UNIVERSIDADE FEDERAL DE MINAS GERAIS

INSTITUTO DE CIÊNCIAS BIOLÓGICAS

DEPARTAMENTO DE MORFOLOGIA

PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA CELULAR

EFEITOS DO TREINAMENTO FÍSICO AERÓBICO NA FUNÇÃO CARDÍACA, CAPACIDADE FUNCIONAL, COMPORTAMENTO DEPRESSIVO E NÍVEIS DE NEUROTROFINAS EM CAMUNDONGOS INFECTADOS PELA CEPA Y DO *Trypanosoma cruzi*

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Tese apresentada ao Programa de Pós-graduação em Biologia Celular como requisito parcial para obtenção do título de Doutor em Biologia Celular.

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LISTA DE ABREVIATURAS E SIGLAS

DCh	Doença de chagas
NF	neurotrofina
GDNF	fator neurotrófico derivado de células gliais
NGF	fator de crescimento neuronal
BDNF	fator neurotrófico derivado do cérebro
TEM	Teste de esforço máximo
FST	Teste do nado forçado
CHC	chagásico sedentário
CHT	chagásico treinado
SC	sedentário controle
TC	treinado controle
SNA	sistema nervoso autônomo
CD	Chagas' disease
RVA	rigth ventricular area
LVA	left ventricular area
SV	stroke volume
EF	ejection fraction
CO	cardiac output
FAC	fractional area change
LV	left ventricle
HF	heart failure

Sumário

RESUMO

A doença de Chagas (DCh) é caracterizada em sua fase crônica pelo alto índice de acometimento cardíaco. O objetivo desse estudo foi investigar, em camundongos infectados pela cepa Y do *Trypanosoma cruzi*, os efeitos do treinamento físico aeróbio de intensidade moderada na função cardíaca, concentrações de neurotrofinas (NF) [fator neurotrófico derivado de células gliais (GDNF), fator de crescimento neuronal (NGF) e fator neurotrófico derivado do cérebro (BDNF)] no coração, plasma, baço e diferentes áreas do cérebro, além de avaliar o comportamento depressivo nesses animais. Camundongos C57BL/6 machos (idade ~55 dias) foram distribuídos em 4 grupos: sedentário controle (SC, n=7), controles treinados (TC, n=7), infectados sedentários (CHC, n=7) e infectados treinados (CHT, n=7). A infecção foi realizada com a injeção intraperitoneal de 10^3 formas tripomastigotas. A parasitemia foi determinada no 7º, 9º, 11º, 13º e 15º dias após a infecção. Os animais foram adaptados à esteira na semana anterior ao começo do treinamento, iniciado 45 dias após a infecção. No último dia de adaptação, bem como ao final da 4ª, 8ª e 12ª semanas de treinamento, o teste de esforço máximo (TEM) foi executado para avaliação da tolerância ao esforço e/ou para determinar os ajustes na velocidade de treinamento, fixada em 70% da velocidade máxima alcançada. Ao final do período de 12 semanas, todos os animais foram avaliados quanto à estrutura e função cardíacas por ecocardiograma e quanto ao comportamento depressivo pelo teste do nado forçado (FST). Os tecidos para análise histológica, bem como para dosagem de NF, foram obtidos após a eutanásia. Não foram encontradas diferenças significativas entre os grupos infectados quanto à cinética parasitêmica. A função cardíaca foi comprometida pela infecção, uma vez que o grupo CHC apresentou maior área do ventrículo direito e volume sistólico final, além de redução na fração de ejeção, débito sistólico e fração de mudança de área do ventrículo

esquerdo. Por outro lado, o treinamento físico foi capaz de atenuar a progressão da doença, visto os melhores parâmetros ecocardiográficos encontrados em CHT e seu melhor desempenho nos TEM. Os níveis de NF no coração e plasma, especialmente o BDNF, foram significativamente aumentados pela infecção, ao passo que o treinamento promoveu um ajuste em sua concentração. No cérebro, as diferentes NF avaliadas adotaram comportamento semelhante, não alterando sua expressão no córtex, diminuindo sua expressão em função da infecção ou do treinamento no estriado e diminuindo sua expressão na associação dos dois estímulos no hipocampo. O FST não foi capaz de identificar comportamento do tipo depressivo nos grupos quanto as variáveis consideradas. Assim, o treinamento físico aeróbio de intensidade moderada executado por 12 semanas preservou a função cardíaca de animais infectados e aumentou a tolerância ao esforço. Além disso, o exercício parece exercer um papel regulatório na expressão de NF nos diferentes tecidos avaliados.

Palavras chave: Cardiopatia chagásica, fator neurotrófico derivado de células gliais, fator de crescimento neuronal, fator neurotrófico derivado do cérebro, função cardíaca, depressão, treinamento físico.

1. INTRODUÇÃO E REVISÃO DA LITERATURA

1.1 Histórico e epidemiologia da Doença de Chagas

Carlos Justiano Ribeiro Chagas, mineiro de Oliveira, biólogo, médico e cientista, destaca-se até os dias atuais como um dos mais brilhantes cientistas do Brasil. Tal fato, deve-se à descoberta, publicada em uma nota prévia datada de 22 de abril de 1909, de uma doença causada por um tripanossomo, a doença de Chagas (DCh). Mais tarde, no mesmo ano, o cientista publicou o trabalho completo, descrevendo o vetor da doença, o popularmente conhecido barbeiro do gênero *Triatoma*, além do ciclo do agente etiológico, o *Trypanosoma cruzi*, e a evolução clínica da doença (Chagas, 1909).

O relato minucioso à comunidade científica aconteceu após uma expedição à pequena cidade do interior mineiro chamada Lassance. Nesse trabalho, o pesquisador informava quanto a detecção em humanos do protozoário *Trypanosoma cruzi*, o agente causador da DCh (Malafaia, Rodrigues, 2010). Esse nome foi dado à doença em função do seu sobrenome, uma prática comum à época quanto a adoção de epônimos.

Ao considerarmos os recursos vigentes na época, comparados aos disponíveis na atualidade, o relato datado de 1909, torna ainda mais robusta a descoberta e aponta para a importância do olhar científico para os detalhes técnicos, mas também para a observação clínica. Esse cenário, além do próprio descobrimento da doença, justifica as honrarias recebidas por Carlos Chagas, como o título de Doutor *Honoris Causa* concedido pela Universidade de Havard, em 1921, mesmo ano em que foi indicado ao Prêmio Nobel de Medicina; Doutor *Honoris Causa* pela Universidade de Paris; o grau de cavaleiro na Legião de Honra da França, dentre outros (Malafaia, Rodrigues, 2010).

É notória que a descoberta da DCh consiste em um dos mais importantes achados no que diz respeito a uma protozoose e, apesar de mais de um século de sua descoberta, ainda é considerada uma das doenças com mais larga distribuição no continente americano.

A DCh, apesar do progresso no controle de sua transmissão, permanece como um dos maiores problemas de saúde pública na América Latina (Rassi Jr., Marin-Neto, 2000), especialmente em dezoito países em desenvolvimento na América Central e do Sul (Rassi *et al.*, 2010; Biolo *et al.*, 2010). De maneira surpreendente, países até então considerados livres de tal mazela, como Japão (Imai *et al*., 2015), Nova Zelândia e Austrália (Pinto *et al.*, 2014; Jackson *et al*., 2014) registraram o aumento no número de casos nos últimos anos. Segundo a *World Health Organization* (WHO, 2012), a DCh apresenta prevalência de 8 milhões de casos em todo o mundo, sendo considerada a maior causa de doença cardíaca infecciosa em países endêmicos.

Apontada como uma das principais causas de insuficiência cardíaca crônica e morte súbita na América do Sul (Marin-Neto *et al*., 2007; Wilson *et al.*, 2005), a DCh impacta negativamente nas contas dos sistemas de saúde pelo alto custo do tratamento.

Dentre as formas crônicas da doença, a forma cardíaca é a mais frequente, assumindo-se que 20 a 30% dos indivíduos infectados desenvolvem algum grau de comprometimento cardíaco no decorrer da infecção. Além disso, é entendida como aquela de maior relevância do ponto de vista médico-social em função da gravidade de suas manifestações (Rassi Jr., Marin-Neto, 2000; Coura, 2007), como no caso do desenvolvimento da cardiopatia chagásica crônica, que é considerada a mais importante e grave manifestação clínica da DCh nesta fase (Dias, 1982; Storino; Milei, 1993).

Todos esses fatos somados reforçam a necessidade de elucidação de lacunas existentes com o investimento em pesquisas relacionadas não só ao controle da

transmissão, mas também ao tratamento dessa doença que permanece negligenciada até os dias atuais.

1.2 Infecção, história natural e manifestações clínicas da Doença de Chagas

A DCh é provocada pelo protozoário hemoflagelado, o *Trypanosoma cruzi*, e transmitida por triatomíneos infectados. A infecção em humanos pode ocorrer a partir do depósito de fezes com a presença das formas tripomastigotas sobre erupção cutânea provocada após a hematofagia e, menos frequentemente, pela ingestão de comida ou bebida contaminadas, da parturiente para a criança, em acidentes laboratoriais ou por transfusão sanguínea (WHO, 2005; Rocha *et al.*, 2007; Rassi Jr. *et al.*, 2010; Ribeiro *et al.*, 2012).

A partir da infecção, instala-se uma doença inflamatória caracterizada por duas fases distintas (Coura, 2007): a fase aguda ou inicial, geralmente assintomática, mas que pode manifestar-se como uma doença febril de curso clínico benigno e autolimitada, com alta parasitemia (Rassi Jr.; Marin-Neto, 2009; Simões *et al.*,2000), e a fase crônica ou tardia, de evolução lenta com baixa parasitemia, que compreende uma forma indeterminada e as consideradas formas clínicas determinadas (cardíaca, digestiva e mista) (Rassi Jr.; Marin-Neto, 2000).

São fatores como a intensidade da infecção inicial pelo *T.cruzi*, a cepa e a sua virulência, a presença de comprometimento miocárdico na fase aguda e o estado imunológico do hospedeiro, que podem explicar o determinismo para uma ou outra forma clínica: cardíaca, digestiva ou mista (Rassi Jr; Marin-Neto; 2000), ou ainda para a evolução em sua forma indeterminada (Coura, 2007; Rocha *et al.*, 2003; Rocha *et al.*, 2007; Rassi Jr.; Marin-Neto, 2000).

Os indivíduos que sobrevivem à fase aguda, em sua grande maioria migram para a forma indeterminada da doença, e até que as manifestações das formas determinadas apareçam, como no caso do desenvolvimento de miocardiopatia, podem permanecer nessa fase indeterminada por 10 a 30 anos (Rassi Jr; Marin-Neto; 2000).

Os sintomas e sinais inerentes à miocardiopatia chagásica crônica derivam essencialmente dos distúrbios arrítmicos, dos fenômenos tromboembólicos e das alterações da função associada à insuficiência cardíaca (Rassi Jr; Marin-Neto; 2000).

Dos distúrbios de condução, os mais frequentes são as taquiarritmias extra-sístoles ventriculares, taquicardia ventricular e fibrilações atriais e ventriculares, e no grupo das bradiarritmias destacam-se a bradicardia sinusal, bloqueio sinoatrial, bem como bloqueios atrioventriculares, dentre outros. A insuficiência cardíaca aparece tardiamente, comparada as arritmias, e apoia-se basicamente na hipossistolia, ou seja, hemodinamicamente manifesta-se como uma miocardiopatia dilatada. A disfunção diastólica também pode estar presente, inclusive antecedendo a disfunção sistólica, mas não constitui o principal mecanismo fisiopatológico da DCh. Nos estágios mais avançados apresenta predominantemente descompensação direita, com claro contraste entre o grau de congestão passiva sistêmica e a pequena intensidade dos sinais e sintomas de descompensação do ventrículo esquerdo (Rassi Jr.; Marin-Neto, 2000).

Quanto aos fenômenos tromboembólicos, são relativamente comuns na DCh e predominam os que afetam o encéfalo, membros inferiores e pulmões, sendo esses mais frequentes em estágios mais avançados da doença (Rassi Jr.; Marin-Neto, 2000).

1.3 Fisiopatologia das alterações cardíacas na Doença de Chagas

A DCh é uma doença inflamatória adquirida, caracterizada por miocardite fibrosante crônica e comprometimento progressivo da função cardíaca contrátil (Coura, 2007). Alterações fundamentais como: inflamação, lesões teciduais e fibrose, podem ser consequências diretas ou indiretas da presença do parasita no miocárdio contrátil, no sistema nervoso autônomo (SNA) intracardíaco e no tecido especializado de condução (Raso *et al.*, 1985).

Em virtude do baixo grau de parasitismo miocárdico e da ausência de correlação topográfica entre os focos inflamatórios e os locais de assentamento das formas amastigotas em miócitos, acreditava-se que o parasita não fosse fator patogênico decisivo na fase crônica da miocardiopatia (Koberle, 1986; Prata *et al.*, 1974; Huguchi *et al.*, 1993). Entretanto, estudos que sucederam as investigações preliminares, observaram a presença de anticorpos dirigidos aos antígenos do *T. cruzi*, comprovando a existência de correlação topográfica entre os focos detectáveis dessas alterações e as formas amastigotas, bem como com as reações inflamatórias (Higuchi *et al.*, 1993; Bellotti *et al.*, 1996).

Ainda que seja baixo o parasitismo na fase crônica, considera-se o mecanismo de ativação antigênica permanente como fator patogênico essencial da ativação das alterações imunológicas na fase crônica (Cunha *et al.*, 1995; Higuchi *et al.*, 1997) e, por consequência, da perpetuação das agressões aos tecidos. Essa linha de pensamento é reforçada por estudos que atribuem ao mecanismo da autoimunidade o papel chave da patogênese da miocardiopatia chagásica crônica, onde haveria um mimetismo antigênico entre o *T. cruzi* e o miocárdio, sugerindo a ocorrência de reatividade imune cruzada entre antígenos do parasita e do próprio miocárdio (Andrade *et al.*, 1994; Santos *et al.*, 1992).

Além dos mecanismos de agressão associados puramente à resposta imunológica, fenômenos isquêmicos transitórios, dependentes de distúrbios microcirculatórios, podem estar relacionados à patogênese da miocardiopatia chagásica crônica (Rassi Jr.; Marin-Neto, 2000). O comprometimento da microcirculação seria secundário às interações lesivas de células inflamatórias com o endotélio vascular (Andrade *et al.*, 1994). Essas interações produziriam como resultado final a rarefação capilar, o edema intersticial, a formação de agregados plaquetários e o espessamento da membrana basal vascular (Rassi Jr.; Marin-Neto, 2000).

Outro achado consistente aponta a depopulação neuronal acentuada em vários estágios da doença. Os achados histopatológicos mostram destruição neuronal difusa e irregularmente distribuída em vários tecidos, fatos esses reforçados pelos achados clínicos que evidenciam depressão do controle autonômico cardíaco, predominantemente no sistema parassimpático (Rassi Jr.; Marin-Neto, 2000).

Entretanto, entende-se que as alterações da microcirculação e do sistema de condução intracardíaco sejam apenas coadjuvantes no processo de instalação da DCh em sua forma cardíaca, ficando a cargo da resposta autoimune os agravos principais da função do órgão.

De maneira resumida, focos inflamatórios e áreas de fibrose no miocárdio podem produzir alterações eletrofisiológicas múltiplas e favorecer o surgimento do fenômeno de reentrada, principal mecanismo das taquiarritmias. Além disso, podem levar a disfunção ventricular pelo processo de destruição e reparo das fibras miocárdicas com a gradual substituição por tecido fibroso. Esse fenômeno desencadeia uma resposta compensatória em que as fibras íntegras são hipertrofiadas e as câmaras cardíacas dilatam de forma a manter o débito cardíaco, a pressão arterial e a perfusão tissular em homeostase (Rassi Jr.; Marin-Neto, 2000).

1.4 Doença de Chagas e o comprometimento da capacidade funcional

A infecção por *T.cruzi* afeta sobremaneira a capacidade funcional e a tolerância à atividade física dos indivíduos acometidos, mas pouco é entendido sobre como o processo infeccioso altera a tolerância ao exercício físico em modelos experimentais e na prática clínica (Novaes *et al.*, 2011). A redução da capacidade funcional em indivíduos com DCh é multifatorial e envolve alterações patológicas graves em órgãos e tecidos como o SNA (condução átrio-ventricular), músculo cardíaco (contratilidade), entre outros (Meiler *et al*., 1987; Montes de Oca, *et al*., 2004; Lima *et al*., 2010; Gallo *et al.*, 1975, Mady *et al.*, 2000).

Apesar de ser uma doença limitante da capacidade funcional, a mesma pode ter sua sintomatologia atenuada pelas alterações morfofisiológicas promovidas pelo treinamento físico (TF). O TF de intensidade moderada contribui para a preservação de neurônios durante a infecção (Moreira *et al.*, 2014b) e também atua preventivamente como um fator de resistência ao desenvolvimento da infecção em modelos animais por estimular a resposta imune (Shebeleski-Soares *et al.*, 2009; Moreira *et al.*,2013; Moreira *et al.*, 2014 a,b). Além disso, deve-se considerar as benéficas e já conhecidas alterações provocadas pelo TF, tais como: redução dos níveis pressóricos e da frequência cardíaca, remodelamento estrutural cardiovascular e melhora da função cardíaca (Chrysohoou *et al*., 2003; Morvan *et al.*, 2013).

1.5 Neurotrofinas e suas relações com a Doença de Chagas

As neurotrofinas (NTs) constituem uma família de proteínas envolvidas em importantes funções do sistema nervoso, incluindo crescimento e reparo axonal,

plasticidade sináptica, diferenciação e mielinização (Chao, 2003). Recentemente, estudos apontaram potenciais atuações em outros sistemas, como o cardiovascular (Caporali, Emanueli, 2009). Esses estudos dão conta da sua expressão em células como macrófagos, linfócitos, plaquetas, endoteliais, musculares lisas de vasos, cardiomiócitos e, até mesmo, musculares estriadas esqueléticas (Ejeri *et al.*, 2005; Lee *et al.*, 2014; Bernd *et al.*, 2004).

Diante disso, um número crescente de pesquisas investigam o padrão de concentração e expressão das NTs frente a várias condições, como na doença arterial coronariana (Ejiri *et al.*, 2005), infarto agudo do miocárdio (Okada *et al.*, 2012), síndromes metabólicas, como obesidade e diabetes (Lee *et al.*, 2014), além de suas aplicações como possíveis marcadores periféricos de índices metabólicos e de homeostase cardiovascular (Golden *et al.*,2010). Esse é o caso do fator neurotrófico derivado de uma linha de células da glia (GDNF), do fator de crescimento neuronal (NGF) e do fator neurotrófico derivado do cérebro (BDNF), que apesar do crescente número de investigações relacionadas às suas funções e padrão de expressão/concentração em várias condições de doenças ou de alterações da homeostasia, poucos relatos são encontrados em relação à DCh.

Partindo do pressuposto de que as terminações nervosas são danificadas na infecção por *T. cruzi* e que uma possível recuperação gradual ocorre após a fase aguda da doença, um estudo em modelo murinho (Martinelli *et al.*, 2006) encontrou pela análise por ELISA um aumento significativo de NGF tanto no átrio quanto no ventrículo no vigésimo dia pós-infecção, momento no qual considera-se haver maior desnervação simpática. Nesse mesmo estudo, não foi observado aumento dos níveis de GDNF neste mesmo período. Esses dados sugerem que o NGF tem importante papel na regeneração subsequente à instalação da miocardite, com possível envolvimento na preservação das

pré-terminações nervosas, e que o GDNF pode ter menor ou transitória participação no processo (Martinelli *et al.*, 2006).

Níveis mais altos de BDNF circulante foram encontrados em indivíduos com DCh, conforme demonstrado por Martinelli e colaboradores (2011). Contudo, nos indivíduos que desenvolveram miocardiopatia dilatada os valores de BDNF foram menores em relação aos assintomáticos e àqueles sem dilatação, sugerindo que a denervação em função da doença estimula a produção desta neurotrofina por cardiomiócitos e que sua progressão para forma dilatada e possível acometimento por fibrose, diminuiriam o número destas células e por consequência a expressão de BDNF (Martinelli *et al.*, 2011). Entretanto, apesar desses achados apontarem para possíveis interações entre as NTs e as manifestações clínicas e alterações morfofisiológicas da DCh, mais estudos são necessários a fim de melhor elucidar esse processo.

1.6 Interações entre neurotrofinas, treinamento físico e Doença de Chagas

É bem estabelecido que o TF promove vários estímulos capazes de alterar o metabolismo e a funcionalidade do organismo em períodos relativamente curtos de treinamento, sendo capaz de mudar a expressão de genes envolvidos no aprimoramento da capacidade funcional. Por exemplo, o estudo pioneiro de Pilc *et al.* (2010) que demonstrou a possibilidade de biogênese mitocondrial em músculo esquelético estimulada por treinamento/atividade física regular (Pilc *et al.*, 2010).

Entretanto, são raros os relatos que associam o TF à expressão/concentração de NTs na DCh. Surpreendentemente, os estudos que o fazem são em sua maioria clínicos e elegem o BDNF como a NT a ser investigada. É o caso de um estudo que avaliou pacientes com a forma cardíaca da DCh submetidos a 12 semanas de treinamento físico

aeróbio. Nesse trabalho foi observada uma melhora no desempenho funcional dos pacientes, principalmente quando os níveis de BDFN pré-treinamento eram baixos e apresentavam uma função autonômica preservada (Lima *et al.*, 2012). Além disso, foi demonstrado que uma única sessão de exercício de intensidade moderada pode não ser um estímulo suficiente para alterar os níveis de BDNF em pacientes com DCh (Costa *et al.*, 2014). Nesse sentido, os níveis de BDNF em pacientes com miocardiopatia dilatada não foram alterados após uma única sessão de exercício máximo, contrastando com os baixos valores obtidos para os indivíduos sem dilatação (Costa *et al.*, 2014).

Dada a carência de dados que tentam esclarecer o mecanismo pelo qual o TF aeróbico de intensidade moderada pode influenciar a expressão e/ou concentração de NTs na presença da DCh, mais estudos são necessários para melhor elucidar essas interações, o que constitui a proposta chave do presente estudo.

1.7 Insuficiência cardíaca e sua associação com a depressão

Apesar do alto índice de associação entre as prevalências da insuficiência cardíaca e da depressão, duas doenças com fisiopatologias bem distintas, a possível interação entre estas permanece pouco elucidada, apesar dos esforços empenhados por estudos mais recentes.

A depressão é de 4 a 5 vezes mais frequente em pacientes com insuficiência cardíaca que na população em geral. Por outro lado, a sua presença pode implicar em um maior risco de desenvolver insuficiência cardíaca e/ou afetar negativamente o prognóstico da insuficiência cardíaca já estabelecida (Ghaemian *et al.*, 2006; Zahid *et al.*, 2018; Rutledge *et al.*, 2006; Lee *et al.*, 2018).

A teoria psicobiológica, que tenta explicar a ligação entre o encéfalo, cognição, emoção e respostas fisiológicas, sugere o estresse como base para o entendimento dos enigmas psicossomáticos. Nesse sentido, a resposta a fatores estressores, como uma doença, parece desempenhar um papel central na interface entre o cérebro, sentimentos, comportamento e efeitos biológicos (Chauvet-Gelinier and Bonin, 2017). A resposta ao estresse envolve a ativação central de sistemas cerebrais responsáveis pela avaliação do meio ambiente. Este mecanismo é importante, uma vez que é a responsável por manter a homeostase diante da exposição a estressores ambientais. Contudo, o estímulo crônico, sobretudo por estressores psicossociais, pode demandar resposta alostática exacerbada, gerando, assim, hiperativação do eixo hipotálamo-hipófise-adrenal e do SNA, o que pode levar ao aumento dos níveis pressóricos e de cortisol, além de estimular uma resposta imuno-inflamatória persistente (Chauvet-Gelinier and Bonin, 2017; Rigas *et al.*,2017). A manutenção desse fenômeno por longo período, seja por adversidade ambiental crônica ou por resposta fisiopatológica sustentada, pode resultar em distúrbios do metabolismo de glicose e lípides, síndrome metabólica e doença cardiovascular (Chauvet-Gelinier and Bonin, 2017). Desse modo, ainda que não completa e robustamente estabelecidas as vias compartilhadas entre as doenças, vinculam-se as manifestações fisiopatológicas da depressão à insuficiência cardíaca, destacando-se a necessidade de mais estudos.

1.8 Alteração dos níveis de neurotrofinas na depressão

Um crescente número de estudos tenta demonstrar a associação entre alterações na expressão de neurotrofinas e outras proteínas com a depressão, com atenção especial voltada ao BDNF (Chen *et al.*,2017; Notaras *et al.*,2017).

Nesse sentido, um estudo recente investigou as alterações na expressão de NF em função do polimorfismo do BDNF Val66Met em associação à susceptibilidade ao estresse e transtornos de afetividade. Neste estudo, animais geneticamente modificados para expressão de BDNF foram submetidos à avaliação comportamental pelo teste do nado forçado. O teste evidenciou um perfil comportamental depressivo em animais $hBDNF^{Met/Met}$ enquanto os camundongos $hBDNF^{Val/Val}$ apresentaram um fenótipo resiliente. Após avaliação comportamental, esses animais foram submetidos a exposição crônica a corticosterona e uma nova avaliação do perfil comportamental revelou a alteração de perfil nos animais $hBDNF^{Val/Val}$, onde estes apresentaram agora um padrão semelhante ao do grupo com comportamento do tipo depressivo ($hBDNF^{Met/Met}$). Alterações no metabolismo de moléculas ao longo do eixo corticohipocampal, como, por exemplo, a redução da expressão de tirosina hidroxilase, poderiam justificar essa mudança de fenótipo, uma vez que sua redução igualaria a expressão de BDNF entre os grupos (Notaras *et al.*,2017).

A redução dos níveis de BDNF também foi observada no hipocampo de ratos submetidos ao isolamento social (Zaletel *et al.,* 2017). Grande parte dos estudos consideram os tratamentos antidepressivos crônicos capazes de restaurar os níveis dessa neurotrofina, e para tanto, atrelam seu restabelecimento à melhora das respostas adrenérgicas e serotoninérgicas, ligadas à regulação neuroendócrina e ao metabolismo de glicocorticoides (Zaletel *et al.,* 2017).

Outro estudo aventou como hipótese que os níveis de NFs poderiam estar alterados em diferentes proporções em função da classificação clínica do quadro depressivo, apontando uma possível ação regulatória da melatonina na expressão das mesmas. Entretanto, apesar das diferenças encontradas nos níveis de melatonina nos grupos com depressão severa e naqueles com depressão leve e moderada, os níveis de

NGF, BDNF e outras NFs foram igualmente diminuídos em todos os indivíduos (Ogłodek *et al.,* 2016). Achados como esses reforçam que a alteração do padrão de expressão de NFs na depressão pode ocorrer em todos os níveis de acometimento.

1.9 Interações entre neurotrofinas, treinamento físico e depressão

Como demonstrado anteriormente, o exercício físico é considerado um estímulo capaz de alterar o padrão de expressão das neurotrofinas. Entretanto, o padrão heterogêneo dos estudos conduzidos até o momento, associando exercício e depressão, contribui pouco para o entendimento dos mecanismos envolvidos nessa associação e na influência deste sobre a depressão.

Não há dúvidas quanto o interesse crescente no uso de exercícios no tratamento da depressão. De maneira geral, a redução nos sintomas depressivos com intervenções de exercícios aeróbicos e não aeróbicos já foi verificada e apoiada por revisões sistemáticas. Em humanos, essas revisões dão conta de evidências para o uso do exercício aeróbico de intensidade moderada, realizado pelo menos três vezes por semana por no mínimo nove semanas, atuando na redução das manifestações clínicas da depressão (Stanton *et al.,* 2014).

Algumas hipóteses aventadas permeiam vias inflamatórias e neuromodulação, como anteriormente mencionado. Nesse sentido, níveis alterados do fator de necrose tumoral (TNF) foram associados ao comprometimento cognitivo na depressão e outras doenças como esquizofrenia e transtorno bipolar (Morgan *et al.*,2018). Por outro lado, é atribuído ao exercício a melhora da cognição e redução da expressão de TNFα via sinalização dos receptores TNFR1 e TNFR2 que parecem mediar os efeitos do exercício na cognição (Morgan *et al.*,2018). É possível que o comportamento depressivo

compartilhe a totalidade ou parte dessa via, porém o potencial do exercício para regular a sinalização e concentração de TNF em humanos, assim como NFs, demanda ainda de maiores investigações.

2. OBJETIVOS

2.1 Objetivo Geral

Avaliar a influência do treinamento físico aeróbio e da infecção chagásica em sua fase crônica sobre a função e morfologia cardíaca, tolerância ao esforço, níveis de neurotrofinas e comportamento do tipo depressivo.

2.2 Objetivos específicos

- Avaliar os efeitos do treinamento físico aeróbio e da infecção pela cepa Y do *Trypanosoma cruzi* sobre a função cardíaca;

- Avaliar os efeitos do treinamento físico aeróbio e da infecção sobre a tolerância ao esforço;

- Avaliar os efeitos do treinamento físico aeróbio e/ou da infecção sobre a concentração de neurotrofinas no plasma, baço e coração, córtex, hipocampo e estriado;

- Avaliar a morfologia cardíaca pelas variáveis ecocardiográficas e morfométricas em função da infecção e do treinamento físico aeróbio;

- Avaliar o comportamento do tipo depressivo em função da infecção e/ou treinamento físico aeróbio utilizando o teste do nado forçado;

Physical training improves exercise tolerance, cardiac function and promotes changes in neurotrophin levels in chagasic mice

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ABSTRACT

Aims: To investigate the effects of moderate aerobic physical training on cardiac function and morphology as well as on the levels of glial cell-derived neurotrophic factor (GDNF), nerve growth factor (NGF) and brain derived neurotrophic factor (BDNF) of animals infected with the Y strain of *Trypanosoma cruzi*. **Main methods:** Twenty-eight male C57BL/6 mice were distributed into 4 groups: sedentary control (SC), trained control (TC), sedentary infected (CHC) and trained infected (CHT). The infection was performed by intraperitoneal injection of trypomastigote forms and the animals were adapted to treadmill in the week before the beginning of the training protocol, initiated 45 days post infection. On the last day of adaptation, as well as at the end of the 4^{th}, 8^{th} and 12^{th} weeks of training, the maximal exercise test (TEM) was performed to establish the adjustments in training speed. At the end of the 12^{th} week, all animals were evaluated for cardiac morphology and function by echocardiography. **Key findings:** CHC group showed a larger area of right ventricle and increased end-systolic volume, as well as reduction in ejection fraction, stroke volume, cardiac output and fractional area change. GDNF level was higher in TC and CHC groups compared to SC in heart and BDNF levels were higher in CHC compared to SC in heart and serum. **Significance:** Physical training ameliorated the cardiac function of infected animals and promotes adjusts in BDNF and GDNF levels suggesting it as possible markers of cardiac dysfunction responsive to exercise stimulus.

Key words: Chagas' disease, Chagasic cardiomyopathy, Exercise training, Cardiac function, Glial cell-derived neurotrophic factor (GDNF), Nerve growth factor (NGF), Brain derived neurotrophic factor (BDNF), Neurotrophins.

Introduction

Chagas' disease (CD) is caused by infection with *Trypanosoma cruzi* trypomastigotes forms[1] and remains as an endemic illness in Latin America. The prevalence of CD is around 8 million worldwide[2] and progressively more number of cases was found in countries previously considered free of this disease.[3-5] It has been considered a major cause of cardiac infectious disease in endemic countries[2] and represents one of the most important health problems with high costs of treatment.

The clinical course of CD is composed by an acute phase, which is in general asymptomatic, characterized by high level of parasitemia.[6] Frequently, people who survived to this phase proceed to the chronic phase in its indeterminate form and could stay on it for several years without clinical manifestations.[7] From this point, infected subjects may develop the cardiac, digestive or both of these forms as clinical manifestation.[6,8,9,10]

Previous studies show that 20% to 30% of infected individuals from endemic areas develop some cardiac damage during the infection.[6,11,12] The most important clinical finding of CD is the chronic chagasic cardiomyopathy[10,13] with progressive impairment of cardiac function. [6]

The cardiac function impairment is a result of multifactorial events which change organs and tissues homeostasis, besides affecting the functional capacity and exercise tolerance. However, it remains unclear how *T. cruzi* infection leads to this scenario.[14] Although the reduction in exercise tolerance observed in CD, physical exercise is recognized as a powerful and beneficial stimulus for cardiovascular remodeling[15], acting as a hypotensive agent [16], which may help to poise or handle the progress of the disease.

Moreover, neurotrophins have been associated with many health problems such as coronary artery disease,[17] myocardial infarction,[18] metabolic syndrome, obesity and diabetes[19] and as peripheral markers of metabolic indexes and cardiovascular conditions.[20] Previous investigations reported that atrial and ventricular levels of nerve growth factor (NGF) significantly increase at the day 20 post inoculation with trypomastigotes forms in rats. In contrast, at the same time point, glial cell line-derived neurotrophic factor (GDNF) did not change, suggesting that NGF plays an important role in the regenerative phenomenon subsequent to a myocarditis and GDNF could have a minor or a more transient participation.[21]

Brain derived neurotrophic factor (BDNF) is also investigated as possible marker of cardiac health in clinical studies of CD. These studies suggested that BDNF levels increase in subjects with CD compared with healthy ones[22] and the improvement of functional capacity after twelve weeks of physical exercise is related to lower levels of BDNF at the baseline.[23]

However, it remains unclear how exercise could affect the expression and/or concentration of neurotrophins in CD, as well as how it could be associated to cardiac function in animal models. Thus, in order to clarify these interactions, here we evaluated the cardiac function and neurothophins levels in hearts and spleens of infected mice after twelve weeks of moderate physical exercise.

Materials and Methods

Ethics Statement

This study was performed in strict accordance with recommendations of the guide for care and use of laboratory animals of the Brazilian National Council of Animal Experimentation and all experiments and procedures were approved by the ethic committee at Federal University of Minas Gerais CEUA/UFMG (protocol number

300/2015). Animals were obtained from CEBIO/UFMG, maintained under pathogen-free conditions, housed in standard clear plastic cages, and kept at 23 ºC with free access to food and water and a 12h light/dark cycle.

Animal Groups and Infection

Twenty-eight C57BL/6 male mice were randomized into four groups: sedentary control (SC; n=7); trained control (TC; n=7); chagasic sedentary control (CHC; n=7); and chagasic trained (CHT; n=7). The Y strain of *T. cruzi* was maintained *in vivo* and used to infect animals with 7-8 weeks of age by the intraperitoneal route with $1x10^3$ trypomastigote forms. Control groups received the same final volume of phosphate-buffered saline (PBS, pH 7.2). The parasitemia was determined from the seventh to the fifteenth day post inoculation on alternate days using 5 µL of blood collected in a tail vein.

Measurement of Exercise Tolerance

Forty-five days after inoculation and at the end of 4^{th}, 8^{th} and 12^{th} weeks of training, all animals were evaluated for exercise tolerance using a maximal exercise test (TEM). The mice were familiarized with a motor-driven treadmill (Goustec®) one week before the first TEM by running at the speed of 8m/min at 5% inclination, 10min/day for 5 consecutive days. The TEM was performed at a constant slope of 5% with the starting speed at 3m/min, and adjusting the velocity in 3m/min every 3 minutes until the mice fatigue, defined as the point on which the animals were no longer able to keep the rhythm with treadmill. The maximum speed reached was used to measure the velocity of training for the following four weeks using 70% of it until the new TEM. The workload (W, in Joules) was calculated multiplying the mice body weight (kg) by the time (minutes) and speed (m/min) for each step reached by the sine θ of treadmill

slope.[24] At the end of twelve weeks of exercise training and before euthanasia, the cardiac function was evaluated by echocardiography.

Cardiac Function Measurements

Cardiac function was evaluated by echocardiography. Animals were anesthetized with isoflurane (2%) in a flow of 1L/min of oxygen and the precordium area was trichotomized. The equipment Vevo 770 (Visual Sonics®, Canada) and a 30MHz transducer were used to record cardiac function data. This equipment was designed for small rodents and shows high quality to assess minor structures. Cardiac geometry was assessed in 2-dimensional mode. The area of the left and right ventricle at the end of diastole was measured in cross-section at the papillary muscles level. The systolic function of the left ventricle was evaluated by ejection fraction calculated according to the Simpson's method, which is able to determine the volume of the left ventricle. Automatically, the software offers values of end-diastolic and end-systolic volumes, as well as the stroke volume and fractional area changes. Animals were evaluated forty-eight hours after the last TEM.

Morphometric Analysis

The euthanasia was performed using intraperitoneal injection of 150 mg/kg BW of thiopental and verified by the absence of the tail reflex. The heart was removed and weighed. The left ventricle was dissected and a fragment was used for histological analysis. The tissue was fixed by immersion in 4% (v/v) glutaraldehyde and 0.05M phosphate buffer. After fixation, cardiac tissue was processed and embedded in plastic (glycolmethacrylate), as described elsewhere.[25] Sections with 4µm thickness were obtained and the distance between these sections was superior to 12µm. The tissue was stained by Toluidine blue 1% sodium borate and mounted on histological slides. The slides were visualized in a light microscope (Olympus BX-53®; Olympus Tokyo, Japan)

and images captured using an objective of 40x by a digital camera (Olympus QColor - 5®, U-CMAD3; Olympus, Tokyo, Japan) connected to it. Fifteen fields from each slide were randomly chosen and used to analyze the diameter of cardiomyocytes nuclei and volume densities of cells of the left ventricle. All these measures were done by using the ImageJ software. The diameter of cardiomyocytes was obtained by the average length of the longer and shorter axis in the nuclei of thirty cells of each animal. The volumetric proportions of cardiac tissue cellular components were determined by a 520 intersections grid placed over each field. Artifacts were not considered in the total number of points used to obtain the volumetric proportion. The components considered were fibroblasts and cardiomyocytes nucleus and its cytoplasm. We used these data to calculate the ratio between cardiomyocytes and fibroblasts, as well as to analyze the cardiomyocytes volume nuclei by using the volume formula of the sphere: $V = 4/3\pi r^3$.

Neurotrophins Levels

Neurotrophins levels were assessed by enzyme-linked immunosorbent assay (ELISA). GDNF and NGF levels were measured in hearts and spleens and BDNF was also measured in serum, according to manufacturer's protocols (R&D Systems, Minneapolis, MN, USA). The concentrations were determined in a spectrophotometer (Spectramax 190) at a 450nm wavelength.

Statistical Analysis

Values are expressed as mean ± SEM and the data were analyzed using one-way or two-way ANOVA followed by the Bonferroni post-test using the software GraphPad Prism (version 5; Graph-Pad Software Inc., San Diego, CA, USA).

Results

There were no significant changes in parasitemia between Y strain infected groups (Fig. 1) comparing the same period from 7th to 15th day post-infection.

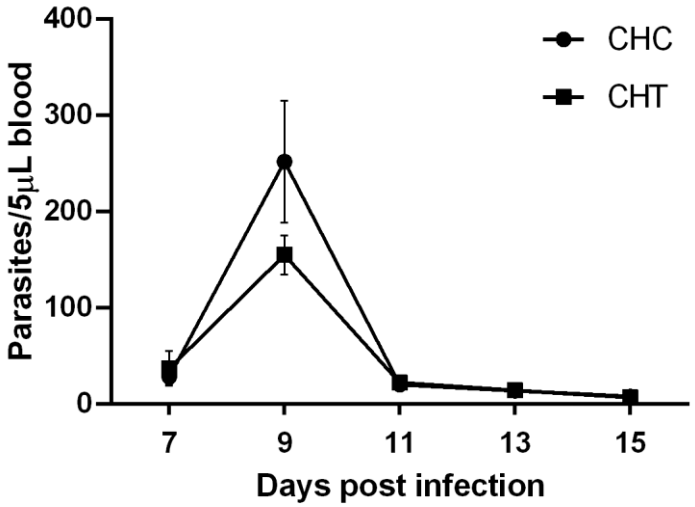

Figure 1 - Parasitemia of mice infected with Y strain of *T. cruzi*. Natural course of *T. cruzi* infection, as measured by parasitemia, of chagasic sedentary animals (CHC, n=6) and chagasic trained mice (CHT, n=7) infected with 1000 trypomastigotes of *T. cruzi*. The data is shown as mean ± SEM of the number of parasites per microliter of blood. Two-way ANOVA followed by the Bonferroni post-test.

The exercise tolerance was assessed by TEM at baseline (TEM 0) and at the end of weeks 4 (TEM 1), 8 (TEM 2) and 12 (TEM 3) and for each period we calculated the Work (W; Joules; Fig. 2). The W was higher in all TEM of trained groups compared with sedentary mice ($p<0.0001$). No significant alterations were found when comparing the groups SC and CHC or TC and CHT, except on TEM 2 when the TEM of CHT was lower compared with TC ($p=0.0416$). In addition, comparing the groups through the all tests, trained groups improved their performance from TEM 0 to TEM 1 in both groups (1.762 J *vs.* 2.792 J in TC and 1.614 J *vs.* 2.372 J in CHT; $p<0.0001$); from TEM 1 to TEM 2 in TC (2.372 J *vs.* 3.174 J; $p=0.0081$) and from TEM 2 to TEM 3 in CHT (2.661 J *vs.* 3.175 J; $p=0.0002$). We did not find any significant difference comparing sedentary groups through the time.

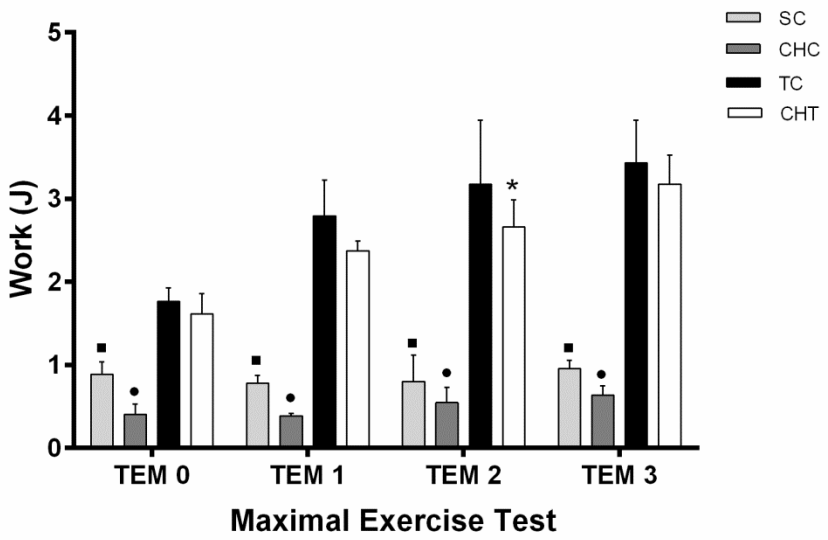

Figure 2 –Maximal exercise tests (TEM) from the baseline (TEM 0) to the end of the 12th week (TEM 3) of training. W (Joules); TC (trained control); CHT (chagasic trained); CHC (chagasic control); and SC (sedentary control). Data are expressed as mean ± SEM ($p<0.05$). Symbols denote statistical differences between groups CHC and CHT (●); TC and SC (■); and TC and CHT (*). One-way ANOVA followed by the Bonferroni post-test.

The cardiac geometry was measured by echocardiography (Fig. 3). The right ventricular area (RVA) was larger in CHC group compared to CHT ($p=0.0004$) and SC ($p<0.0001$). No significant differences in left ventricular area (LVA) were found (Figs. 3A and 3B).

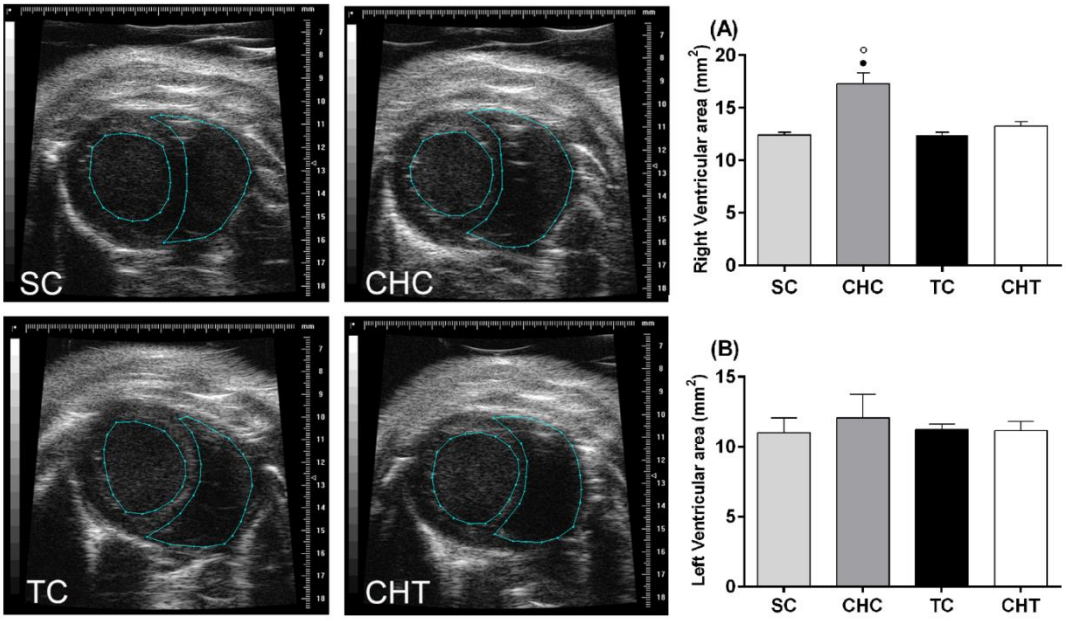

Figure 3 – Representative echocardiographic images of cardiac area of (A) right and (B) left ventricles. TC (trained control); CHT (chagasic trained); CHC (chagasic control); and SC (sedentary control). Data are expressed as mean ± SEM ($p<0.05$). Symbols denote statistical differences between CHC and CHT (●) and SC and CHC (◦). One-way ANOVA followed by the Bonferroni post-test.

The cardiac function measured by echocardiography showed higher ejection fraction in CHT and SC groups compared to CHC, as well as it was reduced when the CHT group was compared to TC (Fig. 4A). The stroke volume and cardiac output also were lower in CHC group compared to SC (Figs. 4B and 4C). No significant differences among the groups were observed in terms of end-diastolic volume (Fig. 4D). However, higher end-systolic volume was found in CHC when compared to SC (Fig. 4E). Fractional area change was higher in CHT and SC compared to CHC (Fig. 4F) and TC group showed lower left ventricular internal diameter and volume compared to SC (Figs. 4G and 4H).

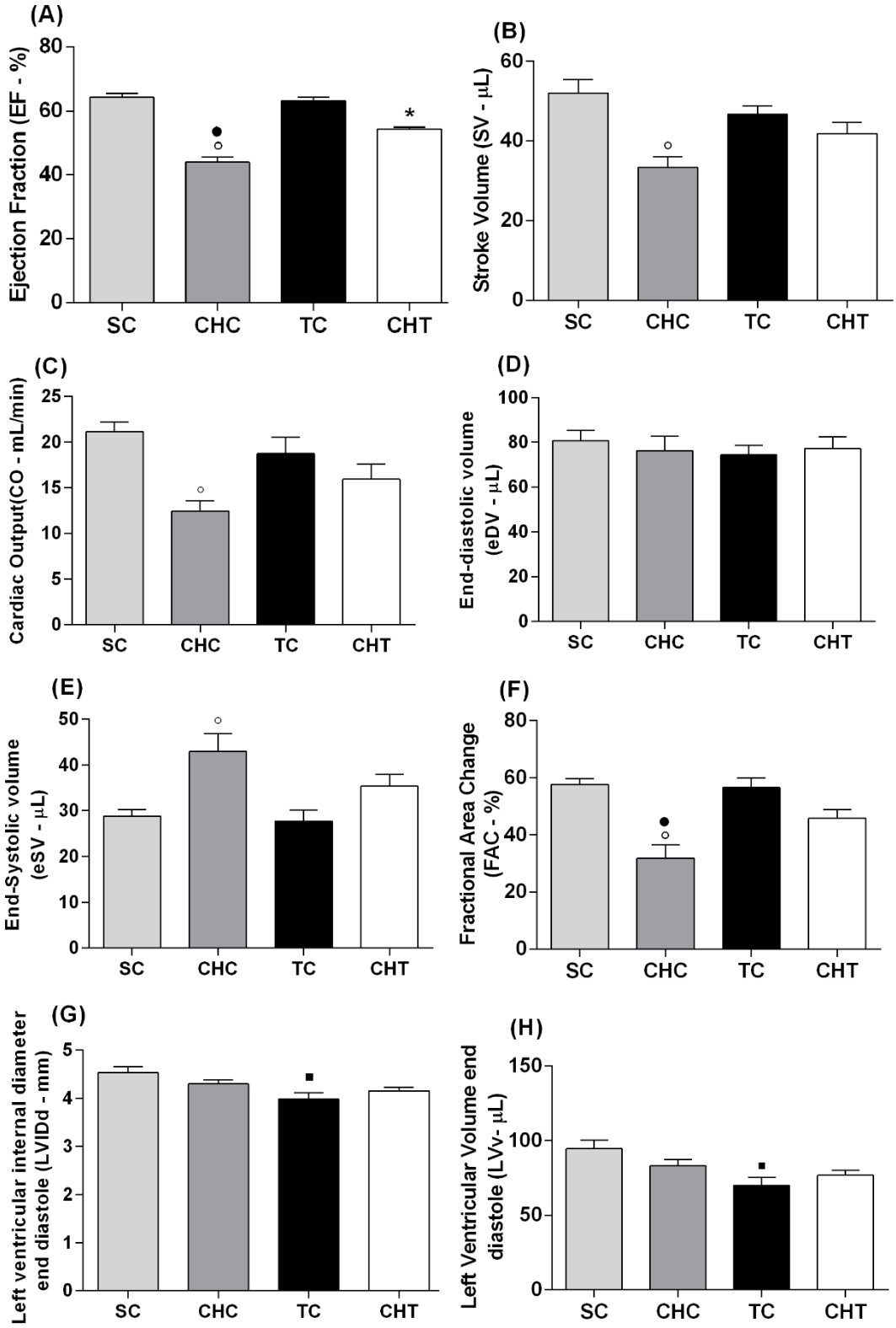

Figure 4 – Cardiac function and morphological measurements. TC (trained control); CHT (chagasic trained); CHC (chagasic control); and SC (sedentary control). Data are expressed as mean ± SEM ($p<0.05$). Symbols denote statistical differences between CHC and CHT (●); TC and SC (■); SC and CHC (◦); TC and CHT (*). One-way ANOVA followed by the Bonferroni post-test.

GDNF level was higher in TC and CHC group compared to SC in heart tissue while lower values for this neurotrophin were found in CHT and SC compared to CHC, and higher value in TC compared to CHT and SC in spleen samples (Fig. 5A). Regarding to NGF levels, in both tissues, TC group showed higher values compared to SC. It was also higher in spleen of CHC mice when compared to SC group (Fig. 5B).

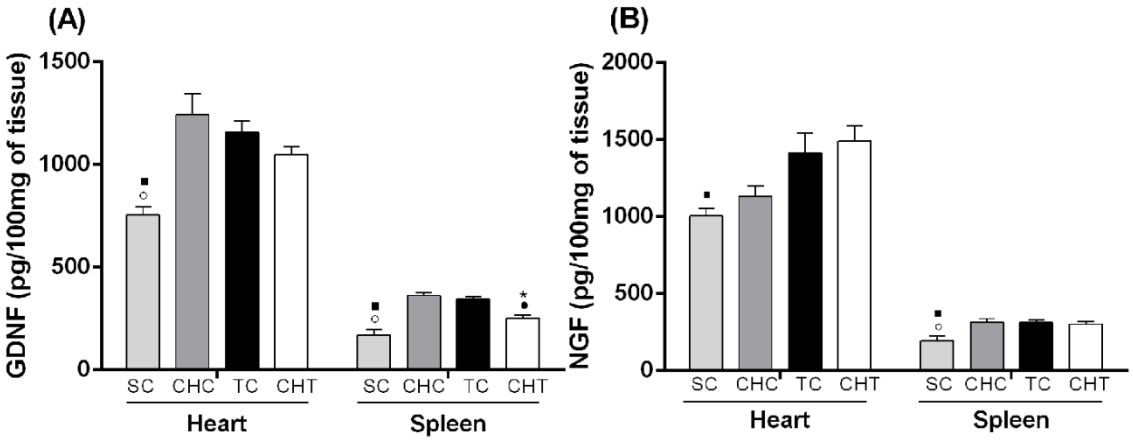

Figure 5– Glial cell line-derived neurotrophic factor (GDNF) and nerve growth factor (NGF) levels in heart and spleen. TC (trained control); CHT (chagasic trained); CHC (chagasic control); and SC (sedentary control). Data are expressed as mean ± SEM ($p<0.05$). Symbols denote statistical differences between CHC and CHT (●); TC and SC (■); SC and CHC (◦); TC and CHT (*). One-way ANOVA followed by the Bonferroni post-test.

Moreover, BDNF levels were higher in CHC compared to SC in heart (Fig. 6A) and serum (Fig. 6B).

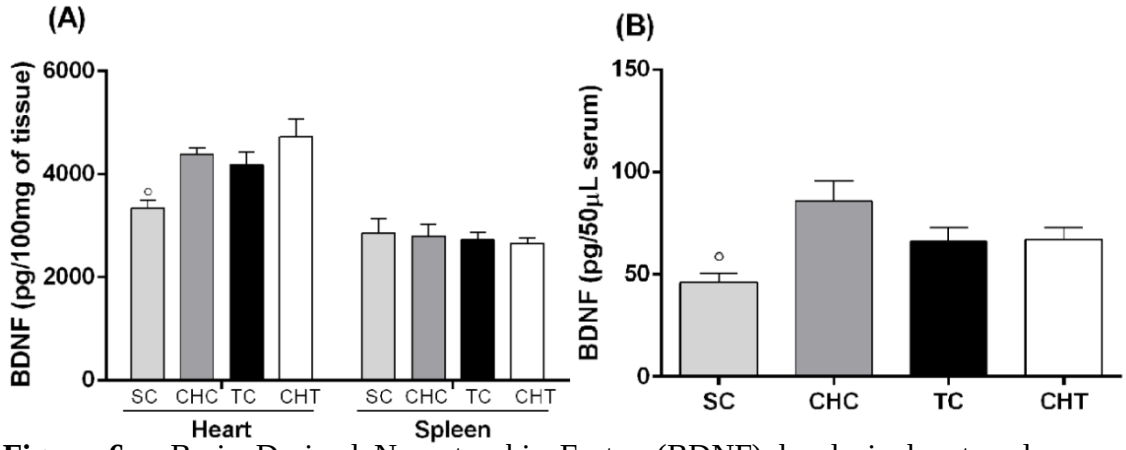

Figure 6 – Brain Derived Neurotrophic Factor (BDNF) levels in heart, spleen and serum. TC (trained control); CHT (chagasic trained); CHC (chagasic control); and SC (sedentary control). Data are expressed as mean ± SEM ($p<0.05$). Symbol denotes statistical differences between SC and CHC (◦). One-way ANOVA followed by the Bonferroni post-test.

Morphometric analyses did not show significant changes among the groups in terms of volume of cardiomyocytes nucleus and ratio between nucleus proportions of cardiomyocytes and fibroblasts (Fig. 7).

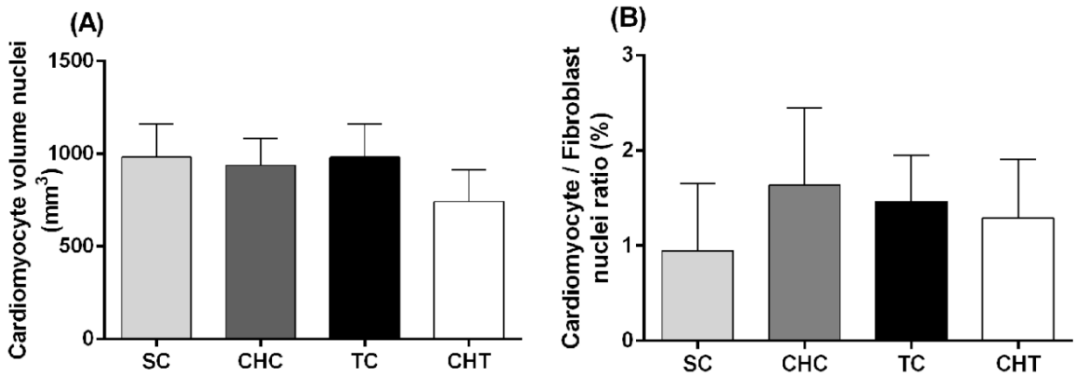

Figure 7 – Morphometric analysis. (A) Cardiomyocyte volume nuclei in mm^3 and nuclei ratio between cardiomyocytes and fibroblasts nucleus (B). TC (trained control); CHT (chagasic trained); CHC (chagasic control); and SC (sedentary control). Data are expressed as mean ± SEM. One-way ANOVA followed by the Bonferroni post-test.

Discussion

These results confirmed that *T. cruzi* infection is able to impair the exercise tolerance and cardiac function, since twelve weeks of moderate exercise training was able to retard the progress of disease, especially keeping the RVA parameters closer to those of non-infected animals and greater FAC in CHT. Furthermore, neurotrophins levels, particularly in terms of BDNF in serum and heart, were higher in CHC.

The parasitemia showed an expected behavior for Y strain similar to other studies.[26] No significant differences in parasitemia between infected groups were viewed, showing that changes in parasitemia were not the reason of the findings observed in other parameters.

Exercise tolerance was measured by TEM and it showed a decrease of the performance in CHC compared to trained mice, as well as between SC and trained animals in all evaluations. These data show that the training protocol was able to

promote gain in exercise tolerance in trained groups, as corroborated by previous studies in rats infected with Y strain.[14,27,28] Despite of differences between trained groups in TEM 2, we did not find significant differences between these groups in TEM 3, indicating that CHT was able to reach similar exercise tolerance of TC. The absence of differences in work between sedentary groups can be explained based on systolic function, which although presenting lower values in CHC, was sufficient to answer to exercise stress similarly to SC.

The larger RVA found in CHC group is in accordance with previous studies using different experimental models[29,30] suggesting the primary involvement of the right ventricle in CD. Many reports observed changes in the physiology of the right ventricle and support the idea that it plays an important role in the development of chagasic cardiomyopathy.[29] Some studies reported right branch block, dilated right ventricular chamber, compromised right ventricular myocyte contractility, and thinning of the right ventricular wall even with the absence of damage in the left ventricle.[31] Others suggested that the right ventricular systolic dysfunction is more commonly associated with the left ventricular systolic dysfunction, although isolated and early right ventricular dysfunction can also be identified.[32] Moreover, the cardiomyopathy progression of CD may depend on the genetic background of the host and/or strain.[33]

We did not find significant differences in the LVA but the systolic function of the left ventricle was affected in infected animals. Our findings suggest that at the end of twelve weeks, besides training, the impairment occurs in both ventricles. However, since we only assessed the cardiac function at the end of twelve weeks, it is possible that RVA was separately affected at preceding periods.

FAC is a measurement that provides an estimate of the global systolic function using chamber area variation within the left ventricle between diastole and systole. The

impairment of myocardial contractility evidenced by lower values of FAC in CHC compared to SC and CHT, may explain the lower CO and SV in CHC when compared to SC, as well as the EF, which showed better values for CHT compared to CHC. It is also suggested by the higher values of the end-systolic volume in CHC compared to SC. Others interesting results were the absence of differences between CHC and CHT in the end-systolic volume and the impairment of EF in CHT compared to TC. Together, this could indicate an initial damage in systolic function of CHT group not enough to reduce the exercise tolerance once we did not observe significant differences between CHT and TC in the TEM 3.

A large amount of clinical studies have been reported the phenotype of cardiac function impairment which shows higher prevalence of only LV or biventricular involvement, not evidencing the presence of isolated right ventricular involvement.[31] Our data showed that CHC presented a larger RVA and systolic deficit, as well as lower EF, while no differences in RVA were observed in CHT group. However, greater dilation of the RV was associated with larger diastolic and systolic diameters of the LV [31], but, in our study, we did not find differences in end-diastolic diameter of LV between infected animals. On the other hand, end-diastolic diameter of LV was different in SC and TC groups, as well as the end-diastolic volume. These differences between non-chagasic groups could indicate a consequence of the physical training on cardiac remodeling and maybe they were more evident in these animals that only faced the adaptive stress of exercise without pathological challenges.

Based on previous studies[22,34], BDNF levels could be changed by infection and may have its levels adjusted by exercise stimulus, so accepting this neurotrophin as a potential protector against cardiac dysfunction.[18] These studies showed that BDNF levels were greater in chagasic subjects compared to non-chagasics, as well as it was

lower in subjects with chagasic dilated cardiomyopathy compared to non-dilated and asymptomatic ones.[22] It suggests that the preservation of cardiac autonomic response is responsible for the greater level of this neurotrophin. It was also confirmed by another investigation that showed better correlation between lower BDNF levels before twelve weeks of aerobic training and the improvement of exercise tolerance after this period mainly when autonomic function was better preserved.[23]

No differences between pre and post one maximal exercise session were evidenced for BDNF in chagasic dilated cardiomyopathy and most subject in this group reached moderate intensity effort. In contrast, non-dilated subjects showed significant decrease in serum BDNF levels and all of them reached high intensity during the session.[34] Thus, it suggests that BDNF levels could be affected by the intensity of the exercise stimulus and that a single session of moderate exercise may not affect its levels.

The higher values found for BDNF in heart and serum in CHC, supports this neurotrophin as a possible marker and protector of cardiac dysfunction in a condition where exercises were not considered. No difference was found between TC and SC, which suggest that twelve weeks of moderate aerobic exercise training may not be enough to change significantly BDNF levels. Taking together the absence of differences in its levels between CHC and CHT and the greater global systolic function in CHT, we can accept that exercise develops an important role adjusting BDNF levels and leading to a higher cardiac tolerance threshold in trained group.

GDNF and NGF expression profiles were investigated in the acute phase of CD. Atrial and ventricular NGF levels increased significantly at the time-point of maximal sympathetic denervation and GDNF levels did not change, indicating an important role for NGF in the regenerative phenomenon subsequent to a myocarditis able to damage

sympathetic nerve endings, while GDNF could have a minor participation on this phase.[21] In our study, NGF levels were not different between chagasic groups at the end of twelve weeks of training. As suggested by previous studies, NGF could develop an important role in acute phase of CD and, even in the presence of training, it was not able to change the levels of this neurotrophin in infected animals in the chronic phase. An explanation for that could be the initial damage in autonomic function in acute phase. As showed by the parasitemia, no differences were found between infected groups, suggesting a similar initial impairment, which could have compromised the response to the exercise. However, NGF level was higher in TC compared to SC in heart and spleen, suggesting that exercise can modulate NGF levels in healthy conditions, probably promoting autonomic adjusts.

The greatest values of GDNF observed in heart and spleen of TC and CHC suggests a role of this neurotrophin in chronic phase and its capacity to answer to exercise stimulus. Also, in spleen, GDNF level was lower in CHT compared to TC and CHC which may suggest that exercise plays an important role up regulating GDNF concentration. On the other hand, in healthy conditions, the higher values found in TC may suggest the better capacity to answer to exercise stimulus in absence of CD and in a scenery where exercise were not considered, the greater values found for CHC may identify a natural response to infection in an attempt to keep cardiac function closely to normal parameters. These results can support GDNF as a possible protector and marker of cardiac function, sensitive to exercise stimulus, placing it by BDNF side.

We did not find any significant changes in morphometric analysis among groups. These results are opposite to previous studies[22] which suggested increases in cardiomyocytes nuclear volume as a possible explanation to higher values of BDNF in CD. We believe that this phenomenon may occurs in the context of an extensive cardiac

function impairment, either by the longer time of disease progression or by strains that have a predilection for cardiac tissue.

To the best of our knowledge, this was the first study that investigated the effects of twelve weeks of exercise training on cardiac function and neurotrophins levels in a chronic experimental model of CD. However, more studies should be considered to better clarify the interaction among infection, neurotrophins levels, cardiac function and exercise tolerance. Adaptations as the time point to beginning the exercise training protocol, the tropism strain, autonomic function evaluation and investigation of neurotrophins pathways in response to exercise stimulus should be considered in future studies.

Conclusion

Twelve weeks of moderate aerobic physical training improved the exercise tolerance and reduced the cardiac function impairment of infected animals mainly keeping better values of RVA and FAC and promoting better balance in neurotrophins levels, especially BDNF and GDNF in the chronic phase. Changes in neurotrophins levels confirmed previous data obtained in clinical studies and suggest these molecules as possible markers of cardiac dysfunction, able to change their expression by exercise stimulus.

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Conflict of interest

The authors declare that there are no conflicts of interest.

REFERENCES

1. Chagas C, Chagas C. Nova tripanozomiaze humana: estudos sobre a morfolojia e o ciclo evolutivo do Schizotrypanum cruzi n. gen., n. sp., ajente etiolojico de nova entidade morbida do homem. Mem Inst Oswaldo Cruz [Internet]. 1909 Aug [cited 2018 Apr 3];1(2):159–218. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0074-02761909000200008&lng=pt&tlng=pt

2. Global Report for Research on Infectious Diseases of Poverty [Internet]. 2012. Available from: www.who.int/tdr/publications/environment

3. Jackson Y, Pinto A, Pett S. Chagas disease in Australia and New Zealand: risks and needs for public health interventions. Trop Med Int Heal [Internet]. 2014 Feb [cited 2018 Apr 4];19(2):212–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24299280

4. Pinto A, Pett S, Jackson Y. Identifying Chagas disease in Australia: an emerging challenge for general practitioners. Aust Fam Physician [Internet]. 2014 Jul [cited 2018 Mar 28];43(7):440–2. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25006603

5. Imai K, Maeda T, Sayama Y, Osa M, Mikita K, Kurane I, et al. Chronic Chagas disease with advanced cardiac complications in Japan: Case report and literature review. Parasitol Int [Internet]. 2015 Oct [cited 2018 Apr 4];64(5):240–2. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25744336

6. Coura JR. Chagas disease: what is known and what is needed - A background article. Mem Inst Oswaldo Cruz [Internet]. 2007 Oct [cited 2018 Apr 4];102(suppl 1):113–22. Available from:

http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0074-02762007000900018&lng=en&tlng=en

7. Rassi A, Marin-Neto JA. Cardiopatia Chagásica Crônica. Vol. 4, Rev Soc Cardiol Estado de São Paulo. 2000. vi-xxxii.

8. Rassi Jr A, Rassi A, Marin-Neto JA. Chagas heart disease: pathophysiologic mechanisms, prognostic factors and risk stratification. Mem Inst Oswaldo Cruz [Internet]. 2009 Jul [cited 2018 Mar 28];104(suppl 1):152–8. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0074-02762009000900021&lng=en&tlng=en

9. Simões MV, Almeida Filho OC de, Pazin-Filho A, Castro RBP de, Schmidt A, Maciel BC, et al. Insuficiência cardíaca na doença de chagas. Rev da Soc Cardiol do Estado São Paulo [Internet]. 2000 [cited 2018 Mar 28];10(1):50–64. Available from: https://bdpi.usp.br/item/001173641

10. Marin-Neto JA, Cunha-Neto E, Maciel BC, Simões M V. Pathogenesis of Chronic Chagas Heart Disease. Circulation [Internet]. 2007 Mar 6 [cited 2018 Apr 4];115(9):1109–23. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17339569

11. DIAS JCO. Doença de Chagas em Bambuí, Minas Gerais, Brasil: Estudo clínicoepidemiológico a partir da fase aguda entre 1940 e 1982 [PhD Thesis]. Belo Horizonte: Universidade Federal de Minas Gerais; 1982. 376pp.

12. Storino R, Milei J. Estudios de seguimiento evolutivo de la enfermedad de Chagas. In: Madoery RJ, Madoery C, Cámera MI (eds). Actualizaciones en la enfermedad de Chagas, Córdoba, p. 67-77, 1993.

13. Rassi A, Rassi A, Marin-Neto JA. Chagas disease. Lancet [Internet]. 2010 Apr 17 [cited 2018 Mar 28];375(9723):1388–402. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20399979

14. Novaes RD, Penitente AR, Gonçalves R V, Talvani A, Neves CA, Maldonado IRSC, et al. Effects of Trypanosoma cruzi infection on myocardial morphology, single cardiomyocyte contractile function and exercise tolerance in rats. Int J Exp Pathol [Internet]. 2011 Oct [cited 2018 Apr 20];92(5):299–307. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21736646

15. Morvan E, Lima NEA, Machi JF, Mostarda C, De Angelis K, Irigoyen MC, et al. Metabolic, hemodynamic and structural adjustments to low intensity exercise training in a metabolic syndrome model. Cardiovasc Diabetol [Internet]. 2013 Jun 18 [cited 2018 Apr 20];12:89. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23777435

16. Lucchetti BFC, Zanluqui NG, de Ataides Raquel H, Lovo-Martins MI, Tatakihara VLH, de Oliveira Belém M, et al. Moderate Treadmill Exercise Training Improves Cardiovascular and Nitrergic Response and Resistance to Trypanosoma cruzi Infection in Mice. Front Physiol [Internet]. 2017 May 18 [cited 2018 Apr 20];8:315. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28572772

17. Ejiri J, Inoue N, Kobayashi S, Shiraki R, Otsui K, Honjo T, et al. Possible Role of Brain-Derived Neurotrophic Factor in the Pathogenesis of Coronary Artery Disease. Circulation [Internet]. 2005 Oct 4 [cited 2018 Apr 20];112(14):2114–20. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16186425

18. Okada S, Yokoyama M, Toko H, Tateno K, Moriya J, Shimizu I, et al. Brain-Derived Neurotrophic Factor Protects Against Cardiac Dysfunction After Myocardial Infarction via a Central Nervous System–Mediated Pathway. Arterioscler Thromb Vasc Biol [Internet]. 2012 Aug [cited 2018 Mar 28];32(8):1902–9. Available from: https://www.ahajournals.org/doi/10.1161/ATVBAHA.112.248930

19. Lee SS, Yoo JH, Kang S, Woo JH, Shin KO, Kim KB, et al. The Effects of 12 Weeks Regular Aerobic Exercise on Brain-derivedNeurotrophic Factor and Inflammatory Factors in Juvenile Obesity and Type 2 DiabetesMellitus. J Phys Ther Sci [Internet]. 2014 [cited 2018 Apr 20];26(8):1199. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4155219/

20. Golden E, Emiliano A, Maudsley S, Windham BG, Carlson OD, Egan JM, et al. Circulating Brain-Derived Neurotrophic Factor and Indices of Metabolic and Cardiovascular Health: Data from the Baltimore Longitudinal Study of Aging. Gaetani S, editor. PLoS One [Internet]. 2010 Apr 9 [cited 2018 Apr 20];5(4):e10099. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20404913

21. Martinelli PM, Camargos ER da S, Azevedo AA, Chiari E, Morel G, Machado CR da S. Cardiac NGF and GDNF expression during Trypanosoma cruzi infection in rats. Auton Neurosci [Internet]. 2006 Dec 30 [cited 2018 Apr 20];130(1–2):32–40. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16854632

22. Martinelli PM, da Costa Rocha MO, Teixeira AL, do Carmo Pereira Nunes M, da Silva Camargos ER. Brain-derived neurotrophic factor is up regulated in

chronic Chagas disease. Int J Cardiol [Internet]. 2011 Jun 2 [cited 2018 Apr 20];149(2):277–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21420187

23. Lima MMO, Nunes MCP, Nascimento B, Costa HS, Sousa LAP, Teixeira AL, et al. Improvement of the functional capacity is associated with BDNF and autonomic modulation in Chagas disease. Int J Cardiol [Internet]. 2013 Sep 1 [cited 2018 Apr 20];167(5):2363–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23232458

24. Balthazar CH, Leite LHR, Rodrigues AG, Coimbra CC. Performance-enhancing and thermoregulatory effects of intracerebroventricular dopamine in running rats. Pharmacol Biochem Behav [Internet]. 2009 Oct [cited 2018 Apr 20];93(4):465–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19549536

25. Leal MC, França LR. The Seminiferous Epithelium Cycle Length in the Black Tufted-Ear Marmoset (Callithrix penicillata) Is Similar to Humans1. Biol Reprod [Internet]. 2006 Apr 1 [cited 2018 Apr 4];74(4):616–24. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16319285

26. Esper L, Utsch L, Soriani FM, Brant F, Esteves Arantes RM, Campos CF, et al. Regulatory effects of IL-18 on cytokine profiles and development of myocarditis during Trypanosoma cruzi infection. Microbes Infect [Internet]. 2014 Jun [cited 2018 Apr 23];16(6):481–90. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24704475

27. Novaes RD, Gonçalves R V., Penitente AR, Bozi LHM, Neves CA, Maldonado IRSC, et al. Modulation of inflammatory and oxidative status by exercise

attenuates cardiac morphofunctional remodeling in experimental Chagas cardiomyopathy. Life Sci [Internet]. 2016 May 1 [cited 2018 Apr 20];152:210–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27040670

28. Novaes RD, Gonçalves R V., Penitente AR, Cupertino MC, Maldonado IRSC, Talvani A, et al. Parasite control and skeletal myositis in Trypanosoma cruzi - infected and exercised rats. Acta Trop [Internet]. 2017 Jun [cited 2018 Apr 20];170:8–15. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28223068

29. Cruz JS, Santos-Miranda A, Sales-Junior PA, Monti-Rocha R, Campos PP, Machado FS, et al. Altered Cardiomyocyte Function and Trypanosoma cruzi Persistence in Chagas Disease. Am J Trop Med Hyg [Internet]. 2016 [cited 2018 Apr 23];94(5):1028. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26976879

30. Andrade ZA, Andrade SG, Andrade ZA, Andrade SG. A patologia da doença de Chagas experimental no cão. Mem Inst Oswaldo Cruz [Internet]. 1980 Dec [cited 2018 Mar 20];75(3–4):77–95. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0074-02761980000200008&lng=pt&tlng=pt

31. Nunes M do CP, Barbosa M de M, Brum VAA, Rocha MO da C. Morphofunctional characteristics of the right ventricle in Chagas' dilated cardiomyopathy. Int J Cardiol [Internet]. 2004 Mar [cited 2018 Apr 4];94(1):79–85. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14996479

32. Moreira HT, Volpe GJ, Marin-Neto JA, Ambale-Venkatesh B, Nwabuo CC, Trad HS, et al. Evaluation of Right Ventricular Systolic Function in Chagas Disease Using Cardiac Magnetic Resonance Imaging. Circ Cardiovasc Imaging

[Internet]. 2017 Mar [cited 2018 Apr 23];10(3). Available from: http://www.ncbi.nlm.nih.gov/pubmed/28289020

33. Andrade SG. Influence of Trypanosoma cruzi strain on the pathogenesis of chronic myocardiopathy in mice. Mem Inst Oswaldo Cruz [Internet]. [cited 2018 Apr 30];85(1):17–27. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2215229

34. Costa HS, Lima MMO, Silva MG, Alencar MCN, Nunes MCP, Camargos ERS, et al. Effect of acute aerobic exercise on serum BDNF levels in patients with Chagas heart disease. Int J Cardiol [Internet]. 2014 Jul 1 [cited 2018 feb 4];174(3):828–30. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24794957

Neurotrophin levels are significantly changed in hippocampus and striatum by *Trypanosoma cruzi* infection and exercise training

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ABSTRACT

Aims: To investigate the interaction between heart failure (HF) in Chagas' disease induced by Y strain and depressive-like behavior assessed by forced swim test (FST). Additionally, to establish how this scenario can be affected by moderate aerobic physical training and affect the levels of glial cell-derived neurotrophic factor (GDNF), nerve growth factor (NGF) and brain derived neurotrophic factor (BDNF) in the mice brain. **Main methods:** Twenty-eight male C57BL/6 mice were distributed into 4 groups: sedentary control (SC), trained control (TC), sedentary infected (CHC) and trained infected (CHT). The maximal exercise test (TEM) was performed to establish the adjustments in training speed at baseline as well as at the end of 4^{th}, ^{8th} and 12^{th} weeks. At the end of the training protocol, all animals were evaluated for cardiac morphology and function by echocardiography and depressive-like behavior by FST. The brain areas (cortex, hippocampus and striatum) were assessed for neurotrophins levels by ELISA. **Key findings:** The infection by Y strain impaired the cardiac function as showed by lower values of ejection fraction (EF), stroke volume (SV) and fractional area change (FAC) in CHC while exercise retarded the disease progress once no differences were found between NT and CHT and trained infected animals presented higher EF compared to CHC. No differences were viewed among groups in FST and neurotrophins levels were significantly decreased by infection and exercise in striatum while in hippocampus only the association between them promoted the same behavior. **Significance:** Twelve weeks of moderate aerobic physical training retard the CD progress keeping better cardiac function parameters. NFs levels were significantly decreased in striatum and hippocampus by infection, exercise or the association between them.

Keywords: Chagas' disease, Chagasic cardiomyopathy, Exercise training, Cardiac function, Glial cell-derived neurotrophic factor (GDNF), Nerve growth factor (NGF), Brain derived neurotrophic factor (BDNF), Neurotrophins, Forced swim test, depressive-like behavior.

Introduction

Heart failure (HF) is a clinical syndrome that results in impairment of the heart function causing unbalance between demand and physiological response. On the other hand, depression might be understand as a disorder characterized by a disturbance of mood, speech, energy and cognition lasting for at least two weeks.[1]Depression is five times more prevalent in HF patients than in general population [2–4] and it can increase morbidity and mortality in these patients.[4]

Chagas' disease (CD) is an acquired inflammatory illness which might be manifested by an impairment of cardiac contractile function. [5] Patients with CD can develop HF during the chronic phase of the disease [6], representing one of the most worrying signs of this infection. Few studies have reported the association between CD and depression [7] and the ever-rising prevalence of HF patients with depression. Thus, it is vital to understand the predictors of depression in association with this clinical condition in order to identify and better manage these patients.

Moreover, a great number of studies investigating the role of neurotrophic factor (NF), especially BDNF, in the development of mood disorders as well as in neurodegenerative diseases and schizophrenia [8] have been reported. In fact, changes in neurotrophins levels were found in different diseases and stages of depression. [9–11] In this scenario, over the past decades, there was a great interest in NF as protective agents against neuronal damages. [9] Also, NF expression can be affected by diseases such as CD [12], diabetes and atherosclerosis. [13]

On the other hand, the effects of physical exercise in NF expression remain little understood, although it is recognized as a potential stimulus able to change the concentration of NF in serum. [14,15] Thus, our aim in this study was to assess the effects of twelve weeks of aerobic physical training on cardiac function of chagasic mice and

correlating the findings with the depressive-like behavior and neurotrophins levels in these animals.

Methods

Ethical statements

This study was performed in accordance with recommendations of the guide to the care and use of laboratory animals of the Brazilian National Council of Animal Experimentation and all experiments and procedures were approved by the ethic committee of the Federal University of Minas Gerais CEUA/UFMG (protocol number 300/2015). Animals were obtained from CEBIO/UFMG, maintained under pathogen-free conditions, housed in standard clear plastic cages, and kept at 23ºC with free access to food and water and a light/dark cycle.

Animals and infection

Twenty eight male C57BL/6 mice with approximately 55 days of life were randomized into four groups: CHC (Chagasic sedentary); CHT (Chagasic trained), SC (Non-chagasic sedentary) and TC (Non-chagasic trained), each one with 7 animals. Chagasic animals were infected using the intraperitoneal route with $1x10^3$ trypomastigote forms of the Y strain. Control groups (non-chagasic mice) received the same final volume of phosphate-buffered saline (PBS, pH 7.2). The parasitemia was performed at 7^{th}, 9^{th}, 11^{th}, 13^{th} and 15^{th} days post-inoculation utilizing 5 µL of blood from the tail vein. A slide was analyzed using optical microscopy through a score of trypomastigote forms in hundred fields.

Exercise training: protocol and measurements of exercise tolerance

Exercise tolerance was measured by a maximal exercise test (TEM) at the baseline (45 days post-inoculation), as well as at the end of the 4^{th}, 8^{th} and 12^{th} weeks of

training. The mice were familiarized with the motor-driven treadmill (Goustec®) one week before the first TEM by running at the speed of 8m/min at 5% inclination, 10min/day for 5 consecutive days. The TEM was performed at a constant slope of 5% with the starting speed at 3m/min, and adjusting the velocity in 3m/min every 3 minutes until the mice fatigue, defined as the point on which the animals were no longer able to keep the rhythm in the treadmill. The maximum speed registered for each mouse was used to calculate the velocity of training (considered as 70% of the maximum) for the following four weeks until the new TEM. The workload (W, in Joules) was calculated multiplying the mice body weight (kg) by the time (minutes) and speed (m/min) for each step reached by the sine θ of treadmill slope. [16]

Cardiac function analysis

At the end of twelve weeks of exercise training and before euthanasia, the cardiac function was evaluated by echocardiography. Animals were anesthetized with 2% isoflurane in a flow of 1L/min of oxygen and the precordium area was trichotomized. The equipment Vevo 770 (Visual Sonics®, Canada) and a 30MHz transducer were used to record cardiac function data. Cardiac geometry was assessed in 2-dimensional mode and area of the left and right ventricle at the end of diastole was measured in cross-sections at the papillary muscles level. The systolic function of the left ventricle was evaluated by ejection fraction calculated according to the Simpson's method which is able to determine the volume of the left ventricle. Automatically, the software offers values of the end-diastolic and end-systolic volumes, as well as the stroke volume and fractional area changes. Animals were evaluated forty-eight hours after the last TEM.

Assessment of depressive behavior

The forced swimming test (FST) was performed to detect depressive behavior. [17] For this test, animals were placed in a clear glass container with 10 cm of heated water (23-25ºC). All tests had as duration 6 minutes but the first two minutes were considered as pre-test and they were not used for analyzes. During the last four minutes, the total time of not moving and the frequency of immobility episodes were assessed using software for tracking these behaviors (EthoVision XT, Noldus Information Technology, Leesburg, VA, USA). The software was set to recognize as an immobility episode speeds lower than 5cm/s to disregard the time when the animal was just floating.

Neurotrophins levels

Neurotrophins levels were assessed by enzyme-linked immunosorbent assay (ELISA). BDNF, GDNF and NGF levels were measured in cortex, hippocampus and striatum, according to manufacturer's protocols (R&D Systems, Minneapolis, MN, USA). The concentrations were determined in a spectrophotometer (Spectramax 190) at a 450nm wavelength.

Statistical analysis

Values are expressed as mean ± SEM and the data were analyzed using one-way or two-way ANOVA followed by the Bonferroni post-test using the software GraphPad Prism (version 5; Graph-Pad Software Inc., San Diego, CA, USA). Statistical significance was set at $p < 0.05$.

Results

To confirm the infection, we performed the parasitemia from 7th to 15th days post inoculation. Y strain infection showed similar values in both groups reaching the peak

of trypomastigotes forms at 9th day. No significant differences between infected groups at the same day were observed (Figure 1).

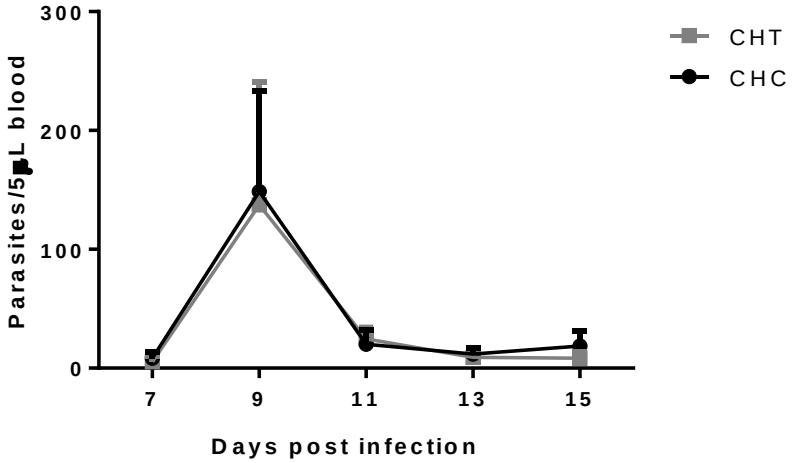

Figure 1 - Parasitemia of mice infected with Y strain of *T. cruzi*. Natural course of *T. cruzi* infection, as measured by parasitemia, of chagasic sedentary animals (CHC, n=6) and chagasic trained mice (CHT, n=6) infected with 1000 trypomastigotes of *T. cruzi*. The data is shown as mean ± SD of the number of parasites per microliter of blood. Two-way ANOVA followed by the Bonferroni post-test.

Responses obtained using TEM presented similar pattern at 4 (TEM 1), 8 (TEM 2) and 12 (TEM 3) weeks, as shown in Figure 2. The infection was able to impair exercise tolerance, as confirmed by significant lower values found for work in CHC compared to SC. Furthermore, the training protocol promoted better work values in the TC group when compared to SC. Importantly, the absence of differences between TC and CHT and the greater values found in CHT when compared to CHC evidence a beneficial effect of the training on CD, since it was able to keep work values closer to those of non-infected trained animals. We did not find significant differences among the groups at the baseline.

Analyzing the groups through the time, the work increased from TEM 0 to TEM 1, TEM 2 and TEM 3 in the SC, TC and CHT groups ($p<0.0001$). However, no significant differences were found from TEM 1 to TEM 2 and from TEM 2 to TEM 3.

In contrast, the CHC group showed a decreased exercise tolerance from TEM 0, TEM 1 and TEM 2 to TEM3 ($p<0.0001$).

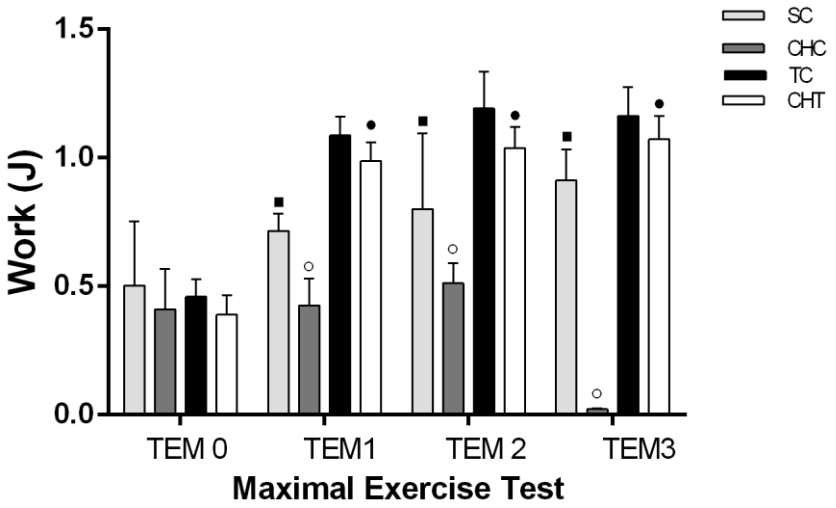

Figure 2 –Maximal exercise tests (TEM) from the baseline (TEM 0) to the end of the 12th week (TEM 3) of training. W (Joules); SC (sedentary control); CHC (chagasic sedentary); TC (trained control) and CHT (chagasic trained). Data are expressed as mean ± SEM ($p<0.05$). Symbols denote statistical differences between groups CHC and CHT (●); TC and SC (■); and SC and CHC (°).One-way and Two-way ANOVA followed by the Bonferroni post-test.

The morphology and cardiac function were assessed by echocardiography. A larger right ventricular area (RVA) was found in CHC compared to SC (Figure 3A) and a smaller left ventricular area (LVA) and end-systolic volume were found in CHT when compared to infected sedentary mice (Figures 3B and 3G). Also, the ejection fraction (EF) was significantly lower in CHC compared to SC and higher in CHT when compared to CHC mice (Figure 3C). The fractional area change (FAC) and stroke volume (SV) were lower in CHC animals compared to SC (Figures 3D and 3E). No significant differences were found among the groups in terms of end-diastolic volume (Figure 3F).

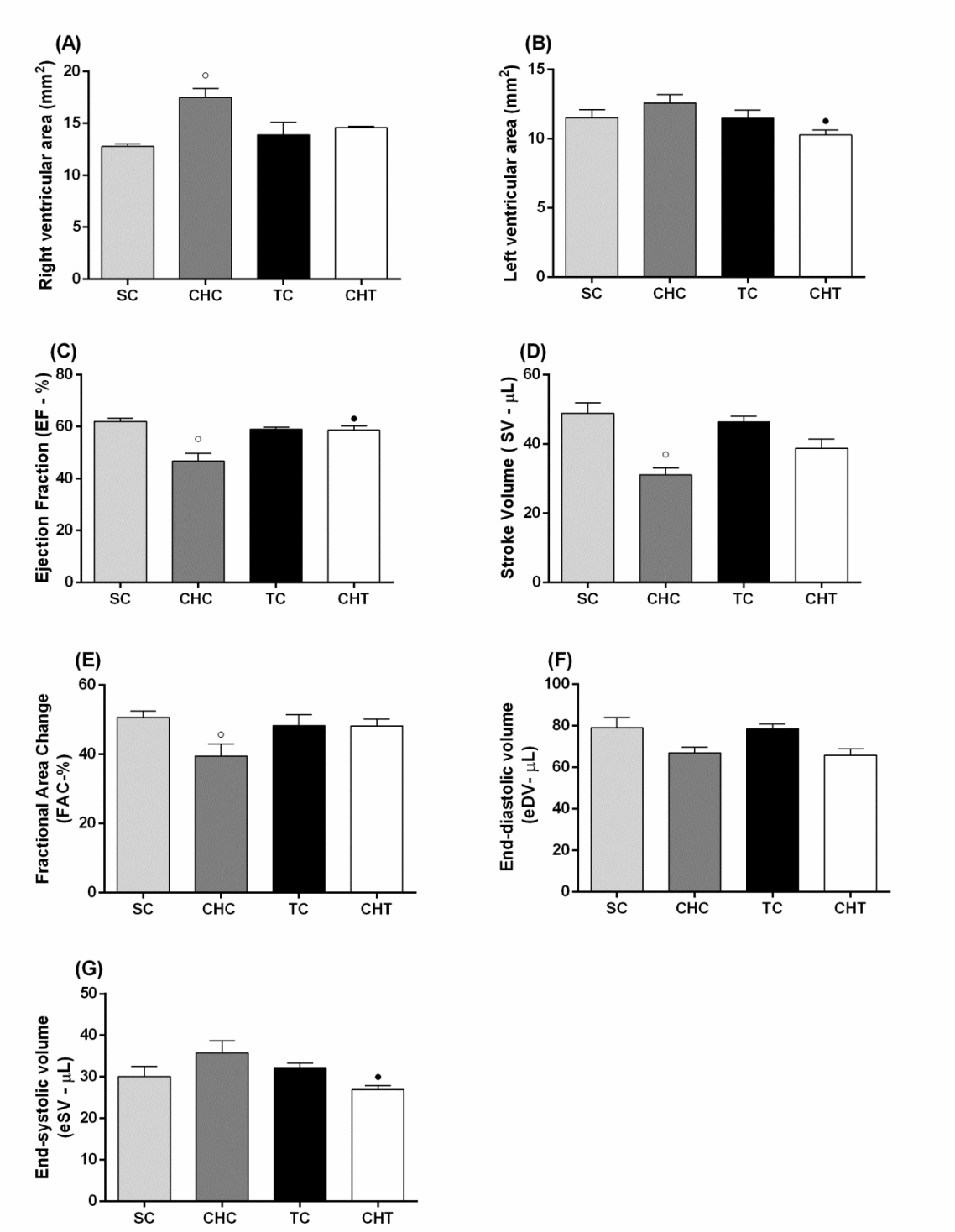

Figure 3 – Cardiac function and morphological measurements. SC (sedentary control); CHC (chagasic sedentary); TC (trained control) and CHT (chagasic trained). Data are expressed as mean ± SEM ($p<0.05$). Symbols denote statistical differences between CHC and CHT (●); SC and CHC (◦). One-way ANOVA followed by the Bonferroni post-test.

The FST was used to assess the depressive-like behavior and no significant differences among the groups in any of the variables evaluated were observed (Figure 4).

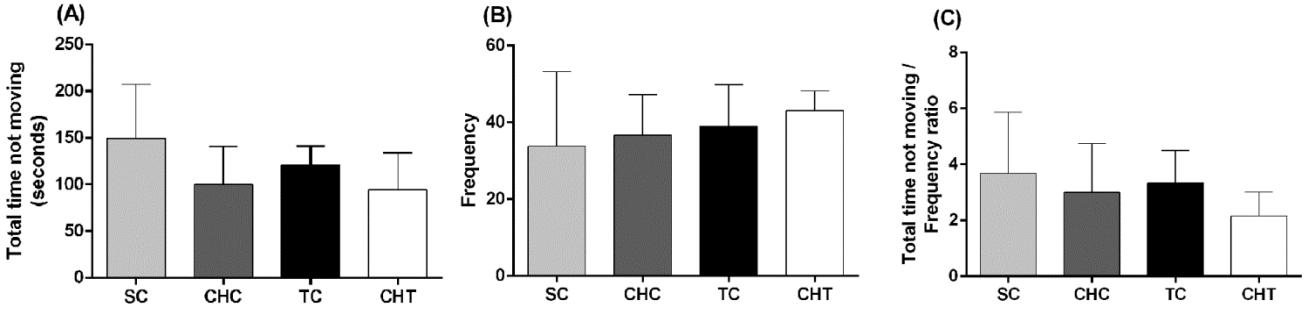

Figure 4 – Forced swimming test (FST). (A) Total time not moving, in seconds; (B) frequency and (C) ratio between total time not moving and frequency. SC (sedentary control); CHC (chagasic sedentary); TC (trained control) and CHT (chagasic trained). Data are expressed as mean ± SEM ($p<0.05$). One-way ANOVA followed by the Bonferroni post-test.

The levels of BDNF, NGF and GDNF showed a similar pattern in cortex, hippocampus and striatum. In the cortex, no significant differences were found among the groups in all neurotrophins evaluated. Lower NF levels were viewed in CHT compared to CHC and TC in hippocampus, while CHC and TC groups showed lower neurotrophins levels compared to SC in striatum (Figure 5).

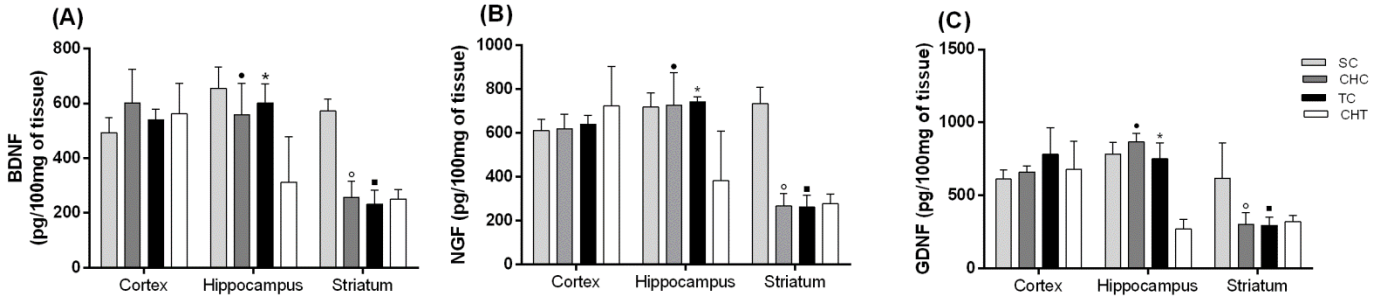

Figure 5 – Brain Derived Neurotrophic Factor (BDNF), Nerve Growth Factor (NGF) and GDNF (Glial cell-derived Neurotrophic Factor) levels in cortex, hippocampus and striatum. SC (sedentary control); CHC (chagasic sedentary); TC (trained control) and CHT (chagasic trained). Data are expressed as mean ± SEM ($p<0.05$). Symbols denote statistical differences between groups CHC and CHT (●); TC and SC (■); SC and CHC (°) and TC and CHT (*). One-way ANOVA followed by the Bonferroni post-test.

Discussion

The main findings of our present study were that the *T. cruzi* Y strain infection was able to impair cardiac function and morphology of right and left ventricles of mice despite of the lack of tropism of this strain to cardiac tissue. Of note, exercise training minimized these deleterious effects. Furthermore, forced swimming test failured to demonstrate any significant depressive behavior in chagasic mice, although significant changes in neurothophins levels were observed in hippocampus and striatum areas.

In keeping to previous studies using *T. cruzi* Y strain[18], we did not find significant differences between infected groups in terms of parasitemia. The Y strain behavior reaches the peak of trypomastigote forms at 9th day post infection followed by a decrease until the 15th day. Importantly, this absence of difference supports similar baseline damages in both infected groups.

The TEM was used to assess the exercise tolerance and our results showed higher values in the TEM 1, TEM 2 and TEM 3 in TC mice compared to SC, suggesting that the training protocol was able to improve the performance under healthy conditions. Previous studies have reported reductions in the total time until fatigue in rats infected by Y strain submitted to physical training. [19–21] Indeed, we found better values of TEM in CHT compared to CHC. This reinforces the capacity of Y strain to impair exercise tolerance and the effectiveness of the training protocol to retard CD progress in infected trained animals. In addition to the absence of differences between TC and CHT groups, our data show a beneficial effect of the training on CD, since it was able to keep work values closer to those of non-infected trained animals.

In accordance with previous studies, the echocardiography analysis evidenced larger RVA in CHC compared to SC, indicating a primary involvement of the right ventricle in chagasic cardiomyopathy, [22–24] for example, right branch block, dilated

right ventricular chamber, impairment of myocyte contractility, and thinning of the right ventricular wall even with the absence of damage in the left ventricle. [24] Noteworthy, the absence of significant differences in RVA between TC and CHT suggests that our training protocol was effectiveness in preserving the RVA in infected trained animals.

The smaller LVA found in CHT when compared to CHC corroborated with the beneficial effects of exercise in CD. This change in LVA may suggest a biventricular involvement in CHC as a consequence of the progression of the disease. In fact, it is recognized that alterations in LV function and structure is the more frequent phenotype of the cardiac CD manifestations. [24]

Furthermore, CHC group showed lower values of EF, SV and FAC compared to SC. These indexes were not different when comparing CHT to TC and the EF was even higher in chagasic trained animals compared to CHC. Altogether, these findings suggest an important role of exercise training in retard the CD progress. This is also corroborated with the lower end-systolic volume observed in CHT when compared to chagasic sedentary mice.

Some studies have associated heart failure (HF) with depression. [2,25,26] HF is characterized by a blood supply which is insufficient to meet the body's demand and can potentially affect the brain. [26] However, the mechanisms underlying these findings remain largely unclear. Physiological factors as endothelial dysfunction, platelet abnormalities, inflammation, autonomic nervous system dysfunction [27] as well as neurohumoral activation are pointed as important features of HF [25] and may establish a possible connection between HF and the depressive-like behavior. Of note, in our study we did not find any significant correlation between chagasic cardiomyopathy and depression. This may suggest that despite the impairment of the ventricular function in the CHC group, the FST was not able to detect any depressive behavior in our protocol.

Recent advances in molecular biology and imaging techniques have permitted a better understanding of the limbic system in the modulation of dysfunctional mechanisms that can lead to depressive behavior in HF. [25] The structural abnormality of the ventral hippocampus was associated to depressive symptoms in HF in rats, [26] as well as changes in neurotrophins signaling in this brain area [10,28] inducing more pronounced depressive behavior in rats vulnerable to stress-induced depression. [28] Furthermore, neurotrophins polymorphisms, as BDNF Val66Met, were supposed to regulate corticohippocampal remodeling and it was associated to behavioral compromising. [11]

It is also recognized the benefits of exercise in depression. A growing number of studies have demonstrated a reduction in depressive symptoms after aerobic and non-aerobic exercise interventions. [29–31] For instance, there is evidence that aerobic exercise undertaken three times weekly at moderate intensity for a minimum of nine weeks induces beneficial outcomes in depression. [29] In addition, exercise is considered a stimulus able to change neurotrophins concentrations in different levels of cardiac impairment. [14]

Our current results showed that the levels of BDNF, NGF and GDNF were similarly affected in hippocampus and striatum. Thus, taking into accounting that the FST showed no differences among the groups, it is reasonable to suppose that these alterations in neurotrophins concentrations were due to *T. cruzi* infection and/or exercise stimulus. Indeed, exercise and infection down-regulated the neurotrophins levels in striatum since both TC and CHC animals presented lower concentration of neurotrophins compared to the SC group. Furthermore, only the association between infection and exercise training reduced the levels of neurotrophins in hippocampus. These data showed that distinct brain regions responded differently to the same stimulus

in terms of NF expressions and more studies are needed to better clarify the pathway and mechanisms involved in these findings.

The impairment of cardiac function in CHC may be worked as a peripheral stimulus to induce lower neurotrophins levels in striatum. In fact, HF has been associated with depressive behavior and decreased NF levels, particularly it was observed that BDNF modulation is involved in the pathogenesis of affective disorders. [10,28,32,33] Also, the lower neurotrophins levels found in striatum of the TC group might be due to the benefits of training in cardiac function, suggesting that the exercise is a stressing stimulus able to promote changes in NF expression.

In line with these speculations, preclinical studies have shown correlations between stress-induced depressive-like behaviors and decreases in hippocampal BDNF levels. The exposure to stressors can significantly down regulate neurotrophins in hippocampus [34–36] and our results showed that the association between infection and exercise decreased the neurotrophins concentration in hippocampus.

Overall, to the best of our knowledge, this is the first study associating *T. cruzi* infection with neurotrophins levels in the brain and depressive-like behaviors. Therefore, future studies should be designed to better understand these correlations, as well as to identify the physiopathological role of the central neurotrophins.

Conclusion

The infection by *Trypanosoma* cruzi Y strain is able to impair cardiac function and twelve weeks of moderated aerobic physical training retard the progress of CD keeping better echocardiographic parameters in infected trained animals. Although the impairment of cardiac function, FST was not able to identify depressive-like behavior in our protocol while exercise and infection promoted changes in NF levels in striatum and the association between them highlighted differences in NF levels in hippocampus.

REFERENCES

1. Rajeswaran T, Plymen CM, Doherty AM. The effect of antidepressant medications in the management of heart failure on outcomes: mortality, cardiovascular function and depression – a systematic review. Int J Psychiatry Clin Pract [Internet]. 2018 Jul 3 [cited 2019 Apr 22];22(3):164–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29172802

2. Ghaemian A, Mohammadpour RA, Hosseini SH ES. Depression in Heart Failure Patients and the Influence of Age, Gender and Severity of Depression on Heart Failure Patients [Internet]. Vol. 2, The Journal of Tehran University Heart Center. Tehran University of Medical Sciences; 2006 [cited 2019 May 11]. p. 31–4. Available from: http://jthc.tums.ac.ir/index.php/jthc/article/view/37

3. Rutledge T, Reis VA, Linke SE, Greenberg BH, Mills PJ. Depression in Heart Failure. J Am Coll Cardiol [Internet]. 2006 Oct [cited 2019 Apr 22];48(8):1527–37. Available from: https://linkinghub.elsevier.com/retrieve/pii/S073510970601905X

4. Zahid I, Baig MA, Ahmed Gilani J, Waseem N, Ather S, Farooq AS, et al. Frequency and predictors of depression in congestive heart failure. Indian Heart J [Internet]. 2018 Dec 1 [cited 2019 May 6];70:S199–203. Available from: https://www.sciencedirect.com/science/article/pii/S0019483218304954?via%3Dihub

5. Coura JR. Chagas disease: what is known and what is needed - A background article. Mem Inst Oswaldo Cruz [Internet]. 2007 Oct [cited 2019 Apr 4];102(suppl 1):113–22. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0074-02762007000900018&lng=en&tlng=en

6. Rassi A, Marin-Neto JA. Cardiopatia Chagásica Crônica. Vol. 4, Rev Soc Cardiol Estado de São Paulo. 2000. vi–xxxii.

7. Suman AC, Costa ÉAPN da, Bazan SGZ, Hueb JC, Carvalho FC de, Martin LC, et al. Evaluating respiratory musculature, quality of life, anxiety, and depression among patients with indeterminate chronic Chagas disease and symptoms of pulmonary hypertension. Rev Soc Bras Med Trop [Internet]. 2017 Mar [cited 2019 Apr 22];50(2):194–8. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0037-86822017000200194&lng=en&tlng=en

8. Castrén E, Kojima M. Brain-derived neurotrophic factor in mood disorders and antidepressant treatments. Neurobiol Dis [Internet]. 2017 Jan [cited 2019 Apr 22];97(Pt B):119–26. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27425886

9. Ferreira RN, de Miranda AS, Rocha NP, Simoes e Silva AC, Teixeira AL, da Silva Camargos ER. Neurotrophic Factors in Parkinson's Disease: What Have we Learned from Pre-Clinical and Clinical Studies? Curr Med Chem [Internet]. 2018 Oct 16 [cited 2019 Apr 22];25(31):3682–702. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29532753

10. Zaletel I, Filipović D, Puškaš N. Hippocampal BDNF in physiological conditions and social isolation. Rev Neurosci [Internet]. 2017 Jan 26 [cited 2019 Apr 22];28(6):675–92. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28593903

11. Notaras M, Du X, Gogos J, van den Buuse M, Hill RA. The BDNF Val66Met polymorphism regulates glucocorticoid-induced corticohippocampal remodeling and behavioral despair. Transl Psychiatry [Internet]. 2017 Sep 19 [cited 2019 Apr

22];7(9):e1233. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28926000

12. Martinelli PM, da Costa Rocha MO, Teixeira AL, do Carmo Pereira Nunes M, da Silva Camargos ER. Brain-derived neurotrophic factor is up regulated in chronic Chagas disease. Int J Cardiol [Internet]. 2011 Jun 2 [cited 2019 Apr 4];149(2):277–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21420187

13. Caporali A, Emanueli C. Cardiovascular actions of neurotrophins. Physiol Rev [Internet]. 2009 Jan [cited 2019 May 11];89(1):279–308. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19126759

14. Costa HS, Lima MMO, Silva MG, Alencar MCN, Nunes MCP, Camargos ERS, et al. Effect of acute aerobic exercise on serum BDNF levels in patients with Chagas heart disease. Int J Cardiol [Internet]. 2014 Jul 1 [cited 2019 Apr 4];174(3):828–30. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24794957

15. Lima MMO, Nunes MCP, Nascimento B, Costa HS, Sousa LAP, Teixeira AL, et al. Improvement of the functional capacity is associated with BDNF and autonomic modulation in Chagas disease. Int J Cardiol [Internet]. 2013 Sep 1 [cited 2019 Apr 4];167(5):2363–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23232458

16. Balthazar CH, Leite LHR, Rodrigues AG, Coimbra CC. Performance-enhancing and thermoregulatory effects of intracerebroventricular dopamine in running rats. Pharmacol Biochem Behav [Internet]. 2009 Oct [cited 2019 Apr 4];93(4):465–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19549536

17. Can A, Dao DT, Arad M, Terrillion CE, Piantadosi SC, Gould TD. The Mouse Forced Swim Test. J Vis Exp [Internet]. 2011 Dec 11 [cited 2019 May

11];(58):e3638. Available from: http://www.jove.com/details.php?id=3638

18. Esper L, Utsch L, Soriani FM, Brant F, Esteves Arantes RM, Campos CF, et al. Regulatory effects of IL-18 on cytokine profiles and development of myocarditis during Trypanosoma cruzi infection. Microbes Infect. 2014;

19. Novaes RD, Gonçalves R V., Penitente AR, Bozi LHM, Neves CA, Maldonado IRSC, et al. Modulation of inflammatory and oxidative status by exercise attenuates cardiac morphofunctional remodeling in experimental Chagas cardiomyopathy. Life Sci [Internet]. 2016 May 1 [cited 2019 Apr 4];152:210–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27040670

20. Novaes RD, Gonçalves R V., Penitente AR, Cupertino MC, Maldonado IRSC, Talvani A, et al. Parasite control and skeletal myositis in Trypanosoma cruzi - infected and exercised rats. Acta Trop [Internet]. 2017 Jun [cited 2019 Apr 4];170:8–15. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28223068

21. Novaes RD, Penitente AR, Gonçalves R V, Talvani A, Neves CA, Maldonado IRSC, et al. Effects of Trypanosoma cruzi infection on myocardial morphology, single cardiomyocyte contractile function and exercise tolerance in rats. Int J Exp Pathol [Internet]. 2011 Oct [cited 2019 Apr 4];92(5):299–307. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21736646

22. Cruz JS, Santos-Miranda A, Sales PA, Monti-Rocha R, Campos PP, Machado FS, et al. Altered cardiomyocyte function and Trypanosoma cruzi persistence in Chagas disease. Am J Trop Med Hyg. 2016;

23. Andrade ZA, Andrade SG, Andrade ZA, Andrade SG. A patologia da doença de Chagas experimental no cão. Mem Inst Oswaldo Cruz [Internet]. 1980 Dec [cited 2019 Apr 4];75(3–4):77–95. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0074-

02761980000200008&lng=pt&tlng=pt

24. Pereira Nunes MDC, De Melo Barbosa M, Andrade Brum VA, Da Costa Rocha MO. Morphofunctional characteristics of the right ventricle in Chagas' dilated cardiomyopathy. Int J Cardiol. 2004;

25. Rigas A, Farmakis D, Papingiotis G, Bakosis G, Parissis J. Hypothalamic dysfunction in heart failure: pathogenetic mechanisms and therapeutic implications. Heart Fail Rev [Internet]. 2018 Jan 19 [cited 2019 May 6];23(1):55–61. Available from: http://link.springer.com/10.1007/s10741-017-9659-7

26. Suzuki H, Sumiyoshi A, Matsumoto Y, Duffy BA, Yoshikawa T, Lythgoe MF, et al. Structural abnormality of the hippocampus associated with depressive symptoms in heart failure rats. Neuroimage [Internet]. 2015 Jan 15 [cited 2019 May 11];105:84–92. Available from: https://www.sciencedirect.com/science/article/pii/S1053811914008660?via%3Dihub

27. Huffman JC, Celano CM, Beach SR, Motiwala SR, Januzzi JL. Depression and Cardiac Disease: Epidemiology, Mechanisms, and Diagnosis. Cardiovasc Psychiatry Neurol [Internet]. 2013 [cited 2019 May 11];2013:1–14. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23653854

28. Serra MP, Poddighe L, Boi M, Sanna F, Piludu MA, Corda MG, et al. Expression of BDNF and trkB in the hippocampus of a rat genetic model of vulnerability (Roman low-avoidance) and resistance (Roman high-avoidance) to stress-induced depression. Brain Behav [Internet]. 2017 Oct [cited 2019 May 11];7(10):e00861. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29075579

29. Stanton R, Reaburn P. Exercise and the treatment of depression: A review of the exercise program variables. J Sci Med Sport [Internet]. 2014 Mar 1 [cited 2019 May 9];17(2):177–82. Available from: https://www.sciencedirect.com/science/article/pii/S1440244013000583?via%3Dihub

30. Krogh J, Speyer H, Gluud C, Nordentoft M. Exercise for patients with major depression: a protocol for a systematic review with meta-analysis and trial sequential analysis. Syst Rev [Internet]. 2015 Dec 2 [cited 2019 May 11];4(1):40. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25875610

31. Morgan JA, Singhal G, Corrigan F, Jaehne EJ, Jawahar MC, Baune BT. TNF signalling via the TNF receptors mediates the effects of exercise on cognition-like behaviours. Behav Brain Res [Internet]. 2018 Nov 1 [cited 2019 May 9];353:74–82. Available from: https://www.sciencedirect.com/science/article/pii/S016643281830216X?via%3Dihub

32. Duman RS, Monteggia LM. A Neurotrophic Model for Stress-Related Mood Disorders. Biol Psychiatry [Internet]. 2006 Jun 15 [cited 2019 May 11];59(12):1116–27. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16631126

33. Larsen MH, Mikkelsen JD, Hay-Schmidt A, Sandi C. Regulation of brain-derived neurotrophic factor (BDNF) in the chronic unpredictable stress rat model and the effects of chronic antidepressant treatment. J Psychiatr Res [Internet]. 2010 Oct [cited 2019 May 11];44(13):808–16. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20172535

34. Lee T, Saruta J, Sasaguri K, Sato S, Tsukinoki K. Allowing animals to bite

reverses the effects of immobilization stress on hippocampal neurotrophin expression. Brain Res [Internet]. 2008 Feb 21 [cited 2019 May 11];1195:43–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18191115

35. Murakami S, Imbe H, Morikawa Y, Kubo C, Senba E. Chronic stress, as well as acute stress, reduces BDNF mRNA expression in the rat hippocampus but less robustly. Neurosci Res [Internet]. 2005 Oct [cited 2019 May 11];53(2):129–39. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16024125

36. Nair A, Vadodaria KC, Banerjee SB, Benekareddy M, Dias BG, Duman RS, et al. Stressor-Specific Regulation of Distinct Brain-Derived Neurotrophic Factor Transcripts and Cyclic AMP Response Element-Binding Protein Expression in the Postnatal and Adult Rat Hippocampus. Neuropsychopharmacology [Internet]. 2007 Jul 13 [cited 2019 May 11];32(7):1504–19. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17164818

5. Conclusões finais

O nosso estudo revelou que o treinamento físico aeróbio de intensidade moderada e realizado por doze semanas foi capaz de retardar a progressão da doença de Chagas, minimizando suas manifestações cardíacas. Nesse sentido, a infecção pela cepa Y do *Trypanosoma cruzi* teve seus efeitos deletérios sobre parâmetros ecocardiográficos atenuados pelo treinamento, uma vez que animais infectados e treinados obtiveram resultados semelhantes ao seu controle não infectado treinado. Índices como débito sistólico, débito cardíaco, fração de mudança de área e fração de ejeção foram consideravelmente maiores nesses animais quando comparados aos do grupo infectado e sedentário que por sua vez também apresentaram maior volume sistólico final, demonstrando novamente o acometimento da função cardíaca.

Outro ponto passível de nota é a capacidade do treinamento físico e/ou a infecção pelo *T. cruzi* modularem a concentração de neurotrofinas em diferentes tecidos. A infecção promoveu, especialmente no coração e plasma, um aumento dos níveis dos fatores neurotróficos. Estes achados sugerem que o aumento da expressão de NFs pode estar relacionado à tentativa de manutenção dos índices de função cardíaca próximo aos parâmetros normais. Nesse mesmo cenário, o exercício pareceu promover um ajuste quanto à concentração das mesmas, demandando menor expressão destas, possivelmente, em função dos benefícios do treinamento sobre a função cardíaca.

No encéfalo, as neurotrofinas apresentaram um padrão semelhante quanto às suas concentrações. Seus níveis não foram alterados no córtex, porém no estriado foram reduzidos em função da infecção e do treinamento e no hipocampo apenas a associação dos dois fatores promoveu a redução dos níveis de BDNF, GDNF e NGF.

O teste de esforço máximo demonstrou a efetividade do protocolo de treinamento. Os animais treinados, infectados ou não, progrediram quanto à tolerância

ao esforço do primeiro para o último teste. Esses achados foram reforçados pelas diferenças encontradas entre os grupos quanto à função cardíaca ao final de doze semanas de treinamento, onde as varáveis ecocardiográficas mimetizaram os resultados do último teste de esforço máximo.

Estudos em continuidade a este devem considerar a investigação dos mecanismos de ativação das vias das neurotrofinas em função da infecção e do treinamento. Para tanto, devem valer-se, por exemplo, de estudos em cultura primária de cardiomiócitos e/ou em animais knockdown para expressão de NFs. Atenção especial deve ser dada ao BDNF em função da sua crescente investigação em estudos clínicos que o sugerem como marcador biológico de disfunção cardíaca. Ademais, pode-se também considerar a infecção por outras cepas do *T. cruzi* que pronunciem as manifestações cardíacas em função do seu tropismo pelo tecido.

REFERÊNCIAS BIBLIOGRÁFICAS

ANDRADE , Z.A., ANDRADE, S.G., CORREA, S., SADIGURSKY, M., FERRANS, V.J. Myocardial changes in acute *Trypanosoma* cruzy infection. **Am J Pathol,** v.144, p 1403-1411, 1994.

BALTHAZAR, C.H, LEITE, L.H, RODRIGUES, A.G, COIMBRA, C.C. Performance-enhancing and thermoregulatory effects of intracerebroventricular dopamine in running rats.**PharmacolBiochemBehav.** v.93, n.4, p. 465-469, 2009.

BELLOTTI, G., BOCCHI, E.A., MORAES, A.V., HIGUCHI, M.L., BARBERO MARCIAL, M., SOSA, E. et *al.* In vivo detection of *Trypanosomacruzy*antigens in hearts of patients with chronic Chagas' disease.**Am Heart J,** v. 131, p. 301-307, 1996.

BERND, P., MILES, K., ROZENBERG, I., BORGHJID, S., KIRBY, M.L. Neurotrophin-3 and TrkC are expressed in the outflow tract of the developing chicken heart. **DevDyn**, v. 230, p.767–772, 2004.

CAPORALI, A.; EMANUELI, C. Cardiovascular actions of neutrophins.**Physiol Rev,** v.89, n.1, p. 279-308, jan. 2009.

Chagas C. Nova tripanossomíase humana. Estudos sobre a morfologia e o ciclo evolutivo do *Schizotrypanum cruzi* n. gen., n. sp., agente etiológico de nova entidade mórbida do homem. MemInstOswaldo Cruz 1909; 1:159-218.

CHAO, M.V., Neurotrophins and their receptors: a convergence point for many signalling pathways. **Nat Rev Neurosci.,** v.4, p. 299-309, 2003.

CHAUVET-GELINIER J-C, BONIN B. Stress, anxiety and depression in heart disease patients: A major challenge for cardiac rehabilitation. **Ann Phys Rehabil Med** [Internet]. 2017 Jan 1 ;60(1):6–12.

CHEN D, MENG L, PEI F, ZHENG Y, LENG J. A review of DNA methylation in depression. **J Clin Neurosci** [Internet]. 2017 Sep 1;43:39–46.

COSTA, H.S., LIMA, M.M.O., SILVA, M.G., ALENCAR, M.C.N., NUNES, M.C.P., CAMARGOS, E.R.S., MARTINELLI, P.M., ROCHA, M.O.C. Effect of acute exercise on sérum BDNF levels in patients with Chagas heart disease. **Internacional Journal of Cardiology,** v.174, p.828-830, 2014.

COURA Jr. Chagas disease: what is known and what is needed background article. **MemInstOswaldo Cruz**, v. 102, n. 1, p. 113-22, out. 2007.

COTMAN, C.W., BERCHTOLD, N.C, CHRISTIE, L.A. Exercise builds brain health: key roles of growth factor cascades and inflammation. **Trends Neurosci.**, v.30, p.464–472, 2007.

CUNHA-NETO, E., KALIL, J. Autoimmunity in Chagas' heart disease.**Rev Paul Med,** v.113, p. 757-766, 1995.

DIAS, J.C.O. **Doença de Chagas em Bambuí, Minas Gerais, Brasil**: Estudo clínico-epidemiológico a partir da fase aguda entre 1940 e 1982. Belo Horizonte, 1982.376p. (Tese) Universidade Federal de Minas Gerais.

DONOVAN, M.J., LIN, M.I., WiIEGN, P., RINGSTEDT, T., KRAEMER, R., HAHN, R., WANG, S., IBANEZ, C.F., RAFII, S., HEMPSTEAD, B.L. Brain derived neurotrophic factor is an endothelial cell survival factor required for intramyocardial vessel stabilization. **Development,** v. 127, p.4531–4540, 2000.

EJIRI, J., INOUE, N., KOBAYASHI, S., SHIRAKI, R., OTSUI, K., HONJO, T. et *al.* Possible role of brain-derived neurotrophic factor in the pathogenesis of coronary artery disease. **Circulation,** v.112, p. 2114-2120, 2005.

ESPER, L.; GOUVEIA, L. U. M. ; SORIANI, F. M. ; BRANT, F. ; ARANTES, R. M. E. ; CAMPOS, C. F. ; PINHO, V. ; SOUZA, D. G. ; TEIXEIRA, M. M. ; TANOWITZ, H.B ; VIEIRA, L. Q. ; MACHADO, F. S. Regulatory effects of IL-18 on cytokine profiles and development of myocarditis during Trypanosoma cruzi infection. **Microbes and Infection**, v.14; p. 1286-4579, 2014.

GALLO, JR. L.; MARIN-NETO, J.A.; MANÇO, J.C.; RASSI, A., AMORIM, D.S. Abnormal heart rate responses during exercise in patients with Chagas' disease.**Cardiology,** v. 60:147-62, 1975.

GHAEMIAN A, MOHAMMADPOUR RA, HOSSEINI SH, EMADI SH. Depression in Heart Failure Patients and the Influence of Age, Gender and Severity of Depression on Heart Failure Patients. **J Teh Univ Heart Ctr** 1 (2007) 31-34.

Global Report for Research on Infectious Diseases of Poverty [Internet]. 2012. Available from: www.who.int/tdr/publications/environment

GOLDEN, E., EMILIANO, A., MAUDSLEY, S., WINDHAM, B.G., CARLSON, O.D. et *al.* Circulating brain-derived neurotrophic factor and índices of metabolic and cardiovascular health: data from Baltimore Longitudinal Study of Aging. **PLoS ONE,** v.5, n.4, april, 2010.

HIGUICHI, M.L., DE BRITO, T., REIS, M.M., BARBOSA, A., BELOLOTTI, G., PEREIRA-BARRETO, A.C., et *al.* Correlation between *T. cruzy*parasitismo and myocardial inflamatory infiltrate in human chronic chagasic myocarditis: ligth microscopy and immunohistochemical findings. **CardiovascPathol,** v.2, p. 101-106, 1993.

HIGUCHI, M.L., RIES, M.M., AIELLO, V.D., BENVENUTTI, L.A., GUTIERREZ, P.S., BELLOTTI, G. et *al.* Association of an increase in CD8+ T cells with the presence of *Trypanosomacruzy*antigens in chronic human chagasic myocarditis. **Am J Trop Med Hyg,** v.56, p. 485-489, 1997.

HOFER, M.M., BARDE, Y.A. Brains-derived neurotrophic factor prevents neuronal death in vivo. **Nature,** v.331, p. 261-262, 1988.

IMAI, K.; MAEDA, T., SAYAMA, Y., OSA, M., MIKITA, K., KURANE, I., et al. Chronic Chagas disease with advanced cardiac complications in Japan: case report and literature review. ***Parasitol. Int.,*** v. 64: 240-242, 2015.

JACKSON, Y.; PINTO, A.; PETT, S. Chagas' disease in Australia and New Zealand: risks and needs for public health intervations. ***Trop. Med. Int. Health.***, v.19, p. 212-128, 2014.

KOBERLE F. Chagas' heart disease and Chagas' syndromes: the patology of American trypanosomiasis. **AdvParasitol,** v.6, p. 63-116, 1968.

LEE, F.S., CHAO, M.V. Activation of Trkneurotrophin receptors in the absence of neurotrophins. **ProcNatlAcadSci,** v.98, p. 3555–3560, 2001.

LEE J, WON M, SON Y-J. Combined Influence of Depression and Physical Frailty on Cognitive Impairment in Patients with Heart Failure. **Int J Environ Res Public Health** [Internet]. 2018 Dec 27;16(1):66.

LEE, S.S., YOO, J.H., KANG, S.H., WOO, J.H., SHIN, K.O., KIM,K.B., CHO, S.Y., ROH, H.T., KIM, Y.I. The effects of 12weeks regular aerobic exercise on Brain-derived neurotrophic factor and inflammatory factors in juvenile obesity and type 2 Diabetes Mellitus.**J PhysTher Sci.,** v. 26, p. 1199-1204, 2014.

LIMA, M.M.O., NUNES, M.C.P., NASCIMENTO, B., COSTA, H.S., SOUSA, L.A.P., TEIXEIRA, A.L., ROCHA, M.O.C., RIBEIRO, A.L.P. Improvement of the functional capacity is associated with BDNF and autonomic modulation in Chagas disease. **International Journal of Cardiology,** v.167, p.2363-2366, 2012.

LUCCHETTI, B.F.C.; ZANLUQUI, N.G.; RAQUEL, R.A.; LOVO-MARTINS, M.I.; TATAKIHARA, V.L.H.; BELÉM, V.O.; MICHELINI, L.C.; ARAÚJO, E.J.A.; PINGE-FILHO, P.; MARTINS-PINGE, M.C. Moderate treadmill exercise training improves cardiovascular and nitrergic response and resistence to *Trypanosomacruzi* infection in mice. ***Front.physiol*** 8:315, 2017.

MADY, C.; IANNI, B.M; ARTEAGA, E.; SALEMI, V.M.C.; FRIMM, C.C. Maximal Functional Capacity in Patients with Chagas1 Cardiomyopathy without Congestive Heart Failure. ***Journal of Cardiac Failure,*** v.6; n.3; p.220-224, 2000.

MALAFAIA, G.; RODRIGUES, A.S.L; Centenário do Descobrimento da Doença de Chagas: Desafios e Perspectivas. **Revista da Sociedade Brasileira de Medicina Tropical**., 43(5):483-485, set-out, 2010.

MARIN-NETO, J.A.; SIMÕES, M.V.; SARABANDA, A.V. Chagas' heart disease. **Arq Bras Cardiol,** v. 72, n. 3, p. 247-280, mar. 1999.

MARIN-NETO, J.A.; CUNHA-NETO, E.; MACIEL, B.C.; Simões, M.V. Pathogenesis of chronic Chagas heart disease.**Circulation,** v.115, n. 9, p.1109-23, mar. 2007.

MARTINELLI, P.M., ROCHA, M.O.C., TEIXEIRA, A.L., NUNES, M.C.P.N., CAMARGOS, E.R.S. Brain-derived neutrophic factor is up regulated in chronic Chagas disease. **International Journal of Cardiology** , v. 149, p. 277-278, 2011.

MARTINELLI, P.M.; CAMARGOS, E.R.S.; AZEVEDO, A.A.; CHIARI, E.; MOREL, G.; MACHADO C.R.S. (2006) Cardiac NGF and GDNF expression during *Trypanosomacruzi* infection in rats. ***Auton. Neurosci.***13-, 32-40.

MORGAN JA, SINGHAL G, CORRIGAN F, JAEHNE EJ, JAWAHAR MC, BAUNE BT. TNF signalling via the TNF receptors mediates the effects of exercise on cognition-like behaviours. **Behav Brain Res** [Internet]. 2018 Nov 1;353:74–82.

MOREIRA,N.M.; SANTOS, F.N.; TOLEDO, M.J.O.; MORAES, S.M.F.; ARAÚJO,E.J.A.; SAN'ANA, D.M.G., ARAÚJO, S.M. Moderate physical exercise reduces parasitaemia and protects colonic myenteric neurons in mice infected with *Trypanosomacruzi*. ***Int. J. Exp. Path.*** v.94; p. 426-435; 2013.

MOREIRA, N.M.; DE MORAES, S.M.; DALALIO, M.M.; GOMES, M.L.; SANT'ANA, D.M.; ARAUJO, S.M. (2014a). Moderate physical exercise protects myenteric metabolically more active neurons in mice infected with *Trypanosomacruzi*. ***Dig. Dis. Sci.***, v. 59, p. 307-314.

MOREIRA, N.M.; ZANONI, J.N.; DE OLIVEIRA DALALIO, M.M.; DE ALMEIDA ARAUJO, E.J.; BRAGA, C.F; ARAÚJO, S.M. (2014b). Physical exercise protects myenteric neurons and reduces parasitemia in *Trypanosoma cruzi* infection. ***Exp. Parasitol.*** v. 141; p. 68-74.

MORVAN, E., LIMA, N.E., MACHI, J.F., MOSTARDA, C.; DE ANGELIS, K.; IRIGOYEN, M.C.; et al. Metabolic hemodynamic and structural ajustments to low intensity exercise training in a metabolic syndrome model.***Cardiovasc.Diabetol.***, 12:89, 2013.

NOTARAS M, DU X, GOGOS J, VAN DEN BUUSE M, HILL RA. The BDNF Val66Met polymorphism regulates glucocorticoid-induced corticohippocampal remodeling and behavioral despair. **Transl Psychiatry** [Internet]. 2017 Sep 19;7(9):e1233.

NOVAES, R.D.; PENITENTE, A.R.; GONÇALVES, R.V.; TALVANI, A.; NEVES, C.A.; MALDONADO, I.R.S.C.; NATALI, A.J. Effects of *Trypanossomacruzi* infection on myocardial morphology, single cardiomyocyte contractile function and exercise tolerance in rats. ***Int. j. exp. Path.,*** v. 92: 299-307, 2011.

NOVAES, R.D.; GONÇALVES, R.V.; PENITENTE, A.R.; CUPERTINO, M.C., MALDONADO, I.R.S.C.; TALVANI, A.; NATALI, A.J. Parasite control and skeletal myositis in *Trypanosomacruzi* infected and exercised rats. ***ActaTropica.*** 2017. (accepted manuscript)

NOVAES, R.D.; GONÇALVES, R.V.; PENITENTE, A.R.; BOZI, L.H.M; NEVES, C.A.; MALDONADO, I.R.S.C; NATALI, A.J.; TALVANI, A. Modulation of inflammatory and oxidative status by exercise attenuates cardiac morphofunctional

remodeling in experimental Chagas cardiomyopathy. ***Life Sciences***. 2016 (accepted manuscript)

OKADA, S.,MASATAKA, Y., HARUHIRO, T., KAURU, T., JUNJI, M., IPPEI, S. et *al*. Brain derived neurotrophic factor protects against cardiac dysfunction after myocardial infarction via a central nervous system-mediated pathway. **ArteriosclerThrombVasc Biol.** v.32, p. 1902-1909, 2012.

OGŁODEK EA, JUST MJ, SZROMEK AR, ARASZKIEWICZ A. Melatonin and neurotrophins NT-3, BDNF, NGF in patients with varying levels of depression severity. **Pharmacol Reports** [Internet]. 2016 Oct 1;68(5):945–51.

PINTOA.; PETT, S.; JACKSON, Y. Identifying Chagas disease in Australia: an emerging challenge for general practitioners. ***Aus. Fam. Physician.*** v. 43, p. 440-442, 2014.

PRATA, A., ANDRADE, Z., GUIMARÃES, A.C. Chagas' heart disease. In: SHAPER, A.G., HUTT, M.S.R., FEJFAR, Z., eds. Cardiovascular Disease in the Tropics. London: British Medical Association; 1974. p.264-281.

PRATA, A. Clinical and epidemiological aspects of Chagas disease.**Lancet,** v. 1, n. 2, p. 92-100, set. 2001.

RASO, P.; CHAPADEIRO, E.; TAFURI, W.L. *et al.* Anatomia patológica da cardiopatia crônica. In: Cançado JR, Chuster M, eds. *Cardiopatia Chagásica*. Belo Horizonte: Fundação Carlos Chagas 1985: 41-53.

RASSI Jr., A.; MARIN-NETO, J.A. Cardiopatia chagásica crônica. **Rev Soc Cardiol Estado de São Paulo.** v. 10, n. 4, p. 6-31, jul./ago. 2000.

RASSI, A. Jr.; RASSI, A.; MARIN-NETO, J.A. Chagas heart disease: pathophysiologic mechanisms, prognostic factors and risk stratification. **Mem Inst Oswaldo Cruz.** v. 104, n. 1, p. 152-158, jul. 2009.

RASSI Jr, A.; RASSI, A.; MARIN-NETO, J. A. Chagas disease. **Lancet**, v. 375, p. 1388-1402, 2010.

RIBEIRO, A. L.; NUNES, M. P.; TEIXEIRA, M. M.; ROCHA, M. O. C. Diagnosis and management of Chagas disease and cardiomyopathy. **Nature Reviews Cardiology**, v. 9, p. 576-589, 2012.

RIGAS A, FARMAKIS D, PAPINGIOTIS G, BAKOSIS G, PARISSIS J. Hypothalamic dysfunction in heart failure: pathogenetic mechanisms and therapeutic implications. **Heart Fail Rev** [Internet]. 2018 Jan 19;23(1):55–61.

ROCHA, M.C.C.; RIBEIRO, A.L.P.; TEIXEIRA, M.M. Clinical management of chronic Chagas cardiomyopathy. **Front Biosci,** v. 8, p. 44-55, 2003.

ROCHA, M.O.C.; TEIXEIRA, M.M.; RIBEIRO, A.L.An update on the management of Chagas cardiomyopathy.**Expert Rev Anti Infect Ther,** v. 5, n. 4, p. 727-743, ago. 2007.

RUTLEDGE T, REIS VA, LINKE SE, GREENBERG BH, MILLS PJ. Depression in Heart Failure. **J Am Coll Cardiol** [Internet]. 2006 Oct;48(8):1527–37.

SANTOS, R.R., ROSSI, M.A., LAUS, J.L., SANTANA-SILVA, J., SAVINO, W., MEGEL, J. Anti-CD4 abrogates rejection and reestablishes long-term tolerance to syngeneic newborn hearts grafted in mice chronically infected with *Trypanosomacruzy*. **J Exp Med,** v.175, p. 29-39, 1992.

SCHEBELESKI-SOARES, C.; OCCHI-SOARES, R.C.; FRAZOI-DE-MORAIS, S.M.; DE OLIVEIRA DALALIO, M.M.; ALMEIDA, F.N., DE ORNELASTOLEDO, M.J., et al. Preinfection aerobic treadmill training improves resistance against *Trypanosomacruzi* infection in mice. ***Appl. Physiol. Nutr. Metab.***, v.34; 659-665, 2009.

SIMÕES, M.V.; ALMEIDA FILHO, O.C.; PAZIN FILHO, A.; CASTRO, R.B.P.; SCHMIDT, A.; MACIEL, B.C. *et al.* Insuficiência cardíaca na doença de Chagas. **Rev Soc Cardiol Estado de São Paulo**, v. 10, n. 1, p. 50-64, jan./fev. 2000.

STANTON R, REABURN P. Exercise and the treatment of depression: A review of the exercise program variables. **J Sci Med Sport** [Internet]. 2014 Mar 1;17(2):177–82.

STERIN-BORDA, L.J. Participation of autonomic nervous system in the pathogenesis of Chagas' Disease. **Act Physiol Pharmacol Thera Latinoan,** v. 44: 109-23, 1994.

STORINO, R.; MILEI, J. Estudios de seguimiento evolutivo em la enfermedad de Chagas. In: Madoery R, Madoery C, Cámera ML, orgs. Actualizaciones em la enfermedad de Chagas. Buenos Aires: **CongressoNacional de Medicina.** p.67-78, 1993.

TANG, W.S., CHU, E., HUI, T., HELMEST, D., LAW, C. Influence of exercise on sérum brain-derived neurotrophic factor concentrations in health human subjects. **Neuroscience Letters,** v.431, p. 62-65, 2007.

WILSON, L. S.; STROSBERGAM, A. M.; BARRIO, K. Cost-effectiveness of Chagas disease interventions in Latin America and the Caribbean: Markov models. **The American Journal of Tropical Medicine and Hygiene**, v. 73, p. 901-910, 2005.

WORLD BANK: THE WORLD BANK. WORLD DEVELOPMENT REPORT 1993. INVESTING IN HEALTH.WORLD DEVELOPMENT INDICATORS. WASHINGTON, OXFORD UNIVERSITY PRESS.

ZAHID I, BAIG MA, AHMED GILANI J, WASEEM N, ATHER S, FAROOQ AS, ET AL. Frequency and predictors of depression in congestive heart failure. **Indian Heart J** [Internet]. 2018 Dec 1;70:S199–203.

ZALETEL I, FILIPOVIĆ D, PUŠKAŠ N. Hippocampal BDNF in physiological conditions and social isolation. **Rev Neurosci** [Internet]. 2017 Jan 26;28(6):675–92.

ZOLADZ, J.A.; PILC, A. The effect od physical activity on the brain derived neurotrophic factor: from animal to human studies. **Journal of Physiology and Pharmacology,** v.61, n.5, p. 533-541, 2010.