UNIVERSIDADE ESTADUAL PAULISTA - UNESP
CÂMPUS JABOTICABAL

AVALIAÇÃO CARDÍACA DE EQUINOS SUBMETIDOS AO CONDICIONAMENTO EM ESTEIRA GUIADO PELA CONCENTRAÇÃO DE LACTATO

Maíra Moreira Santos
Médica Veterinária

2023

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Maíra Moreira Santos

Orientador: Prof. Dr. José Corrêa de Lacerda-Neto

Tese apresentada à Faculdade de Ciências Agrárias e Veterinárias – Unesp, Câmpus de Jaboticabal, como parte das exigências para a obtenção do título de Doutor em Medicina Veterinária, área: Clínica Médica.

2023

S237a

Santos, Maíra Moreira
Avaliação cardíaca de equinos submetidos ao condicionamento em esteira guiado pela concentração de lactato / Maíra Moreira Santos. -- Jaboticabal, 2023
46 p. : tabs.

Tese (doutorado) - Universidade Estadual Paulista (Unesp), Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal
Orientador: José Corrêa de Lacerda-Neto

1. Adaptação cardíaca. 2. Cavalo. 3. Ecocardiografia. 4. Fisiologia do exercício. 5. Treinamento. I. Título.

Sistema de geração automática de fichas catalográficas da Unesp. Biblioteca da Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal. Dados fornecidos pelo autor(a).

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Impacto potencial desta pesquisa: Espera-se com esta pesquisa, maior compreensão do remodelamento cardíaco do equino após o treinamento físico, visto que a espécie é susceptível aos problemas cardiovasculares, os quais afetam a saúde e o bem-estar, além de causarem redução do desempenho atlético.

Potential impact of this research: Through this research, a greater understanding of equine cardiac remodeling after physical training is expected, considering that the species is susceptible to cardiovascular problems which affect health and welfare, in addition to causing a reduction in athletic performance.

CERTIFICADO DE APROVAÇÃO

TÍTULO DA TESE: AVALIAÇÃO CARDÍACA DE EQUINOS SUBMETIDOS AO CONDICIONAMENTO EM ESTEIRA GUIADO PELA CONCENTRAÇÃO DE LACTATO

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Jaboticabal, 08 de maio de 2023

DADOS CURRICULARES DO AUTOR

Maíra Moreira Santos, nascida em Ubiratã - Paraná, em 04 de agosto de 1991. Médica Veterinária formada pela Universidade Estadual de Londrina em 2014. Durante a graduação participou de diversos trabalhos de pesquisas e realizou três projetos de iniciação científica. Na mesma instituição, concluiu a residência em Clínica Médica, Cirúrgica e Reprodução de Grandes Animais em 2017, e obteve o título de mestre em Ciência Animal em 2019. No mesmo ano, ingressou no Programa de Pós-Graduação em Medicina Veterinária, da Faculdade de Ciências Agrárias e Veterinárias da Universidade Estadual Paulista "Júlio de Mesquita Filho", câmpus de Jaboticabal.

“Mais inteligente é aquele que sabe que não sabe”

Sócrates

AGRADECIMENTOS

A Deus, por todas as bênçãos concedidas, pois sem Ele nada é possível.

Aos meus familiares, por todo apoio nas minhas decisões.

Ao prof. Dr. José Corrêa de Lacerda-Neto, pela oportunidade.

Às demais pós-graduandas do Laboratório de Medicina Esportiva Equina, que colaboraram na realização do projeto.

Aos meus colegas de pós-graduação, Gabriel Vieira Ramos e Isabela Marques de Figueiredo, que trabalharam com seriedade na execução do projeto, sendo imprescindíveis para o seu desenvolvimento.

Às residentes de grandes animais do HV da FCAV - UNESP, Angélica Cristina Titotto, Julia Maria Falavigna Romanini, Thayná da Cruz Paduan Silva e Theodora Giovanna Totti Ribeiro, por todo auxílio com os animais.

Ao funcionário do setor de equideocultura Odair Oian, por ter cuidado tão bem das "potras", com todo carinho e atenção, assim como pelo trabalho de doma realizado, o qual tornou possível o desenvolvimento do experimento.

Aos alunos do Colégio Técnico Agrícola, os quais ajudaram muito durante o período de estágio, principalmente no treinamento dos animais.

Aos colegas do LAFEQ, Nathali Adrielli Agassi de Sales, Thayssa Littiere e Guilherme Barbosa da Costa, que auxiliaram no trabalho em algumas ocasiões.

Aos proprietários Auroxochitl Monteiro Pinheiro e Almir José Ribeiro, que gentilmente emprestaram os animais para a pesquisa.

À Associação Brasileira dos Criadores do Cavalo Árabe (ABCCA), pela colaboração.

Ao Programa de Pós-Graduação em Medicina Veterinária da FCAV - UNESP, pela oportunidade e suporte acadêmico.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq: bolsa de pós-graduação 140407/2020-0), pela bolsa concedida.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) Código de Fincanciamento 001, pelo apoio financeiro.

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001.

À Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (processo nº 2020/11223-4), pelo auxílio à pesquisa.

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UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Câmpus de Jaboticabal

CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o projeto de pesquisa intitulado **"Adaptações musculoesqueléticas e cardíacas em eqüinos submetidos a treinamento guiado pelo lactato"**, protocolo nº 012601/19, sob a responsabilidade do Prof. Dr. José Corrêa de Lacerda Neto, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao Filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da lei nº 11.794, de 08 de outubro de 2008, no decreto 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), da FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS, UNESP - CÂMPUS DE JABOTICABAL-SP, em reunião ordinária de 10 de outubro de 2019.

Vigência do Projeto	01/11/2019 a 01/07/2022
Espécie / Linhagem	Puro sangue árabe
Nº de animais	12
Peso / Idade	360 kg / quatro à nove anos
Sexo	Machos ou fêmeas
Origem	Serão adquiridos de criatório da raça e alojados junto ao Setor de Equinocultura da Faculdade de Ciências Agrárias e Veterinárias/ UNESP, câmpus de Jaboticabal

Jaboticabal, 10 de outubro de 2019.

Fabiana Pilarski

Prof.ª Dr.ª Fabiana Pilarski
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AVALIAÇÃO CARDÍACA DE EQUINOS SUBMETIDOS AO CONDICIONAMENTO EM ESTEIRA GUIADO PELA CONCENTRAÇÃO DE LACTATO

RESUMO – O remodelamento cardíaco que ocorre com o treinamento físico pode ser identificado pela ecocardiografia, a qual permite avaliar a morfologia e a função cardíaca. Objetivou-se avaliar em equinos, o efeito do condicionamento na velocidade correspondente à concentração de 2 mmol/L de lactato, sobre variáveis ecocardiográficas. Foram treinados durante seis semanas em esteira, 12 potros Puro Sangue Árabe (09 fêmeas e 03 machos castrados), com idade de 28,42 ± 3,75 meses e peso corporal de 312,17 ± 17,37 kg, com base na velocidade em que a concentração do lactato plasmático foi igual 2 mmol/L (V_2), determinada em teste de exercício incremental (TEI). No mesmo teste, determinou-se a velocidade em que o lactato sanguíneo atingiu 4 mmol/L (V_4). Foram realizadas ecocardiografias em repouso nos modos-B e M, Doppler pulsátil de fluxo e Doppler tecidual. A partir de medidas do modo M, foram calculadas a espessura média (EMP) e relativa (ERP) da parede livre do ventrículo esquerdo (VE), massa do VE (mVE), e as frações de encurtamento (FE) e de ejeção (FEj) do VE. As ecocardiografias foram realizadas antes (AT) e depois (DT) do período de treinamento físico, assim como o TEI para a determinação do nível de condicionamento. Empregaram-se o teste t para a comparação ao nível de 5% de significância e a análise por componentes principais (ACP). Todos os animais completaram o programa de condicionamento sem intercorrências. Houve aumento na V_2 (AT: 5,2 ± 0,3; DT: 6,7 ± 0,4 m/s) e V_4 (AT: 5,8 ± 0,4; DT: 7,6 ± 0,5 m/s) (p < 0,001). O diâmetro diastólico (AT: 9,11 ± 0,72; DT: 9,87 ± 0,75 cm) (p = 0,007), a massa (AT: 1.690,86 ± 372,64; DT: 1.886,90 ± 269,57 g) (p = 0,011) e o volume ejetado (VEj) (AT: 444,60 ± 129,62; DT: 538,40 ± 100,43 mL) (p = 0,042) do ventrículo esquerdo se elevaram. A parede livre do VE em diástole (PLVEd) (AT: 1,87 ± 0,27; DT: 1,84 ± 0,30 cm) e em sístole (PLVEs) (AT: 3,36 ± 0,22; DT: 3,40 ± 0,24 cm) não se modificou (p = 0,654) e (p = 0,617), respectivamente. A ACP reduziu a dimensionalidade de 24 variáveis selecionadas em sete componentes, os quais explicam 84% da variabilidade total. No componente um, o qual explica 22% da variabilidade total, as variáveis que apresentaram maior contribuição foram: átrio esquerdo (AE), débito cardíaco (DC), diâmetro interno do VE em diástole (DIVEd) e sístole (DIVEs), EMP, PLVEd, mVE, espessura do septo interventricular em diástole (SIVd), VEj, FEj, FE, velocidade máxima do enchimento ventricular inicial do fluxo transmitral (Emáx) e sua relação com a velocidade final (E:A). O protocolo de condicionamento empregado foi eficiente, pois melhorou a capacidade aeróbia dos animais. Ocorreu remodelamento cardíaco induzido principalmente pelo treinamento físico, com aumento da massa ventricular e do volume ejetado.

Palavras-chave: adaptação cardíaca, cavalo, ecocardiografia, fisiologia do exercício, treinamento

CARDIAC EVALUATION OF HORSES SUBMITTED TO LACTATE CONCENTRATION-GUIDED CONDITIONING ON A TREADMILL

ABSTRACT – Physical training-induced cardiac remodeling can be identified by echocardiography in wich allows to evaluate the heart's morphology and function. This study evaluated the effect of conditioning based on the velocity corresponding to a concentration of 2 mmol/L of lactate on echocardiographic measurements of horses. Twelve Purebred Arabians horses (09 filly and 03 geldings) with 28,42 ± 3,75 months old and body weight 312,17 ± 17,37 kg were trained during six weeks, based on the velocity wich plasmatic lactate concentration was 2 mmol/L (V_2) determined in an incremental exercise test (IET). Also, the velocity that blood lactate reached 4 mmol/L (V_4) was determined in the same test. Echocardiographic exams were performed at rest in the B and M-mode, pulsed Doppler, and tissue Doppler imaging. From M-measurements were calculated: the mean (MWT) and relative (RWT) free wall thickness of the left ventricle (LV), LV mass, fractional shortening (FS), and ejection fraction (EF) . The exams were performed before (BT) and after (AT) the physical training period as well as IET for conditioning level determination. The t-test was employed for comparison at a significance level of 5%, along with principal components analysis (PCA). All animals completed the training program with no intercurrence. The velocities increased V_2 (BT: 5.2 ± 0.3; AT: 6.7 ± 0.4 m/s) e V_4 (BT: 5.8 ± 0.4; AT: 7.6 ± 0.5 m/s) ($p < 0.001$). End-diastolic left ventricular internal diameter (LVIDd) (BT: 9.11 ± 0.72; AT: 9.87 ± 0.75 cm) ($p = 0.007$), LV mass (BT: 1690.86 ± 372.64; AT: 1886.90 ± 269.57 g) ($p = 0.011$), and stroke volume (SV) (BT: 444.60 ± 129.62; AT: 538.40 ± 100.43 mL) ($p = 0.042$) increased. There were no changes in left ventricular free wall at end-diastole (LVFWd) (BT: 1.87 ± 0.27; AT: 1.84 ± 0.30 cm) and end-systole (LVFWs) (BT: 3.36 ± 0.22; AT: 3.40 ± 0.24 cm) ($p = 0.654$) and ($p = 0.617$), respectively. The PCA reduced the dimensionality of 24 cardiac variables into seven components, which account for 84% of the total data variability. In component one, which explains 22% of the total variability, there was a greater contribution of left atrium (LA), cardiac output (CO), LVIDd, end-systolic left ventricular internal diameter (LVIDs), MWT, LVFWd, LV mass, end-diastolic interventricular septum thickness (IVSd), SV, EF, FS, the peak E wave (Emax) of the transmitral flow, and the E_{max} to A_{max} ratio (E_{max}:A_{max}). The conditioning protocol was effective to improve the aerobic capacity of the animals. There was cardiac remodeling induced mainly by physical training, with increase of left ventricular mass and stroke volume.

Keywords: cardiac adaptation, echocardiography, equine, exercise physiology, training

LISTA DE ABREVIATURAS

AE	Átrio esquerdo
AE/Ao	Relação átrio esquerdo artéria aorta
Am	Velocidade diastólica final do átrio esquerdo
$A_{máx}$	Velocidade máxima do enchimento ventricular final
Ao	Diâmetro aórtico
DC	Débito cardíaco
DIVEd	Diâmetro interno do ventrículo esquerdo em diástole
DIVEs	Diâmetro interno do ventrículo esquerdo em sístole
Em	Velocidade diastólica inicial
$E_{máx}$	Velocidade máxima do enchimento ventricular inicial
EMP	Espessura média de parede
ERP	Espessura relativa de parede
FC	Frequência cardíaca
FE	Fração de encurtamento
FEj	Fração de ejeção
ITV	Integral tempo-velocidade
IS	Índice sitólico
MVE	massa do ventrículo esquerdo
PLVEd	Parede livre do ventrículo esquerdo em diástole
PLVEs	Parede livre do ventrículo esquerdo em sístole
PPE	Período pré-ejeção
SIVd	Septo interventricular em diástole
SIVs	Septo interventricular em sístole
Sm	Velocidade de contração
TEj	Tempo de ejeção
TEji	Tempo de ejeção indexado à frequência cardíaca
TPP	Tempo para o pico
TRIV	Tempo de relaxamento isovolumétrico
Vcf	Velocidade média de encurtamento de fibras circunferenciais
$V_{máx}$	Velocidade máxima
$\dot{V}O_{2máx}$	Consumo máximo de oxigênio

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CAPÍTULO 2 – Cardiac changes after lactate-guided conditioning in young purebred Arabian horses.

CAPÍTULO 1 – CONSIDERAÇÕES GERAIS

1. Introdução

O treinamento físico a longo prazo induz a formação do "coração de atleta", o qual inclui adaptações morfológicas, elétricas e funcionais, tanto em humanos quanto em animais (Shave et al., 2017; Mahjoub et al., 2019). Sabe-se que em equinos há correlação positiva entre diâmetro ventricular e capacidade aeróbia (Young et al., 2002) e portanto, entre tamanho cardíaco e desempenho atlético, conforme constatado em cavalos de corrida e de enduro (Sleeper et al., 2014; Fintl et al., 2020). O "coração de atleta" pode ser avaliado por meio da ecocardiografia, a qual é uma técnica não-invasiva, que auxilia no diagnóstico e prognóstico de doenças cardíacas (Schwarzwald, 2019).

A literatura médica acerca do assunto é abundante, pois o desafio é diferenciar o "coração de atleta" de condições patológicas, uma vez que as características morfológicas e eletrocardiográficas são similares (Jakubiak et al., 2021). Entretanto, na medicina esportiva equina, apesar de haver trabalhos que comprovem que os equinos também são propensos ao remodelamento observado em atletas humanos, assim como à queda de desempenho por problemas cardíacos, pouco se sabe sobre o seu desenvolvimento (Shave et al., 2017; Navas de Solis, 2019).

Muitas pesquisas foram realizadas com cavalos de centros de treinamento, e portanto, sem padronização da carga do condicionamento, o qual foi realizado de forma empírica (Buhl et al., 2005; Gehlen et al., 2019). Visto que o grau de adaptação cardíaca é dependente da intensidade, volume e duração do treinamento (Buhl et al., 2004a; D'Andrea et al., 2018), a realização de estudos padronizados são fundamentais para a compreensão do remodelamento cardíaco nessa espécie. Além do mais, a prescrição do treinamento físico de equinos pode ser realizada de forma objetiva, a fim de elevar o ganho no condicionamento e prevenir lesões esportivas (De Maré et al., 2022).

O objetivo deste estudo foi avaliar a adaptação cardíaca em equinos jovens da raça Puro Sangue Árabe, submetidos ao programa de condicionamento físico baseado na velocidade em que a concentração de lactato plasmático atinge 2 mmol/L, durante seis semanas, por meio da ecocardiografia.

2. Revisão de literatura

2.1 Adaptação cardiovascular ao treinamento

O treinamento consiste no exercício físico regular, com elevação progressiva da intensidade, o qual desencadeia adaptações estruturais e funcionais nos sistemas músculo-esquelético e cardiovascular (Poole e Erickson, 2014). A notável capacidade atlética do equino se deve às respostas cardiovasculares durante o exercício máximo, como a elevação da frequência cardíaca (FC) de 20 a 240 batimentos por minuto e do volume ejetado (VEj), que passa de 2-2,5 mL/kg em repouso, para 3-4 mL/kg. Essas alterações associadas à grande reserva esplênica de células vermelhas, aumentam o débito cardíaco consideravelmente e otimizam o transporte de oxigênio (Young, 2003; Poole e Erickson, 2014). Além disso, a massa cardíaca do cavalo é maior do que de espécies que não são atléticas, podendo atingir de 0,9 a 1% da massa corpórea, e de 1,1 a 2% em cavalos de elite (Poole e Erickson, 2014).

A longo prazo, o treinamento físico intenso induz alterações cardíacas estruturais, funcionais e elétricas, as quais são conhecidas como “coração de atleta” em humanos (Morganroth et al., 1975), cães (Restan et al., 2022) e cavalos (Young et al., 2005; Buhl e Ersbøll, 2012; Sleeper et al., 2014). A primeira observação de que humanos atletas apresentam coração maior foi determinada por meio de percussão torácica em 1899 (Henschen, 1899). O remodelamento cardíaco pode ser avaliado por meio de exames de imagem, sendo a ecocardiografia o método mais acessível e empregado em equinos (Young et al., 2002; Young et al., 2005). De modo geral e simplista, são descritas duas morfologias cardíacas em humanos, a depender do tipo de treinamento predominante.

Com o treinamento de resistência, isto é, exercício dinâmico, a sobrecarga de volume causada pelo débito cardíaco elevado, leva ao aumento do volume e da espessura da parede do ventrículo esquerdo (VE). Quando a relação entre diâmetro e espessura de parede do VE é mantida, a alteração é classificada como hipertrofia excêntrica. Por outro lado, quando o componente de força prevalece (exercício estático), a hipertrofia é concêntrica, ou seja, há aumento da espessura da parede provocada pela sobrecarga de pressão, com alterações mínimas no diâmetro (Morganroth et al., 1975).

Em cavalos de corrida (exercício de duração curta e intensidade alta) e de enduro (duração longa e intensidade moderada) são observados aumento da câmara ventricular e da espessura média da parede do VE, porém sem alteração da espessura relativa, o que condiz com a adaptação ao treinamento de resistência (Buhl, 2008; Sleeper et al., 2014; Gehlen et al., 2019). No entanto, em equinos Puro Sangue Inglês foi constatada hipertrofia mista, com aumento tanto do diâmetro ventricular quanto da espessura relativa da parede (Young, 1999).

Além do aumento do diâmetro do VE e da espessura de parede, ocorre dilatação do átrio esquerdo em humanos e cães atletas, a qual é compensatória ao aumento do volume do VE (D'Andrea et al., 2018; Restan et al., 2022). No entanto, os estudos com cavalos objetivam avaliar o VE, e até o momento, pouca atenção foi dada à adaptação do AE. Ademais, em humanos que realizam treinamento de resistência, o diâmetro e a espessura da parede do ventrículo direito (VD) também se elevam, de modo semelhante ao VE (D'Andrea et al., 2018). Em cavalos de corrida, também foi observado aumento do diâmetro do VD. Contudo, devido às suas peculiaridades anatômicas, que dificultam a obtenção das imagens e a repetibilidade das mensurações, há poucos estudos do ventrículo direito nessa espécie (Lightfoot et al., 2006; Young et al., 2006).

Quanto ao tamanho cardíaco, constatou-se em cavalos de corrida por meio da ecocardiografia, correlação positiva entre massa ventricular, a qual tem relação com a espessura de parede e diâmetro diastólico, e consumo máximo de oxigênio ($VO_{2máx}$). Ou seja, quanto maior a câmara cardíaca, maior a capacidade aeróbia (Young et al., 2002). Além do mais, foi observada relação entre diâmetro ventricular, função sistólica e desempenho em corridas (Seder et al., 2003; Young et al., 2005). Em cavalos de enduro, também constatou-se relação entre diâmetro do VE e desempenho atlético (Sleeper et al., 2014).

Após o condicionamento físico, no equino com aptidão aeróbia, pode haver aumento de 10-25% do consumo de oxigênio, com elevação do volume ejetado e da capacidade de difusão muscular de O_2 durante o exercício. Ademais, a frequência cardíaca máxima e a pressão arterial média não se alteram, mas há redução da resistência vascular periférica. A sensibilidade cardíaca aos mediadores vasoativos se eleva, assim como na musculatura esquelética (Poole e Erickson, 2014).

Ao contrário do que é relatado em humanos (Bahrainy et al., 2016) e cães (Restan et al., 2022), a frequência cardíaca do cavalo em repouso pode não se alterar após o condicionamento (Gehlen e Schlaga, 2019; Lindner et al., 2020). Porém, pode haver redução da FC durante o exercício submáximo. Na maior parte dos trabalhos com equinos em treinamento, não foi constatada alteração da FC em repouso, enquanto que em alguns estudos houve redução. Estas divergências podem ser atribuídas aos diferentes programas de condicionamento empregados. Entretanto, na espécie equina, ainda é necessária investigação no âmbito genético, visto que em humanos, múltiplos genes estão envolvidos com a adaptação da FC ao treinamento (Lindner et al., 2020).

Ao se avaliar as medidas cardíacas deve-se considerar que os valores de referências podem variar entre raças, idade, crescimento, estado de treinamento e gestacional, tamanho corporal, e possivelmente entre gênero (Seder et al., 2003; Buhl et al., 2005; Young et al., 2005; Al-Haidar et al., 2013; Al-Haidar et al., 2017). Sabe-se que o tipo, a intensidade, o volume e a duração do treinamento ao longo dos anos, e a predisposição do indivíduo, parecem influenciar na resposta adaptativa (Prince et al., 2002; Young et al., 2005; Mahjoub et al., 2019; Lindner et al., 2020). De modo geral, o remodelamento cardíaco fisiológico, decorrente do condicionamento, difere do patológico. Entretanto, o exercício intenso e prolongado pode levar ao remodelamento com efeitos adversos e complicações (Bernardo e McMullen, 2016).

Com o remodelamento cardíaco induzido pelo treinamento físico, insuficiências valvulares com regurgitação e arritmias podem se desenvolver (Buhl e Ersbøll, 2012; Sleeper et al., 2014). Essas alterações podem ser consideradas fisiológicas, dependendo do grau da regurgitação e do tipo de arritmia (Gehlen e Schlaga, 2019; Massie et al., 2021). Nas regurgitações fisiológicas, as valvas encontram-se normais e não há alteração cardíaca secundária (Buhl e Ersbøll, 2012). Por outro lado, quando patológicas, estão associadas às alterações estruturais e hemodinâmicas, as quais podem causar redução do desempenho e elevar o risco de morte súbita (Hövener et al., 2021; Navas de Solis, 2016; Chope, 2019).

Quanto às arritmias, não são necessariamente relacionadas à hipertrofia induzida pelo exercício, sendo resultantes, provavelmente, do desequilíbrio entre sistema nervoso simpático e parassimpático (Buhl et al., 2013). Além das alterações ventriculares e valvares, a sobrecarga de volume induzida pelo treinamento leva ao aumento atrial, o qual é um processo dinâmico e reversível. Entretanto, em atletas humanos de elite, o remodelamento atrial a longo prazo pode estar associado ao risco elevado de fibrilação atrial (D'Andrea et al., 2018).

Em um estudo retrospectivo com 262 atletas humanos de exercícios de resistência, participantes de competições nacionais e internacionais, foram observadas na eletrocardiografia, alterações adaptativas e anormais em 63% e 3,8%, respectivamente. No exame ecocardiográfico, detectou-se que 98% apresentavam adaptação ao treinamento e em 26%, as alterações cardíacas excediam o que se considera normal para o atleta. De acordo com os pesquisadores, os achados de adaptação na eletrocardiografia e os anormais na ecocardiografia, estavam associados com a idade, volume de treino semanal e anos de treinamento (Jakubiak et al., 2021).

Por outro lado, as pesquisas realizadas até o momento na medicina esportiva equina não permitem a definição do que é normal e anormal no remodelamento cardíaco (Shave et al., 2017). Ademais, há escassez de estudos controlados, sobretudo com a padronização da carga do treinamento e realização de programa de condicionamento guiado por parâmetros fisiológicos, os quais ajudariam na compreensão do remodelamento em cavalos. Com isso, seria possível identificar o limiar de "toxicidade" do treinamento intenso e qual o programa de treinamento ideal para conferir proteção cardiovascular e resistência (Mahjoub et al., 2019).

2.2 Ecocardiografia

A ultrassonografia cardíaca é frequentemente utilizada para o diagnóstico de doenças cardíacas congênitas e adquiridas, bem como para o monitoramento da resposta ao tratamento e da progressão da doença (Boon, 2011). Além disso, o exame auxilia no estabelecimento do prognóstico e utilização do cavalo para o esporte, uma vez que detecta problemas que podem desencadear arritmias ventriculares, embora a sua sensibilidade para esse fim seja incerta (Navas de Solis, 2020). A correlação positiva entre tamanho cardíaco e capacidade aeróbia do cavalo atleta foi constatada há muito tempo (Buhl et al., 2005; Young et al., 2005). Entretanto, ainda não foi esclarecido se a ecocardiografia pode ajudar na seleção de equinos com maior aptidão atlética ou na orientação do condicionamento físico, devido à escassez de estudos delineados para essas finalidades (Shave et al., 2017).

A ecocardiografia transtorácica é uma técnica não invasiva que permite avaliar o tamanho, morfologia e função cardíaca (Long et al., 1992; Boon, 2011). Dentre as principais indicações para a realização do ecocardiograma no cavalo estão: suspeita de defeitos congênitos ou de doença do miocárdio, abafamento de sons cardíacos à auscultação, suspeita de endocardite ou pericardite, febre de origem desconhecida, doença respiratória grave, episódio de fraqueza, intolerância ao exercício, redução do desempenho atlético ou colapso, casos de arritmia clinicamente relevante e sopro cardíaco (Marr e Patteson, 2010; Schwarzwald, 2019). Quanto ao sopro cardíaco, embora o grau à auscultação não esteja diretamente relacionado à gravidade (Hövener et al., 2021) e a ecocardiografia seja recomendada em casos a partir do grau 3 (3/6), a necessidade do exame ecocardiográfico em graus menores deve ser considerada com base no histórico e exame clínico (Schwarzwald, 2019).

Diferentes métodos de ecocardiografia têm sido empregados em cavalos, tais como modo bidimensional (modo-B), unidimensional (modo-M), tridimensional (modo-3D), Doppler pulsátil, Doppler tecidual e *speckle tracking* bidimensional (Schwarzwald, 2019). Apesar de várias opções disponíveis, ainda é necessário estabelecer a relevância clínica de alguns desses métodos para a espécie equina (Decloedt et al., 2017; Koening et al., 2017). Além do mais, a ecocardiografia em cavalos apresenta algumas limitações fisiológicas, anatômicas e técnicas, as quais serão destacadas a seguir.

Para realizar a ecocardiografia em cavalos adultos, é necessário um equipamento com transdutor setorial com frequência que varia de 1,3 a 3,5 MHz e profundidade de penetração de 25 a 30 cm. Para as mensurações do fluxo sanguíneo, é imprescindível a obtenção do eletrocardiograma simultâneo, para determinar corretamente os eventos do ciclo cardíaco (Schwarzwald, 2019). As mensurações podem ser feitas *off-line* a partir das imagens e vídeos salvos, entretanto, a avaliação qualitativa das estruturas é feita em tempo real. É importante que o exame seja feito por um ultrassonografista experiente, pois a baixa qualidade das imagens, mensurações incorretas e mal alinhamento do feixe para se obter o Doppler, podem levar à interpretação errônea (Buhl et al., 2004b).

Além disso, para se avaliar a evolução das alterações em exames seriados, a repetibilidade das medidas é essencial. Em virtude da influência fisiológica e da técnica nas medidas ecocardiográficas, foram realizados diversos estudos de repetibilidade (intraobservador), reprodutibilidade (interobservador) e variação entre diferentes dias de obtenção das imagens ou realização das medidas (Voros et al., 1991; Young e Scott, 1998; Buhl et al., 2004b; Schwarzwald et al., 2007; Schwarzwald et al., 2009a; Schwarzwald et al., 2009b). Apesar das limitações de cada método ecocardiográfico, é possível empregar a ecocardiografia em cavalos, desde que haja o devido conhecimento da variabilidade das medidas, a fim de garantir a interpretação correta (Buhl et al., 2004b). Ademais, é importante ressaltar que o ecocardiograma deve ser interpretado juntamente com o histórico do animal e achados da auscultação (Reef et al., 2014).

Os métodos tradicionais de exame ecocardiográfico consistem nos modos-B, M e Doppler, os quais são utilizados tanto no diagnóstico de doenças quanto para avaliar as alterações induzidas pelo treinamento físico (Buhl et al., 2004b). Os modos B e M são utilizados para avaliar o tamanho das estruturas cardíacas e quantificar parâmetros funcionais. Inicialmente, a avaliação cardíaca é feita em modo-B, em que é possível escanear o coração e os grandes vasos em diferentes planos de imagem obtidos pela janela paraesternal direita. Em tempo real, são observados o diâmetro das câmaras ventriculares e atriais, as valvas e o padrão de movimentação das estruturas de forma geral (Long et al., 1992). Além disso, podem ser obtidas imagens para a mensuração das estruturas. A avaliação da função das valvas pode ser complementada com a aplicação do Doppler colorido, sendo de grande valia para a diferenciação das regurgitações fisiológicas e patológicas, uma vez que o equino apresenta prevalência elevada de regurgitação fisiológica (Hövener et al., 2021).

O modo-M, por sua vez, permite avaliar a movimentação das estruturas ao longo do tempo. As imagens obtidas por esse método não são limitadas pela taxa de quadros, que é a velocidade em que as imagens são adquiridas e exibidas por segundo. Portanto, as imagens do modo-M apresentam alta resolução temporal, permitindo uma análise detalhada e precisa do movimento ao longo do ciclo cardíaco (Schwarzwald, 2019). Além do mais, a angulação do cursor do modo-M poder ser corrigida, uma vez que é guiada pela imagem do modo-B, gerando mensurações mais precisas do que aquelas realizadas na imagem bidimensional (Long et al., 1992). No entanto, a desvantagem do modo-M é o seu alinhamento fixo com a direção do feixe de ultrassom, o que impossibilita a visualização das estruturas em outras dimensões. Ademais, podem ocorrer artefatos de movimento que resultam em imprecisões nas medidas. Apesar disso, as medidas no modo-M, em conjunto com o modo-B, apresentam maior reprodutibilidade do que as obtidas pelo Doppler (Buhl et al., 2004b).

A partir da imagem do modo M, são realizadas as mensurações rotineiras, que incluem o diâmetro dos ventrículos, a espessura das paredes e do septo interventricular, entre outras (Schwarzwald, 2019). Com as medidas da câmara ventricular, é possível calcular alguns índices de função sistólica, que refletem a capacidade de contração e de ejeção do sangue pelo ventrículo. Em cavalos, os índices de função sistólica mais utilizados são o volume ejetado (VEj), fração de ejeção (FEj), fração de encurtamento (FE) e débito cardíaco (DC) (Poole e Erickson, 2014). Com a adaptação do cardíaca induzida pelo treinamento, que envolve sobrecarga de volume e remodelamento, espera-se melhoria no desempenho cardíaco (D’Andrea et al., 2018). No entanto, os resultados dos índices de função em cavalos treinados são controversos, sendo observado aumento, redução ou ausência de alteração (Patteson, 1993; Young, 1999; Gehlen et al., 2019). Porém, sabe-se que o método de cálculo pode influenciar o VEj, FEj e FE, além desses índices serem sensíveis à frequência cardíaca, pré e pós-carga e contratilidade (Patteson, 1993; Boon, 2011; Poole e Erickson, 2014).

A função cardíaca também pode ser avaliada por meio do Doppler pulsátil e tecidual, que mensuram a velocidade do fluxo sanguíneo e dos tecidos, respectivamente. Para a obtenção do Doppler pulsátil, é fundamental o alinhamento do feixe de ultrassom o mais paralelo possível com o fluxo sanguíneo (Boon, 2011). O melhor alinhamento para avaliação do fluxo transmitral e da movimentação do VE é obtido pela imagem apical cinco câmaras esquerda, a qual é realizada em pequenos animais. No entanto, a verdadeira imagem apical esquerda não é possível de ser obtida em cavalos adultos, devido à limitação anatômica (Boon, 2011). De qualquer forma, as imagens do fluxo transmitral e aórtico de cavalos podem ser obtidas pela janela paraesternal esquerda (Schwarzwald, 2019).

A partir do Doppler pulsátil, obtêm-se os intervalos de tempo sistólicos, os quais complementam os índices da fase de ejeção, fornecendo informação da função sistólica global do ventrículo (Blissit e Bonagura, 1995). Com o Doppler tecidual, é possível avaliar a função regional do miocárdio, a função sistólica e diastólica ventricular. Entretanto, no equino, o Doppler tecidual permite avaliar apenas a movimentação radial, desconsiderando a movimentação complexa do VE, uma vez que essa avaliação é feita na imagem de eixo curto do VE obtida pela janela paraesternal direita (Schwarzwald et al., 2009a). Além disso, os índices obtidos pelo Doppler são influenciados pelas condições de carga, contratilidade e frequência cardíaca (Boon, 2011).

Para maior compreensão do efeito do treinamento na função do miocárdio de cavalos, é interessante associar diferentes técnicas. No entanto, até o momento, foi encontrado apenas um estudo que empregou o Doppler tecidual e o *speckle tracking*, para avaliar a velocidade e os parâmetros de deformação do miocárdio de cavalos atletas, concluindo que esses métodos podem ser utilizados em pesquisas semelhantes (Gehlen et al., 2019).

Ao realizar o exame ecocardiográfico, devem-se considerar todos os fatores relacionados à técnica e aqueles inerentes ao cavalo, como raça, idade, sexo, gestação e nível de treinamento (Buhl et al., 2004a; Buhl et al., 2005; Al-Haidar et al., 2013; Al-Haidar et al., 2017) Além disso, a dimensão do coração está associada ao tamanho corporal, o que impede a utilização de um único valor de referência das medidas cardíacas para todas as raças (Fintl et al., 2020). Em virtude disso, as medidas ecocardiográficas de dimensão, devem ser indexadas às medidas morfométricas, como circunferência torácica e peso corporal (Al-Haidar et al., 2017). A medida comumente empregada é o peso corporal, sendo comum a indexação em cavalos com peso médio de 500 kg (Trachsel et al., 2016; Schwarzwald, 2019). No entanto, na literatura consultada, não foram encontrados trabalhos com cavalos menores, como os da raça Árabe, que tivessem os valores ecocardiográficos indexados. Em relação aos valores de referência, o emprego de equações de regressão para predizer o valor de normalidade dentro de cada raça vem sendo estudado, o que eleva a precisão das medidas ecocardiográficas no diagnóstico de doenças cardíacas (Al-Haidar et al., 2017; Fintl et al., 2020).

2.3 Condicionamento físico baseado na velocidade correspondente à concentração fixa de lactato

O principal fator limitante do desempenho do cavalo atleta pode ser a eficiência do programa de condicionamento (Von Wittke et al., 1994). Mediante a realização de teste de exercício incremental, o qual pode ser realizado tanto em laboratório quanto a campo, obtém-se dados do desempenho atlético, que servem para seleção de cavalos e embasamento do programa de condicionamento (Evans et al., 1993; Von Wittke et al., 1994; Lindner, 2010a).

Com isso, o treinamento físico deixa de ser empírico e possibilita a maximização do desempenho (Campbell, 2011). Ademais, prepara o animal para a competição, atrasa o início da fadiga, promove melhoria das habilidades e capacidade do cavalo, enquanto mantém o entusiasmo para o exercício e a integridade física (Castejon-Riber et al., 2017). Além disso, o início do treinamento de animais imaturos é controverso e causa apreensão, pela crença de que pode impactar negativamente na longevidade e desempenho do cavalo. Contudo, visto que o exercício pode ser benéfico para o desenvolvimento das estruturas músculo-esqueléticas, há necessidade de se estabelecer a carga externa ideal de treinamanento (Logan e Nielsen, 2021).

Um dos métodos de avaliação do potencial atlético é a curva lactato-velocidade, a qual fornece parâmetros para definir a carga do exercício, a fim de produzir estímulo apropriado para o condicionamento (Von Wittke et al., 1994; Campbell, 2011; Ferraz et al., 2022). A relação lactato-velocidade é empregada em diversos métodos de treinamento em humanos, cães e equinos (Lindner et al., 2009; Orozco et al., 2021;

De Maré et al., 2022; Restan et al., 2022; Santos et al., 2022). Durante o exercício, o ponto de início do desequilíbrio entre produção, remoção e metabolização do lactato é conhecido como limiar de lactato. Portanto, o limiar de lactato é utilizado na avaliação da capacidade aeróbia (Ferraz et al., 2022) e parece ser a carga de treinamento ideal (Lindner, 2010b; De Maré et al., 2022).

A máxima fase estável do lactato (MFEL) é o teste padrão ouro para a avaliação da capacidade aeróbia em humanos (Hering et al., 2018), cães (Ferraz et al., 2022) e equinos (Lindner, 2010b; Soares et al., 2014). A MFEL representa a velocidade máxima que pode ser sustentada entre 5 e 25 min de exercício, sem que a concentração de lactato sanguíneo aumente mais que 1 mmol/L (Lindner, 2010b). No entanto, para a definição da MFEL, um único animal deve ser submetido entre quatro e seis sessões de exercício em dias diferentes, inviabilizando seu emprego na rotina. Em virtude disso, métodos alternativos são estudados a fim de se predizer o limiar de lactato por meio de testes mais simples (Lindner, 2010b; Soares et al., 2014; De Maré et al., 2022).

Para se determinar o limiar de lactato do cavalo, diversos modelos com concentrações fixas de lactato têm sido pesquisados (Werkmann et al., 1996; Lindner et al., 2009; Lindner, 2010b). Em cavalos, sabe-se que a velocidade da MFEL se aproxima da $V_{1,5}$ e V_2, ou seja, velocidade em que a concentração sanguínea de lactato atinge 1,5 e 2 mmol/L (Gondim et al., 2007; Lindner, 2010b). Além da intensidade, a duração das sessões do programa de condicionamento também interfere nas respostas benéficas vistas na musculatura esquelética (Gansen et al., 1999; Rivero et al., 2007). Diante disso, o treinamento guiado pela $V_{1,5}$ ou $V_{2,}$ as quais predizem o limiar de lactato do equino com maior acurácia, pode fornecer estímulo apropriado para melhoria da capacidade aeróbia.

Por muito tempo a V_4 foi considerada um bom fator de predição da capacidade aeróbia e relacionada ao desempenho de equinos (Couroucé et al., 1997; Couroucé et al., 2000; Sommer et al., 2015). Entretanto, essa informação foi extraída de pesquisa com humanos e há várias evidências apontando, que na maioria das vezes, a V_4 superestima a MFEL e, consequentemente, o limiar de lactato do cavalo (Heck et al., 1985; Lindner, 2010b, Miranda et al., 2014; Soares et al., 2014). Portanto, a V_4 representa intensidade muito alta, e não deve ser empregada em programa de condicionamento, que tem a finalidade de melhorar a capacidade aeróbia (Soares et al., 2014). Além do mais, todos os métodos que utilizaram valores fixos de lactato parecem não predizer a MFEL de maneira confiável (Gondim et al., 2007; De Maré et al., 2022). Apesar disso, esses métodos são empregados na determinação do condicionamento, pois protocolos de exercício adequados para a avaliação de equinos de diversas modalidades esportivas, ainda precisam ser estabelecidos (De Maré et al., 2022).

Devido à necessidade de maior compreensão da fisiologia cardíaca de cavalos atletas, realizou-se uma pesquisa, a qual será abordada no capítulo seguinte. Nesse estudo, equinos jovens que não tinham realizado nenhum tipo de exercício controlado foram submetidos ao treinamento físico, com carga interna estabelecida individualmente. O programa de treinamento foi baseado na velocidade em que a concentração de lactato atingiu 2 mmol/L, o qual foi adaptado de um estudo previamente publicado (Trilk et al., 2002). Ao término do programa de treinamento, foram constatadas melhoria na capacidade aeróbia e alterações cardíacas morfológicas e funcionais, detectadas por meio da ecocardiografia.

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CAPÍTULO 2 – Cardiac Changes after Lactate-Guided Conditioning in Young Purebred Arabian Horses [1]

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Simple Summary: Horses can develop "athlete's heart" in a similar way to humans and dogs after physical training. The purpose of this study was to assess by echocardiography the cardiac changes of young purebred Arabian horses after performing a lactate-guided conditioning program. The conditioning program was established individually, according to the physical fitness of each animal, as determined by an incremental exercise test (IET). The IET and echocardiograms were carried out before and after six weeks of treadmill conditioning. At the end of the conditioning period, the horses showed improved aerobic capacity and structural and functional heart changes associated with "athlete's heart."

Abstract: Cardiac adaptation to conditioning in horses was evaluated after empirical training based on trainers' experience. Twelve purebred Arabian horses, aged (mean ± SD) 28.42 ± 3.75 months, which did not perform any type of exercise prior to the research, were submitted to treadmill conditioning for six weeks. The conditioning

[1] Este capítulo corresponde ao artigo científico submetido à revista 'Animals'.

program was based on the velocity run by the horse at which the blood lactate concentration, determined in an incremental exercise test (IET), reached 2 mmol/L (V_2). The velocity at which the blood lactate concentration reached 4 mmol/L (V_4) was also determined. The echocardiograms were performed at rest with pulsed-wave and tissue Doppler imaging in B- and M-modes. All procedures were carried out before and after the conditioning period. The results showed increases in V_2 (from 5.2 ± 0.3 to 6.7 ± 0.4 m/s) and V_4 (from 5.8 ± 0.4 to 7.6 ± 0.5 m/s) ($p < 0.0001$). There were also increases in the left ventricle internal diameter at diastole (LVIDd), left ventricle mass (LV mass), and stroke volume (SV), while no changes were observed in the LV free wall thickness and mean and relative wall thicknesses. The conditioning protocol, which was completed by all horses, proved to be safe and efficient, as it improved the aerobic capacity of the animals. Finally, the cardiac remodeling that occurred was mainly associated with the effect of physical training.

Keywords: athlete's heart; cardiology; cardiovascular adaptation; echocardiography; equines; exercise physiology

1. Introduction

Cardiac remodeling in horses can be assessed by echocardiography, a procedure that accurately identifies training-associated changes [1–5]. Long-term intensive training leads to a cardiac adaptation known as "athlete's heart," which comprises two distinct morphologies [6]. In horses, the most common change found is eccentric hypertrophy, characterized by an increased left ventricular volume and the proportional increase in the myocardial wall thickness, thus maintaining the relative thickness. This adaptation observed in racing and endurance horses resembles the heart of human athletes who perform aerobic sports [2–4,7]. Concentric hypertrophy, on the other hand, is determined by wall thickening, relatively small ventricular volume, and increased relative thickness [6]. However, an increase in both ventricular diameter and relative wall thickness has been detected in thoroughbred horses, suggesting that these animals can also develop mixed hypertrophy [8].

Improvements in aerobic capacity induced by conditioning are largely related to changes in heart morphology and stroke volume (SV), which mainly occur in the first few months of training [9]. Nevertheless, similar to what is observed in human athletes, some studies on the effects of conditioning in horses showed varied results in structural and functional cardiac indexes. This was explained by the different characteristics of the training program, as well as its intensity, volume, and duration [2,6–8,10–13].

Additionally, disparities in body size, age, gender, and possibly between breeds make it difficult to extrapolate results and comparisons [2,5,8,14–16].

In human athletes, the “athlete’s heart” is extensively investigated in order to distinguish it from pathological conditions [17]. However, despite numerous studies showing the relationship among athletic performance, left ventricular diameter, and left ventricle mass in horses, there is still no detailed understanding of the cardiac structure and function in affected horses. Moreover, considering that these animals may have more homogeneous changes in their physical conditioning than humans because of the genetic selection for a particular sport, it is still necessary to investigate whether the cardiac adaptation induced by conditioning can assist in the selection of animals with greater athletic potential [16].

Although some studies employed echocardiography to evaluate the effect of conditioning in horses submitted to track [1,2,4,7–9,11] or treadmill training [10], they did not report the use of any objective method to guide the conditioning. So far, only one study, more specifically a doctoral thesis, investigated cardiac adaptation after lactate-guided treadmill conditioning. However, as the horses used were kept inactive prior to the research, potentially being considered detrained, this may have contributed to the absence of significant changes, except for a tendency towards hypertrophy in the studied period [12].

To the best of our knowledge, no research on heart changes resulting from conditioning guided by physiological parameters has been carried out using untrained horses. Blood lactate concentration is an indicator of anaerobic energy metabolism and has been used to assess the fitness of horses and guide conditioning intensity [18,19]. The velocity–blood lactate relationship helps to determine the conditioning load [20]. Thus, the prescription of conditioning load based on physiological parameters such as blood lactate concentration is essential to improving the performance of horses and avoiding sports injuries [20,21].

Therefore, this study aimed to assess by echocardiography the cardiac changes in young untrained horses. The animals, which did not perform any type of controlled exercise prior to the research, were submitted to a lactate-guided conditioning program for six weeks. We hypothesized that the prescribed conditioning method would increase the aerobic capacity of the horses and lead to a cardiac adaptation known as “athlete’s heart.”

2. Materials e Methods

2.1. Animals

The experimental procedures were approved by the Ethics Committee on the Use of Animals of the School of Agricultural and Veterinary Studies (FCAV) of São Paulo State University (UNESP), protocol no. 012601/19. A total of 12 naive purebred Arabian horses (nine females and three geldings), aged (mean ± SD) 28.42 ± 3.75 months, were used. The animals belonged to breeders who allowed the application of the proposed conditioning program. They were housed in the equine breeding area of the School of Agricultural and Veterinary Studies of UNESP, vaccinated against influenza, eastern/western encephalomyelitis, and tetanus (Fluvac Innovator® EWT, Zoetis, SP, BR), and dewormed every three months (Equest® Pramox, Zoetis, SP, BR). The horses were kept grouped together on a 100 × 70 m^2 Tifton 85 grass paddock provided with water ad libitum. During the conditioning period, they were fed coast cross hay corresponding to 2% of body weight (BW) and supplemented with concentrate (1% of BW) divided into two daily portions. The nutrients and energy needed were provided according to the horses’ growth and moderate exercise requirements [22]. The supplementation was introduced fifteen days before the training period.

The physical and cardiovascular system examinations were performed to confirm if the horses were healthy. Cardiac auscultation did not detect significant murmurs or arrhythmias. BW was measured using a scale, while height and thoracic circumference were determined using a tape measure. BW was measured four times: in the first and second echocardiographic examinations, which had an interval of 5.5 months, and at the beginning and end of the conditioning period, which lasted six weeks. Between the first echocardiographic examination and the beginning of the conditioning period, the animals were submitted to rational taming, which was performed without physical exercise to facilitate handling in a horse stock and on a treadmill (Figure 1). Adaptation to treadmill exercise was carried out over three consecutive days and consisted of different speeds so that the horses could develop all gaits. This included 1 min at an inclination of 0% and 1 min at an inclination of 3% for each speed, i.e., 1.6, 3.4, 6, and 1.6 m/s.

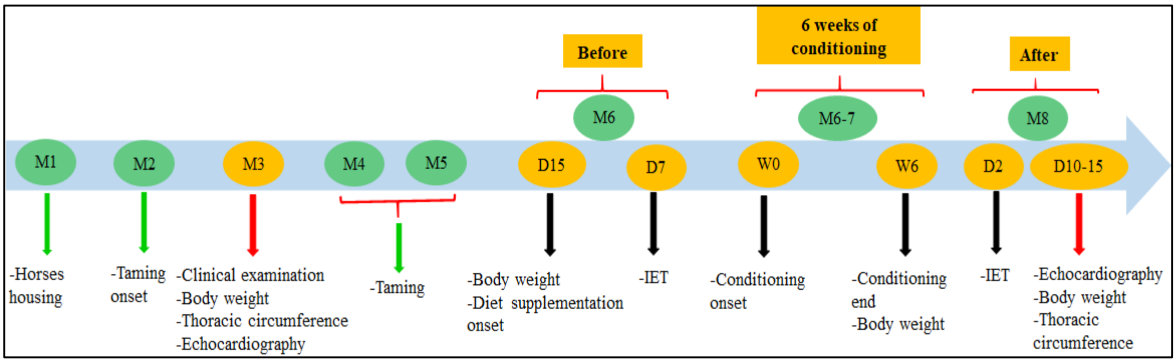

Figure 1. Experiment timeline. The naïve horses were housed at the university and submitted to rational taming. During this period, the experimental procedures were carried out. The incremental exercise test (IET) was performed before and after six weeks of conditioning. The echocardiographic images were obtained before and after the 5.5 month interval, including the conditioning period. M, month; D, day; W, week.

2.2. Incremental Exercise Test (IET)

The incremental exercise test and physical training were performed on a treadmill (Galloper®, Sahinco, Palmital, SP, Brasil) housed at the Equine Sports Medicine Laboratory of UNESP, Jaboticabal, Brazil, where the animals were previously adapted to the equipment and laboratory environment. First, the IET was used to determine the conditioning level of the horses and establish the individual training program. After six weeks of training, the same IET protocol was applied to assess the conditioning evolution. During the tests and training programs, the temperature of the treadmill room was maintained at 20 °C.

The IET consisted of three stages: warm-up, incremental effort, and cool-down. The horses remained at each speed for 5 min. During the warm-up, the speeds were 1.6 and 3.4 m/s at an inclination of 0%. Then, the inclination angle of the treadmill was increased to 3% and the speed was adjusted to 5 m/s, followed by speed increments of 0.5 m/s until a maximum speed of 8.5 m/s. At the end of this stage, the cool-down was performed at a speed of 3.4 and 1.6 m/s and an inclination of 0%. Between each speed of the incremental effort stage, the treadmill was turned off for 1 min (including the time to stop) for blood sample collection (Figure 2). The maximum velocity of each horse was the speed at which the blood lactate concentration reached or exceeded 4 mmol/L (V_4), ending the incremental effort stage [21]. The horses were submitted to this IET before the training program and two days after its end.

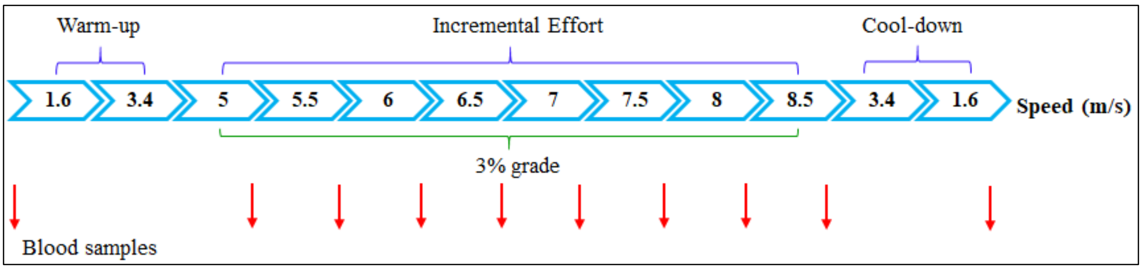

Figure 2. Schematic representation of the incremental exercise test (IET). The IET was performed before and after the conditioning period to determine the velocity run by the horse at which the blood lactate concentration reached 4 mmol/L (V_4). The horses remained at each speed for 5 min. At the end of each speed of the incremental effort stage, the treadmill was turned off for 1 min for blood sample collection. After obtaining the lactate–velocity curve, it was possible to determine the velocity at which the blood lactate concentration reached 2 mmol/L (V_2).

2.3. Blood Sample Collection and Laboratory Analysis

Prior to the IET, the horses were catheterized with a 14G central venous catheter (Biomedical®, Equipamentos e Produtos Médico-Cirúrgicos Ltda., SP, Brasil) in the left jugular vein. An extender tube was attached to this catheter, and in order to maintain the access, the system was filled with heparinized solution. Blood samples of 4 mL were collected and placed in vacuum tubes containing EDTA and sodium fluoride (Becton Dickinson Indústrias Cirúrgicas LTDA, MG, Brasil) until analysis. Before collection, 15 mL of blood was removed and discarded to clean the system.

Blood samples were collected at rest, before catheterization, directly from the jugular vein, while other samples were obtained from the extensor tube at the end of each speed of the incremental effort stage (when the treadmill stopped) and at the end of the cool-down. Immediately after collection, the blood lactate concentration was measured using an electroenzymatic methodology (Yellow Springs Instrument®, YSI 2300, OH, USA). After the determination of the venous blood lactate concentration, the samples were refrigerated until centrifugation for plasma separation (1500 × *g* for 10 min), which occurred within 30 min of collection. At the end of the IET, the lactate concentration was measured in the refrigerated fresh plasma. The blood lactate concentration was used to determine the maximum velocity at which each animal should run during the IET (i.e., the velocity at which the lactate concentration was ≥4 mmol/L). In addition, the lactate concentration obtained in the plasma was used to establish the physical conditioning level of the horse and the conditioning program.

2.4. Conditioning Program

The intensity of the conditioning program was established based on V_2, i.e., the velocity at which the plasma lactate concentration reached 2 mmol/L throughout the IET [21]. From the plasma lactate values determined in the incremental effort stage, it was possible to obtain the lactate–velocity curve, in which the exponential regression analysis was applied to determine the V_2 of each horse. The physical training was carried out for six weeks and consisted of 5 sessions, each with 14 days, totaling 15 sessions. During the week, the animals rested for one day between the sessions.

In the first three sessions, the treadmill training intensity was 80% of the individually calculated V_2 at an inclination of 3%. From the fourth to the sixth session, the animals were trained at 100% of the V_2 and an inclination of 3%. From the seventh session onwards, the inclination was increased to 5%. In each session, the horses exercised for 55 min, with 10 min of warm-up (5 min at 1.6 m/s and 5 min at 3.4 m/s at 0% inclination), 40 min of training, and 5 min of cool-down (1.6 m/s at 0% inclination). To avoid repetitive strain injury, the 40 min training was divided and interspersed until the sixth session, as follows: 12 min at the speed and inclination described above and 2 min of canter at an inclination of 0% and the lowest possible speed, which was adjusted on an individual basis. From the seventh session onwards, the horses cantered at the training speed, interspersed with 2 min of trotting at 4 m/s and 0% inclination (Figure 3). Two days after the end of the six weeks of training, the animals were submitted to the IET to determine the level of conditioning through V_4 (determined from plasma lactate concentration).

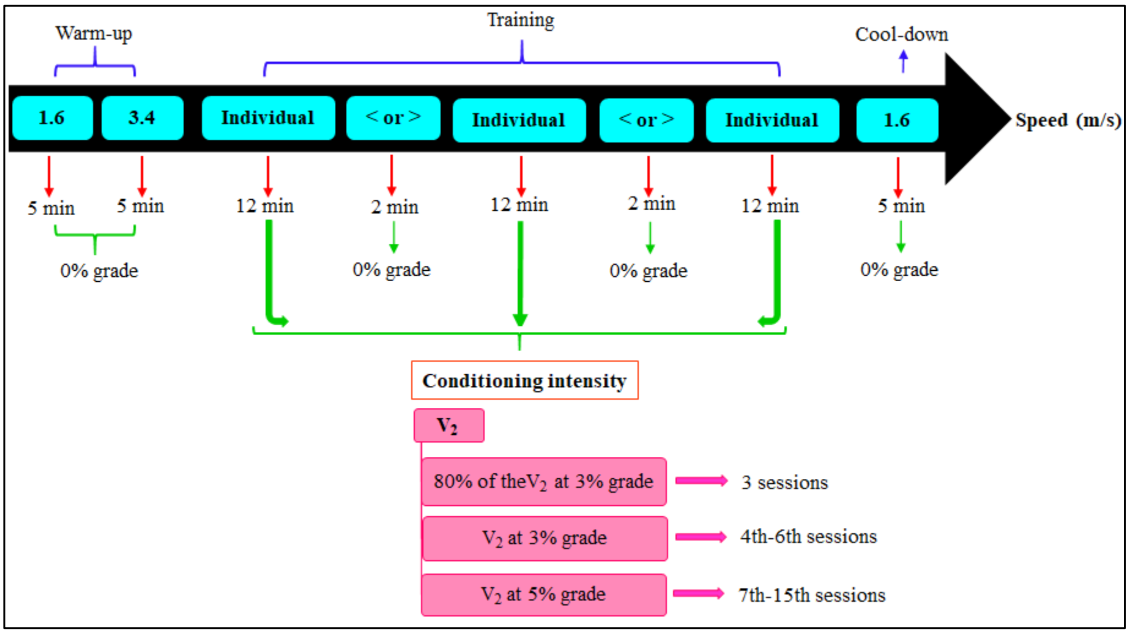

Figure 3. Schematic representation of the conditioning program performed on the treadmill. The intensity of the conditioning program was established based on V_2 (velocity at which the plasma lactate concentration reached 2 mmol/L during the incremental exercise test). The conditioning intensity was increased every three sessions. Each session consisted of the following stages: warm-up, training, and cool-down. The training speeds were individualized, and to avoid repetitive strain injury, they were interspersed with 2 min of speed reduction or increase up to the sixth session. From the seventh session onwards, the horses cantered at the training speed, interspersed with 2 min of speed reduction to trot.

2.5. Echocardiography

The echocardiographic examination was performed at the Equine Sports Medicine Laboratory using a portable device (Esaote®, MyLab Alpha, GE, Italy), a 1-4 MHz probe, and simultaneous electrocardiogram. Firstly, the animals were acclimatized for 15 min in a climate-controlled room (20 °C) before examination. Then, they were kept at rest and in a horse stock without sedation. Afterwards, the animals had their right and left parasternal regions shaved for the application of the transmission gel. The standardized images were obtained in B- and M-modes using pulsed-wave and tissue Doppler imaging. Initially, the qualitative assessment of the cardiac structures and their dimensions was performed in the right parasternal window in B-mode and real time [23]. The measurements were made offline based on the images and videos recorded. The average of three cardiac cycles was obtained, excluding cycles containing blocks and the following cycle. The same examiner (MMS) performed all echocardiographic examinations and measurements. All measurements were made according to the inner edge and leading edge-to-leading edge method in B- and M-modes, respectively [23].

The examination of the untrained animals was carried out before the IET. After the conditioning program, images were acquired between 10 and 15 days, during which the animals remained in the stall.

2.6. B-Mode Measurements

The maximum diameter of the left atrium (LA) was obtained at the end of systole, one frame before the mitral valve opening, from the 4-chamber long-axis image focusing on the LA in the right parasternal window, while the diameter of the aorta artery (Ao) was measured at the end of diastole in the sinus of Valsalva from the image of the left ventricle (LV) outflow tract before the QRS. From these measurements, it was possible to calculate the LA/Ao ratio [24]. To determine the stroke volume, the aortic diameter was measured in the valve annulus at the peak of systole [25].

2.7. M-Mode Measurements

The maximum diameter of the left atrium (LA) was obtained at the end of systole, one frame before the mitral valve opening, from the 4-chamber long-axis image focusing on the LA in the right parasternal window, while the diameter of the aorta artery (Ao) was measured at the end of diastole in the sinus of Valsalva from the image of the left ventricle (LV) outflow tract before the QRS. From these measurements, it was possible to calculate the LA/Ao ratio [24]. To determine the stroke volume, the aortic diameter was measured in the valve annulus at the peak of systole [25].

2.8. Pulsed Doppler Measurements

Measurements of the transmitral and aortic flows were performed in the left parasternal window [26]. From the transmitral flow it was possible to measure the peak E (E_{max}) and A (A_{max}) wave velocities and calculate the E_{max}-to-A_{max} ratio (E_{max}:A_{max}) [29], while from the aortic flow it was possible to obtain the pre-ejection period (PEP), the ejection time (ET), the PEP-to-ET ratio (PEP/ET), the time to peak (TTP), the maximal velocity of the aortic flow (V_{max}), and the velocity time integral (VTI). Afterwards, the following values were determined: ejection time index (ETI) corrected to heart rate (HR), stroke volume (SV), cardiac output (CO), stroke index (SI), and mean velocity of circumferential fiber shortening (Vcf) (Table S1) [25].

The tissue Doppler imaging was performed with the region of interest in the LVFW in the short-axis of the LV, in the right parasternal window. The following values were then obtained: PEP, ET, PEP/ET, isovolumic relaxation time (IVRT), early (Em) and final (Am) diastolic velocities, and ejection velocity (Sm) [24].

2.9. Statistical Analysis

All analyses were carried out using Rstudio software (v. 4.2.1) at a significance level of 5%. The data were evaluated using the Shapiro–Wilk normality test and expressed as the mean and standard deviation. The paired t-test was applied to determine differences before and after the training program. To establish the relationship between the measurements and some cardiac indices, body weight, and thoracic circumference, the Pearson's correlation coefficient was verified. Principal component analysis was used in order to reduce data dimensionality and understand the behavior of each variable in relation to the principal components. To decide the number of factors, the Kaiser criterion was adopted, which suggests that the choice of factors takes into account eigenvalues equal to or greater than 1 [30].

3. Results

All 12 horses completed the six weeks of conditioning uneventfully. There were increases in both V_4 (from 5.8 ± 0.4 to 7.6 ± 0.5 m/s) (p < 0.0001) and V_2 (from 5.2 ± 0.3 to 6.7 ± 0.4 m/s) (p < 0.0001) after the training program (Figure 4). The mean values of BW, age, thoracic circumference, and height are listed in Table 1. These data were measured in the first and second echocardiographic examinations, which had an interval of 5.5 months (during which the six-week conditioning was performed). After the conditioning, no increase in BW was observed (before: 309.67 ± 18.05 kg; after: 314.75 ± 25.15 kg) (p = 0.575).

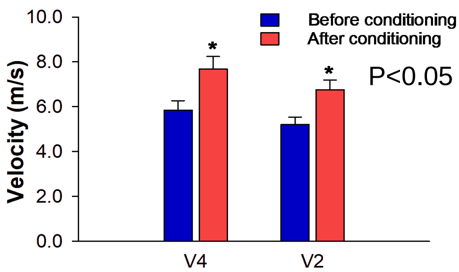

Figure 4. Mean ± standard deviation of V_4 and V_2 obtained in the incremental exercise test (IET). The graph represents the velocities reached in the test before and after the conditioning program. * Significant increase after conditioning compared to before conditioning (p < 0.05).

Table 1. Mean and standard deviation values of age, body weight, thoracic circumference, and height of purebred Arabian horses before and after a 5.5 month interval, during which six weeks of conditioning was performed.

Parameter	Before	After	*p*-Value
Age (months)	28.42 ± 3.75	34.42 ± 3.75	-
Body weight (kg)	312.17 ± 17.37	321.33 ± 19.13	0.232
Thoracic circumference (cm)	155.08 ± 3.78	158.25 ± 4.08	0.061
Height (m)	1.43 ± 0.02	1.44 ± 0.03	0.276

In the qualitative assessment of the heart by echocardiography, no macroscopic differences were observed. As for the echocardiographic indices obtained in B- and M-modes, there were increases of 8.34% in LVIDd ($p = 0.007$) and 17% in LV mass ($p = 0.011$), whereas HR decreased ($p = 0.003$). No significant changes in LVFW, MWT, RWT, IVS, FS, EF, LA, Ao, and LA/Ao were verified (Table 2). The correlations between BW, thoracic circumference, and HR obtained by echocardiography and some selected cardiac variables are shown in Figure 5. In general, HR during the echocardiographic examination showed significant moderate negative correlations with thoracic circumference (r = −0.44), LA (r = −0.47), SV (r = −0.52), and aortic flow ET (r = −0.53). The other variables had no significant correlation with HR.

Table 2. Mean and standard deviation values of echocardiographic measurements of purebred Arabian horses obtained at rest, before and after the 5.5 month interval, during which six weeks of conditioning was performed.

B-Mode			
Parameter	**Before**	**After**	***p*-Value**
HR (bpm)	42.24 ± 6.78	35.50 ± 2.73 *	0.003
LA (cm)	8.92 ± 0.68	9.16 ± 0.61	0.129
Ao (cm)	5.81 ± 0.44	6.09 ± 0.44	0.061
LA/Ao	1.56 ± 0.16	1.51 ± 0.17	0.275
M-Mode			
IVSd (cm)	2.15 ± 0.24	2.29 ± 0.20	0.089
IVSs (cm)	3.56 ± 0.25	3.75 ± 0,20	0.062
LVIDd (cm)	9.11 ± 0.72	9.87 ± 0.75 *	0.007
LVIDs (cm)	5.41 ± 0.71	5.59 ± 0.58	0.349
LVFWd (cm)	1.87 ± 0.27	1.84 ± 0.30	0.654
LVFWs (cm)	3.36 ± 0.22	3.40 ± 0.24	0.617
MWT (cm)	2.01 ± 0.19	2.07 ± 0.16	0.350
RWT	0.44 ± 0.04	0.42 ± 0.05	0.188
LV mass (g)	1,610.86 ± 372.64	1,886.90 ± 269.57 *	0.011
FS (%)	40.79 ± 4.04	43.40 ± 3.59	0.067
EF (%)	69.17 ± 4.79	72.01 ± 4.14	0.081

HR, heart rate; LA, left atrium; Ao, aortic diameter; LA/Ao, left atrium-to-aorta ratio; IVS, interventricular septum thickness; d, measurements at end-diastole; s, measurements at peak systole; LVID, left ventricular internal diameter; LVFW, left ventricular free wall thickness; MWT, mean wall thickness; RWT, relative wall thickness; LV mass, left ventricular mass; FS, fractional shortening; EF, ejection fraction. * Significant difference before and after conditioning ($p < 0.05$).

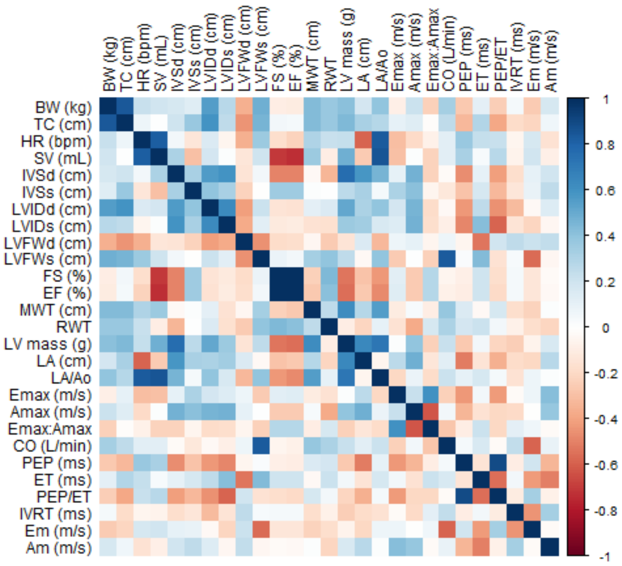

Figure 5. Heat map showing the correlation matrix between echocardiographic and body measurements. The color gradient indicates the correlation between BW, body weight; TC, thoracic circumference; and HR heart rate during echocardiography and other variables obtained and calculated from B-mode, M-mode, mitral flow Doppler (E_{max}, A_{max}, E_{max}:A_{max}), aortic flow Doppler (PEP, ET, PEP/ET) and tissue Doppler imaging of LVFW (IVRT, Em, Am). Refer to Tables 2 and 3 for abbreviations.

During the evaluation of the transmitral flow by pulsed-wave Doppler imaging, it was possible to observe a decrease in A_{max} (p = 0.047), while no changes in E_{max} and E_{max}:A_{max} were noted (Table 3). The parameters of the aortic flow showed increases in ET (p = 0.029), VTI (p = 0.021), and SV (p = 0.042). No differences in PEP, ETI, PEP/ET, TTP, V_{max}, CO, SI, and Vcf were found. In the second examination by tissue Doppler imaging, only ET increased (p = 0.011), with no significant changes being found in other periods and velocities (Table 3).

Table 3. Mean and standard deviation values obtained by pulsed-wave and tissue Doppler imaging of purebred Arabian horses at rest, before and after a 5.5 month interval, during which six weeks of conditioning was performed.

Transmitral Flow			
Parameter	**Before**	**After**	***p*-Value**
E_{max} (m/s)	0.72 ± 0.08	0.68 ± 0.13	0.349
A_{max} (m/s)	0.41 ± 0.05	0.36 ± 0.05 *	0.047
E_{max}:A_{max}	1.78 ± 0.24	1.90 ± 0.44	0.433
Aortic flow			
PEP (ms)	96.39 ± 12.73	99.77 ± 23.34	0.437
ET (ms)	437.16 ± 48.32	472.89 ± 34.40 *	0.029
ETI (ms)	460.39 ± 46,72	492.41 ± 34.03	0.068
PEP/ET	0.22 ± 0.03	0.21 ± 0.06	0.604
TTP (ms)	130.97 ± 14.83	120.00 ± 17.62	0.113
V_{max} (m/s)	0.91 ± 0.09	0.96 ± 0.13	0.318
VTI (cm)	27.25 ± 4.42	31.58 ± 4.14 *	0.021
SV (mL)	444.60 ± 129.62	538.40 ± 100.43 *	0.042
CO (L/min)	18.47 ± 5.34	19.09 ± 3.65	0.682
SI (mL/kg)	1.42 ± 0.41	1.72 ± 0.26	0.050
Vcf (ms)	0.94 ± 0.15	0.92 ± 0.08	0.635
Tissue Doppler imaging of LV free wall			
PEP (ms)	117.11 ± 13.99	121.11 ± 16.18	0.383
ET (ms)	390.00 ± 58.47	434.22 ± 25.80 *	0.011
PEP/ET	0.31 ± 0.08	0.28 ± 0.04	0.220
IVRT (ms)	43.11 ± 21.96	41.33 ± 11.84	0.794
Em (m/s)	0.29 ± 0.05	0.28 ± 0.04	0.465
Am (m/s)	0.10 ± 0.01	0.09 ± 0.02	0.171
Sm (m/s)	0.11 ± 0.02	0.11 ± 0.01	0.627

E_{max}, peak E wave velocity; A_{max}, peak A wave velocity; E_{max}:A_{max}, E_{max}-to-A_{max} ratio; PEP, pre-ejection period; ET, ejection time; ETI, ejection time index (corrected for HR); PEP/ET, PEP-to-ET ratio; TTP, time to peak; V_{max}, maximal velocity of the aortic flow; VTI, velocity time integral; SV, stroke volume; CO, cardiac output; SI, stroke index; Vcf, mean velocity of circumferential fiber shortening; IVRT, isovolumic relaxation time; Em, early-diastolic velocity; Am, late-diastolic velocity; Sm, ejection velocity. * Significant difference before and after conditioning ($p < 0.05$).

The principal component analysis reduced the dimensionality of 24 cardiac variables into seven components with eigenvalues > 1.0, explaining 84% of the total data variability (Figure 6). The contribution of each variable within its component can be seen in Figure 7.

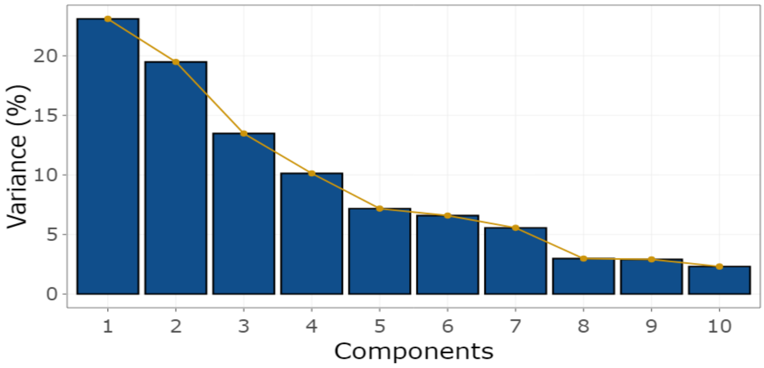

Figure 6. Percentage variation of principal components. The first seven components explained 84% of the variability of the echocardiographic variables. As can be observed, the degree of explainability was low from component eight onwards, justifying the presentation of only ten components.

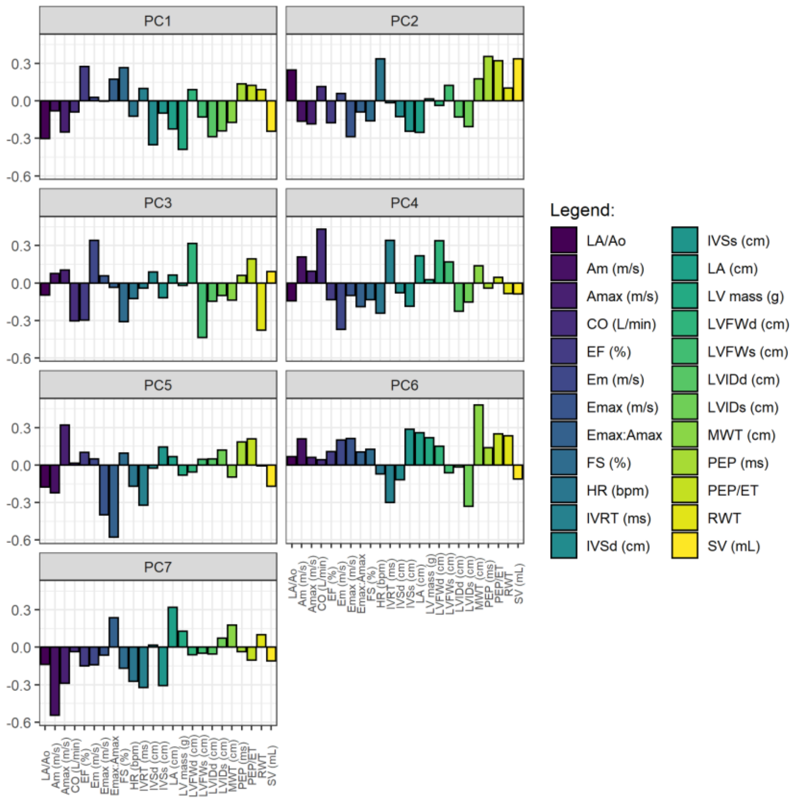

Figure 7. Principal components. In the two-dimensional image, it is possible to visualize the weight of each echocardiographic variable within each component. The variables selected for analysis were: HR, heart rate during echocardiography, and other variables obtained and determined in B- and M-modes with mitral flow Doppler (E_{max}, A_{max}, E_{max}:A_{max}), aortic flow Doppler (PEP, ET, PEP/ET), and tissue Doppler imaging of LVFW (IVRT, Em, Am). See legends in Tables 2 and 3.

4. Discussion

To the best of our knowledge, this is the first study to employ a lactate-guided physical conditioning program in naive young horses that did not perform any type of controlled exercise prior to the research in order to assess their cardiac changes by echocardiography. The animals were kept in a paddock; however, the voluntary activity had no relevant effect on fitness, given that it was not a regularly repeated exercise and the workload did not increase. The physical training employed was considered efficient since V_4 increased by 31%, a value greater than that observed in other studies [18,21,31,32]. Furthermore, there was also an increase of 28.84% in V_2. The conditioning program used in the present research proved to be suitable for young Arabian horses because of their enhanced aerobic capacity and absence of lameness, which represents a reduced risk of training-associated injury. It is also worth noting that the increases in both V_2 and V_4 were higher than those observed in a similar study [21], in which six-week training was applied to horses at 100% of V_2 together with an IET every two weeks to adjust the training intensity to the new V_2. In this study, V_4 increased by 17% and the animals showed irritability and reluctance, which were associated with the onset of overtraining.

Given the difficulty of performing several IETs under practical conditions, the program tested in the present study can be successfully applied to young horses due to its safety and effectiveness in improving their endurance capacity. Nevertheless, the difference observed in the conditioning enhancement between the present work and that developed by Trilk et al. [21] may be related to the breed used. While the latter employed different breeds with a wide age range (4–20 years), the present study used a homogeneous group of young purebred Arabian horses, a breed that seems to have a greater oxidative capacity resulting from the high number of type I and IIa muscle fibers in relation to others [14]. On the other hand, when 90 day training was applied to purebred adult (8.6 ± 3.3 years old) Arabian horses at 80% of V_4 with monthly velocity adjustment, no significant increase in V_4 was observed in animals that did not receive supplementation, a parameter that was being evaluated in the referred study. However, the authors used horses that were kept inactive in a paddock for eight months [32]. Some degree of previous conditioning might have contributed to the insignificant increase in V_4 since the intensity and period of conditioning were greater than those applied in the present study and the work conducted by Trilk et al. [21].

Even though the maximal lactate steady state (MLSS) was not determined in the current study, the increase in V_4 was similar to that in V_2–a parameter that approximates the MLSS velocity of horses [19]. V_4 is not a reliable parameter to estimate the aerobic capacity of horses, as it overestimates the MLSS velocity [19,20,33]. The gold standard for estimating aerobic capacity is the MLSS test; however, it is not practical to perform this test with the number of animals used in this work. In addition, there is still no standardized alternative exercise test for appropriately assessing the anaerobic–aerobic threshold before and after a conditioning period [20].

In addition to improving the aerobic capacity, the conditioning program employed had a significant effect on the hearts of horses. Significant adaptations, such as increased LVIDd, LV mass, and SV, likely occurred due to the high preload caused by the expanded plasma volume resulting from physical training [34]. The increases in these variables corroborated the findings traditionally seen in humans [6,35] and dogs [36] after endurance training. Changes in LVID and LV mass are considered relevant, as they have strong positive correlations with aerobic capacity and are associated with better performance in thoroughbred horses [1]. The relationship between heart size and race performance was addressed in several studies [1–3,5]. Moreover, similar to what was found in racing breeds, higher LVIDd and SV in Arabian horses may lead to improved performance [4].

The fact that the classic “athlete’s heart,” characterized by increased thickness of the LVFW, did not develop may be attributed to the short duration of the conditioning program. Similarly, significant hypertrophy of the LVFW was also not observed in Arabian horses after training for four months [12] and in thoroughbred and standardbred horses after six months of training [10,11]. However, with the extension of the conditioning period, there could be an increase in the wall thickness of the left ventricle in order to reduce the tension in the myocardial wall caused by the enlargement of the ventricular chamber, according to Laplace’s law [35]. In addition to the duration, the intensity of the conditioning program also plays an important role in cardiac adaptation [11]. When comparing animals that performed training at different intensities, some authors observed higher LVID and LV mass in those trained at a higher intensity [2,4]. Furthermore, in contrast to the previously mentioned studies, wall hypertrophy was identified in thoroughbred horses after four and a half months of training [8].

It is known that LVIDd, LV mass, and MWT can increase with normal heart growth, as seen in standardbred horses [2,11]. Additionally, increases in both LA and LV were reported in purebred and crossbreed Arabians horses aged between four and six years [15]. Nonetheless, considering that the interval between echocardiograms was only 5.5 months, it is unlikely that the results of the present study can be solely attributed to normal growth. Another point to consider is that no expressive body development was verified during this period, according to the thoracic circumference and BW results.

To differentiate conditioning effects from normal heart growth, a control group with animals of the same age and gender is required [4,7]. However, as it is difficult to find young untrained horses, only two studies used this resource [10,11]. Although differences in some echocardiographic measurements were found between trained and untrained groups before conditioning, cardiac adaptation was observed after six months of treadmill conditioning [10]. Nonetheless, no specific method was used to guide the training, and the changes were smaller than those observed in another study after 4.5 months of conditioning in a training center with horses of the same breed [8]. In another work, increases in BW, LVIDd, LV mass, and MWT were verified after six months of low-intensity conditioning in both trained and control groups due to normal growth [11]. Additionally, the percentage increase in ventricular diameter in young standardbred horses [2] after six months of conditioning was found to be greater than that reported by other researchers after ten months of training using animals of the same breed [7]. These divergences highlight the importance of carrying out a controlled study, as is the case in the present work, which despite not having included a control group, employed an individualized lactate-guided conditioning protocol according to the physical fitness of each animal. Therefore, this is the first study to evaluate cardiac adaptation in horses without any previous physical exercise after conditioning with standardized intensity.

Cardiac dimensions and function indices are influenced by HR, load, and contractility [23]. Despite the decreased HR in the second echocardiographic examination, it is unlikely that this parameter significantly influenced the results. In the first echocardiographic examination, the HR was slightly above the value commonly observed in horses at rest. This probably occurred because the horses were at the beginning of taming, which was carried out without the use of any physical exercise, and had not yet been fully adapted to the facilities and procedure. Thus, the HR obtained by echocardiography likely did not represent the true HR of the horses, and therefore could not be used to assess the effect of conditioning [2,8,10,11].

In humans, HR variations of 10% have minimal influence on cardiac dimensions and function indices [37]. In contrast, a study on the reliability and repeatability of echocardiographic measurements in horses indicated that an HR variation of 27% triggered the occurrence of significant changes in cardiac dimensions [38]. In addition, when comparing exercise stress echocardiograms with those obtained at rest, some researchers found differences in LVIDs and LVIDd only for HRs greater than 80 and 100 bpm, respectively [39]. Thus, the elevated LVIDd and SV observed in the present study may be explained by the volume overload caused by resistance training [40]. Moreover, among the cardiac variables that changed, only SV showed a moderate correlation with HR obtained by echocardiography.

With respect to SV, there are few studies in the literature on the effect of physical training in horses [41]. Additionally, the results reported and the forms of calculation are diverse, which can compromise a more accurate comparison [10,42]. The increase in SV at rest is a common finding in human athletes [13,40] and dogs with an absence of wall hypertrophy after endurance conditioning [37]. In the present work, although no wall hypertrophy was observed after conditioning, the SV increased by 26%, a value similar to that observed in young thoroughbreds after six months of track training [9]. When evaluating the physiological adaptations to conditioning after six and ten months, the researchers found a greater increase in aerobic capacity through maximum oxygen consumption ($\dot{V}O_{2max}$) after six months of training.

Likewise, a greater increase in SV was observed in treadmill-trained thoroughbred horses in the initial conditioning period, that is, after three months [10]. The main factor that led to an elevated $\dot{V}O_{2max}$ was the SV, which was determined both by echocardiography and by the values obtained from the Fick principle (since other variables involved in oxygen transport did not change) [9]. This result reinforced the significant gain in conditioning obtained herein.

Only a few studies in the literature evaluated the effect of physical training on both FS and EF at rest. Regarding FS, the results in human athletes and horses are conflicting. In horses, FS either increased [10], was reduced [2,8] or did not change

[7], as occurred in the present study. It is known that these indices are influenced by loading, myocardial contractility, and HR [23]. In the present study, despite the increase in SV, HR during echocardiography decreased, which may have masked the change in FS, since this parameter showed a tendency to increase. On the other hand, in addition to the differences observed in HR at rest after training [8,10], the method used to determine FS and EF may also contribute to divergences in the results. In human athletes, there are also controversies about EF, given that this index has low sensitivity for the assessment of LV function in the presence of structural changes (as it does not consider geometric changes) [40]. After training, it is expected that systolic function would be preserved or increased [6]. However, as adaptation of LV contractility may not be detected due to the method used [40], further studies using more sensitive methods of assessing systolic function in horses are still needed to help differentiate pathological from physiological changes induced by exercise [43].

Even though the clinical application of tissue Doppler imaging for the assessment of LV systolic and diastolic functions in horses has been widely investigated [44], there is only one study in the literature evaluating its use in measuring cardiac adaptation to physical training. In the present study, no changes were detected in Em, Am, and Sm of the horses, corroborating the results reported in dogs after eight weeks of conditioning [36], despite the different type of measurement made, that is, short-axis versus apical plane images. In addition, while tissue Doppler imaging in horses assesses only the radial movement of the LV wall, it measures the longitudinal movement in dogs. The absence of changes in the present study and that by Restan et al. [36] may be related to the intensity and duration of the conditioning program.

Unlike in the present study, when assessing the myocardial function of standardbred horses after ten months of conditioning using tissue Doppler imaging, some researchers observed a reduction in IVRT and an increase of 21.32% in the Sm wave of LVFW. The increased LVFW systolic velocity was attributed to the training effect since the animals developed an “athlete’s heart” [7]. As alterations in LV systolic function may occur after changes in the myocardial wall, this may justify why this function did not change in the present study, given the increased cardiac chamber and SV. Although tissue Doppler imaging is limited to the study of LVFW radial motion and, consequently, regional cardiac function, the proposed method is promising to understand cardiac adaptation in athletic horses [7].

In the "athlete's heart" condition, LA remodeling tends to be common in humans [45] and dogs [36] after intense endurance training. In this sense, increases in both LV preload and volume are crucial for atrial remodeling since the volume of blood reaching the atrium rises along with atrial pressure in order to maintain the ventricular filling pressure [6,45]. Although in the present study there was an increase in the ventricular diameter, no significant change was found in LA. When comparing the endurance of Arabian horses classified as elite and non-elite, Sleeper et al. [4] observed no difference in atrial diameter despite an enlarged LV in elite animals. Furthermore, atrial enlargement seems to be dependent on the intensity and duration of conditioning in humans [6,45], which may explain the lack of alteration in horses at the beginning of their athletic career [15].

Even though there are numerous studies in medicine investigating the characterization and differentiation of physiological and pathological LA remodeling in humans, studies with horses are scarce. Only one study that evaluated the effect of conditioning in horses reported information on their LA, which increased after ten months [7]. Different from physiological remodeling, a pathological increase in LA elevates the risk of atrial fibrillation in elite athletes [45]. However, what is known about LA remodeling in horses is restricted to atrial fibrillation, which in horses may occur due to the chronic stretching of LA, triggered by valvular regurgitation [46]. The fact that horses may have regurgitation of multiple valves associated with adaptation to training [3] can make it difficult to clarify whether this species experiences physiological atrial remodeling induced by exercise, as is the case with humans.

Endurance sport athletes may have normal or supranormal diastolic function at rest with an increased contribution from early diastolic filling, which in turn elevates E_{max}/A_{max} [6]. Likewise, after eight weeks of aerobic conditioning in dogs, increases in both LVIDd and E_{max}/A_{max}, were verified, along with a reduction in A_{max} of the transmitral flow [36]. Nevertheless, although A_{max} decreased in the current work, E_{max}/A_{max} did not change. The lack of more consistent results on the improvement of diastolic function may be attributed to the short period of conditioning, the different HRs obtained between the tests due to the dependence of HR function indices, and the pre- and post-load and contractility conditions [23].

When a large number of variables is involved in a study, it is pertinent to select a smaller dataset [47]. In light of this, we opted to use principal component analysis, which proved capable of identifying and discriminating the echocardiographic variables that most accounted for the total variance. According to the contribution of each variable to the formation of the components, it was found that in component one, which explained 22% of the total variability, there was a greater contribution of LA, CO, LVIDd, LVIDs, MWT, LVFWd, LV mass, IVSd, SV, EF, FS, transmitral flow variables, E_{max}:A_{max}, and E_{max}. This subset of variables expressed the maximum amount of information contained in the original variables and are therefore the most relevant to be explored in echocardiographic studies involving horse conditioning. Indeed, most of these variables changed after six and ten months of conditioning in horses [7,10] and eight weeks of conditioning in dogs [36].

In spite of the promising results, the present study had some limitations. The absence of a control group prevented the estimation of the total effect of conditioning on the cardiac adaptation of the horses. The fact that there may have been some influence of normal growth cannot be ruled out. Although both female and gelding horses were used, the number of geldings was small and the body development of the animals was homogeneous, pointing to an unlikely gender interference. However, further research should be developed to clarify whether the effect of training on the heart after conditioning at the same intensity differs between genders. Furthermore, considering that this study investigated the effect of a single conditioning program and only in purebred Arabian horses, different cardiac and conditioning adaptations may occur in other breeds submitted to different types of programs. Despite this, the aerobic feature of the conditioning protocol employed herein makes it a suitable foundation for any sport, since endurance improvement is a fundamental aspect regardless of the equestrian discipline. In addition, although cardiac assessment was performed at rest, the cardiac function can be better assessed after exercise by echocardiography due to the direct relationship between exercise intensity and oxygen demand [41]. Finally, an extension of the conditioning period is necessary since physiological changes can overlap pathological ones after intense and long-term training in human athletes.

5. Conclusions

Six weeks of aerobic training was considered efficient to improve the conditioning of horses and promote their cardiac adaptation, with an increase in ventricular diameter and stroke volume. Additionally, the conditioning program used proved to be safe for young horses, serving as a basis for future research.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Table S1: Formulae used for the calculated parameters.

Author Contributions: J.C.L.-N. conceived and designed the experiment, and obtained the funding. M.M.S., G.V.R., I.M.d.F., and T.C.B.V.S. designed and performed the study. I.M.d.F. and T.C.B.V.S. carried out the laboratory analysis. M.M.S. performed the image analysis. M.M.S. and G.V.R. carried out the data analysis. J.C.L.-N. and M.M.S. prepared the original draft of the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the São Paulo Research Foundation (FAPESP) [grant nº. 2020/11223-4], in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-Brasil (CAPES)-Finance Code 001, and the National Council for Scientific and Technological Development (CNPq) [grant nº. 140407/2020-0].

Institutional Review Board Statement: The animal study protocol was approved by the Ethics Commission on Animal Use of the Faculty of Agricultural and Veterinary Sciences at São Paulo State University (CEUA-FCAV) (protocol code 01206/19, 10 October 2019).

Informed Consent Statement: Informed consent was obtained from the horses' owners involved in the study.

Data Availability Statement: The data that support the findings of this study are available upon request from the corresponding author.

Acknowledgments: We would like to thank the breeders Mrs. Auroxochitl Monteiro Pinheiro and Mr. Almir José Ribeiro for lending their horses for the research. We are also grateful to the Brazilian Association of Arabian Horse Breeders (ABCCA) for their collaboration. Lastly, we thank the Laboratory of Pharmacology and Equine Exercise Physiology (LAFEQ) for providing the lactate analyzer and for their technical support.

Conflicts of Interest: The authors declare no conflict of interest.

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APÊNDICE

Tabela complementar ao artigo **“**Cardiac Changes after Lactate-Guided Conditioning in Young Purebred Arabian Horses”.

1A - **Table S1**: Formulae used for the calculated parameters.

Parameter	**Equação**
MWT (cm)	(LVFWd + IVSd)/2
RWT	(LVFWd + IVSd)/LVIDd
LV mass (g)	1,04 * ([LVIDd + LVFWd + IVSd]^3 – LVIDd^3) – 13,6
FS (%)	([LVIDd – LVIDs]/LVIDd) * 100
LVVd (cm^3)	(7 * [LVIDd^3]/2,4 + LVIDd)
LVVs (cm^3)	(7 * [LVIDs^3]/2,4 + LVIDs)
EF (%)	(LVVd –LVVs)/LVVd * 100
CSA	CSA = π * (Ao * 0.5)^2 (cm)
ETI (ms)	ET + (0,55 * HR)
SV (mL)	VTI * CSA
SI (mL)	SV/BW
CO (L/min)	HR * SV/1000
Vcf (mm/s)	(LVIDd-LVIDs)/(LVIDd * ET/1000)