishmaniasis, caused by *L. chagasi* (15). Study cases of chronic myocarditis on canines naturally infected with *L. chagasi* showed the development of cardiac muscle alterations with clinical and pathological consequences. Such involvement seems to be related to the tissue's response to *Leishmania*, and not *Leishmania* itself (19).

Providing further evidence of immunological dysfunction, Silva-Almeida et al. demonstrated that a mouse strain susceptible to *Leishmania* infection (BALB/c) showed a high number of infected macrophages among muscle fibers at the onset of disease. Importantly, the major *Leishmania* infection susceptibility in BALB/c mice strain is related to their genetic background which favors/leads the development of a predominant Th2 type response, with the production of high levels of IL10 and IL4 cytokines, and a defective expression of CXCR3 chemokine receptors in T cells upon activation (46). These genetic susceptibilities contribute to explain the large non-healing lesions in BALB/c mice. On the other hand, C57Bl/6 and C3H.He strains present favorable outcomes for *Leishmania* infection, because of their ability for mounting a protective IL-12 induced Th1-type response. In the C3H.He *Leishmania* resistant mouse strain, muscle fibers are found mostly spared, though some parasitized macrophages were discovered (26). In line with the previous works, the major pathophysiological effect of *Leishmania* on the musculature appears to be related with immunological alterations in humans, rodents and canine hosts and as infected macrophages were observed, likely inflammatory (14, 15, 17, 19, 26, 45).

Turning towards muscle performance, the plantaris, soleus, and diaphragm muscles from *L. donovani* infected hamsters showed both direct and indirect effects on skeletal muscle performance when comparing the effects of simple calorie restriction and chronic leishmaniasis infection. Both scenarios produced similar reductions in body weight and muscle cross-sectional area. Caloric restriction alone could explain the reductions in muscle cross sectional areas and tetanic tension of the diaphragm and soleus muscles of the infected animals, suggesting nutritional interventions to offset some of these deleterious effects (47).

Relative caloric reduction to muscle tissues may be a key piece of the pathogenic mechanism (48). Leishmaniasis is known to cause latent infections (49), whose agents stay viable as inactive forms in adipose tissue, adipose tissue-derived mesenchymal stem cells, and fibroblasts (50). Pre-adipocytes can develop macrophage-like phagocytic characteristics (51), and when dormancy is disturbed by an exogenic stimulus, such as caloric restriction (e.g., dieting), *Leishmania* can migrate to other sites, including muscle, in search of nutrients (48).

Case reports on the detrimental effects of *Leishmania* parasites on human skeletal muscles in children document marked loss of mass in posterior muscles, suggesting that weakness accompanying *Leishmania* infection results not only from loss of muscle mass, but also from the impaired contractile performance of the remaining muscle (52, 53). A 1993 case report demonstrated the dangers of ignoring conflicting pathologic testing concerning leishmaniasis. A nine-year-old child with fever and severe muscle dysfunction produced no antibodies against numerous bacterial, viral, or parasitic diseases. However, serological testing for *L. infantum*

and *L.donovani* showed high circulating antibody levels. *Leishmania* amastigotes were not found in bone-marrow aspirate. Increased serum creatine kinase (CK) and muscle biopsy findings confirmed myositis. As bone-marrow smears were negative for leishmania amastigotes, pathologists incorrectly considered the serological findings to be falsely positive. The child started on prednisone and saw improvements in strength and pain within a week of treatment (53).

Eight months later, the child was readmitted to the hospital with return of symptoms and hepatosplenomegaly. VL was confirmed by the presences of leishmania amastigotes in bone-marrow smears. After correcting the treatment with pentavalent antimony derivative there was a significant improvement in hematological findings and regression of hepatosplenomegaly occurred shortly thereafter (53). Despite the paucity of case reports, *Leishmania* spp. should be considered as a possible cause of human myositis, particularly when other etiologies are dismissed.

2. Apicomplexa phyla

Apicomplexa is a taxonomic group of protozoa of about 5,000 species, grouped on the presence of an apical complex in the sporozoite and merozoite stages (54). Many species of this group cause severe diseases in humans, such as babesiosis, malaria, toxoplasmosis, and coccidiosis. *Plasmodium* spp. and *Toxoplasma gondii* are the main apicomplexan protozoans that cause direct and indirect detrimental effects on skeletal and cardiac muscle. Numerous studies have demonstrated apicomplexan infection-related skeletal and cardiac muscle damage which will be discussed.

2.1. Toxoplasmosis affecting skeletal and cardiac muscles

Toxoplasma gondii is one of the most medically relevant protozoan parasites, affecting avian species, reptiles, amphibians, and mammals. Toxoplasmosis is the most prevalent parasitic infection in humans, with an impressive infection rate of 25 to 30% in the world population (55). Although most toxoplasmosis infections are asymptomatic, infection can be devastating in immunosuppressed or immunocompromised individuals (56). For example, toxoplasmosis is the most common secondary nervous system infection in individuals with acquired immune deficiency syndrome (AIDS) (57). Higher frequencies of muscle effects from *T. gondii* have been found in persons with AIDS with 0.5-4% of *T. gondii* and AIDS-diagnosed persons showing toxoplasma cysts, greater than those with *T. gondii* only. Muscular *T. gondii* involvement results in greater weakness, myalgias, and elevated CK, suggesting the need for muscular biopsies for persons with AIDS in areas with endemic *T. gondii* activity (58).

The bradyzoite stage primarily affects brain and muscle tissue which, innately, have limited immune response capability (59). Toxoplasmic pneumonitis is responsible for pulmonary complications related to AIDS that can lead to septic shock (60, 61). Myocarditis caused by *T. gondii* is a rare but serious complication that can result in SCD (56, 62-65). Cardiac effects are more pronounced in heart-transplant recipients; a retrospective study found that of the 1.3% of persons that developed *T. gondii* infections in the post-operative period following transplantation, 50% died (66).

Gomes et al. investigated the ramifications of *T. gondii* infection on myogenesis in a skeletal muscle model (67, 68). Macroscopically, infected myoblasts saw significant myogenic inhibition, increased infection risk, and a complete

inability to fuse into myotubes. In addition, cadherin expression was down-regulated (67) and intracellular lipid bodies increased, providing nutrient storage for *T. gondii*, redirecting nutrients intended for the muscle to itself (68). Towards myokines, infected cells showed increased IL-12, IFN-γ, prostaglandin E2 (PGE2), and cyclooxygenase 2 (COX-2) (68). Chronic *T. gondii* infections present a life-long struggle with systemic inflammation, gradually worsening as parasitic cysts develop in the brain and skeletal muscle (69). This eventually leads to complex myositis and associated muscle damage from the chronic, extensive accumulation of pro-inflammatory macrophages (70). One therapy option is the reduction of regulatory T cells during infection, rescuing macrophage homeostasis and allowing skeletal muscle regeneration. This suggests that a significant portion of the muscular symptoms of *T. gondii* infection directly results from the person's own immune response (71).

Muscle is not defenseless, however. SkMCs infected with IFN-γ and/or TNF-α-activated *T. gondii* triggers the expression of inducible nitric oxide synthase (iNOS), leading to elevated intracellular nitric oxide (NO) levels. This significantly inhibits the replication of intracellular *T. gondii* parasites, as seen by the increase in immune-related GTPases (IRG) within parasitic vacuoles. This, ultimately, does not prevent tachyzoite-bradyzoite transformation and can only slow, not stop, the progression of the infection (72).

2.2. Effect of *Plasmodium* spp. infection in skeletal and cardiac muscles

Malaria is one of the most prevalent and severe protozoan diseases worldwide. Approximately 219 million cases and 435,000 deaths were reported in the year 2017, with children and pregnant women remaining the most affected group. Disappointingly, little if any progress was made in the 2015-2017 period with new infections and deaths stabilizing, and over 3.2 billion potentially exposed to malaria worldwide (73).

Plasmodium parasites have never been detected inside muscle fibers, preferring indirect damage related to their pathologic mechanisms. Although the most serious effects of malaria infection in skeletal and cardiac muscles have been related to the species *P. falciparum*, other *Plasmodium* species, including the human *P. vivax*, have been shown to damage heart and muscles (74-77). In the case of *P. falciparum*, the bulk of systemic damage derives from the agglutination and subsequent microvasculature sequestration of erythrocytes leading to hypoxia (77), rather than any currently recognized effect on the erythrocytes themselves (10, 78). The resulting skeletal muscle necrosis and related rhabdomyolysis has been reported in persons with severe *P. falciparum* (10, 79, 80). This effect can be detected via increased CK levels, indicating an increase in skeletal and cardiac muscle damage (79, 81).

These effects extend to protein- and functional-level testing as well. Infected rat muscle showed lower protein transcription and protein levels when compared to non-muscular tissue and non-infected rats (82). Our group demonstrated this effect with *P. berghei* infected mice, halving extensor digitorum longus (EDL) and soleus (SOL) muscle contractile force and significantly impaired fatigue recovery capacity following exercise (83). These effects were associated with the significant decrease in contractile proteins (e.g., myosin and troponin) in the muscle fibers of infected rodents, corroborating with prior findings of detectable serum contractile proteins following plasmodium infection (79, 81, 84, 85). Elevated muscle protein levels were observed in the plasma of children with cerebral malaria, indicating muscle-specific

proteins in plasma as a potential indicator of advanced malaria (86).

Case studies have demonstrated numerous *P. vivax* infection-related cardiac complications including tachycardias, arrhythmias, and CHF (87-89). A decent part of these effects may stem directly from antimalarial therapies themselves. EKG reports from *Plasmodium* infected persons have demonstrated cardiac arrhythmias as a leading cause of SCD in severe infection cases (87, 89). *P. chabaudi*, *P.vinckei*, *P. petteri*, and *P. yoeliinigeriensis* produced irreversible lesions on the heart and other organs following acute and chronic infection in mice (84). Humanized Balb/c mice infected with *P. falciparum* from human blood revealed high levels of peripheral parasitemia and the deaths of 10 out of 25 inoculated mice. Post-mortem examination revealed extensive cardiac sequestration and inflammatory mediator presence (88).

Several serum biomarkers can be used to measure the severity of plasmodium infection-induced myocardial injury and necrosis. N-terminal pro-brain natriuretic peptide (NT-proBNP) and heart-type fatty acid-binding protein (H-FABP) were significantly elevated in complicated, but not uncomplicated, plasmodium cases in a case-control study with 63 participants (90). Similar results were seen in a separate study with 400 African children with mild-to-severe *P. falciparum* infection. Significantly elevated levels of myoglobin and CK were seen in children with severe- and fatal-stage infection respectively (91).

The release of Troponin T, the most sensitive and selective biomarker for myocardial damage (92), occurs when the myocardium is damaged by a variety of conditions, such as necrotic tissue injury (93, 94). Analysis of sera samples from *P. falciparum* persons showed that the myocardia damage detected by high Troponin T levels is a rare event (92). Glycosyl phosphatidylinositol (GPI) has many functions, including acting as a toxin during malaria falciparum pathogenesis. Cardiomyocytes treated with purified falciparum GPI showed upregulated genes related to apoptosis and myocardial damage. This effect has also been seen in human case studies (95).

3. Conclusions

The parasitic diseases discussed here affect human muscles both directly and indirectly. While only *Leishmania* spp., *Trypanosoma cruzi*, and *Toxoplasma gondii* have been detected within muscle fibers, *Plasmodium* spp. causes indirect damage to muscle tissue. Other protozoan parasites (not discussed in this review) operate indirectly and in more discreet ways, such as *Giardia* spp., *Cryptosporidium* spp., and *Balantidium* spp. (**Table 1**), causing diarrhea and dysentery, leading to dehydration and malnutrition sufficient to inflict loss of muscle mass. The direct and indirect effects of protozoan infections on muscles are summarized in **Figure 1.** Direct damage can come from resultant inflammation, such as with Leishmaniasis infection, or from the pathologic mechanism of the parasite itself, seen in the structural mitochondrion changes seen in Chagasic infections. None of the diseases discussed here limit themselves to just the muscle; *Plasmodium* spp. invades hepatocytes and erythrocytes, *Trypanosoma cruzi* the heart, esophagus, intestines, and macrophages.

The diseases caused by the protozoan parasites reviewed here induce an intense inflammatory response, with oxidative stress and elevated production of pro-inflammatory molecules (cytokines and lipid mediators), leading to detrimental effects on cardiac and skeletal muscles. It is noteworthy that pathologies caused by these protozoan parasites may also affect the activity of the infected vertebrate, potentially exacerbating

the loss of muscle mass and function. Further study into the effects of parasitic diseases on vertebrate hosts will advance efforts to find additional therapies and care strategies, with antioxidant and/or anti-inflammatory molecules to minimize muscle injury and help the millions of people with these diseases.

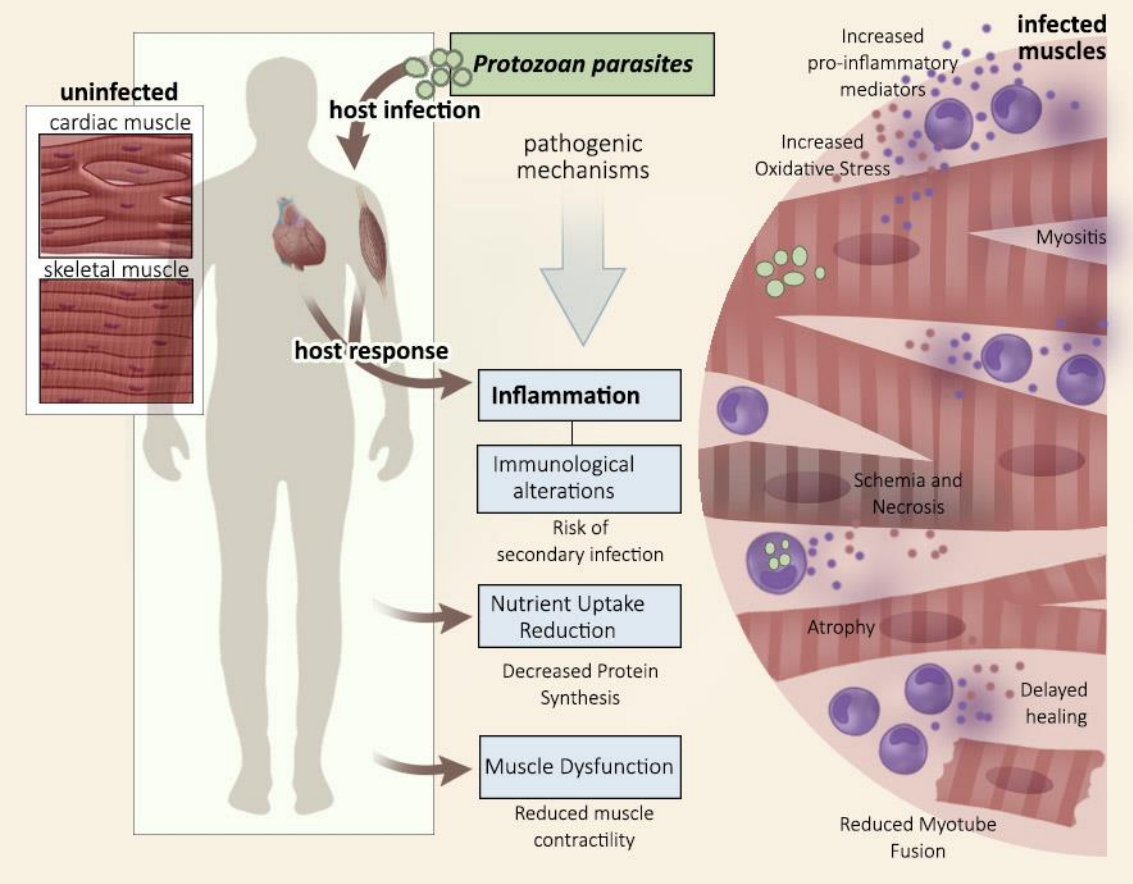

Figure 1. Proposed schematic model of the direct and indirect pathogenic effects of protozoan infections on cardiac and skeletal muscles. After the host infection by protozoan parasite [e.g. *Trypanosoma cruzi* (Chagas disase), *Leishmania* spp. (leishmaniasis), *Toxoplasma gondii* (toxoplasmosis), and *Plasmodium* spp (malaria)]. These protozoans can induce an intense inflammatory response, with the associated oxidative stress and pro-inflammatory cytokine production leading the establishment of positive feedback loop. Some species of protozoans (e.g. *Leishmania* spp.), can infect different tissues and cells, such as macrophages, but muscle fibers can also be infected (eg. leishmaniasis and Chagas). Infected macrophages can release more toxic substances to skeletal muscles. Reduction in caloric intake associated to these pathogens also contributes to decreased protein synthesis, delayed healing and muscle atrophy. In cases of severe myosistis (as observed in Chagas disease), there is rapid degeneration and necrosis of muscle fibers, which significantly reduces skeletal muscle contractility. In the magnified representation of infected muscles, the fibers are morphologically equivalent to cardiomyocytes, but the representation of pathological mechanisms is considered for both, skeletal and cardiac muscles.

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