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FISIOLOGIA ANIMAL COMPARADA

A influência do exercício físico de moderada intensidade e da suplementação com ácido lipóico sobre a função cardíaca, a bioquímica sanguínea e a angiogênese em miocárdio de Rattus norvegicus

Dissertação defendida no âmbito do Programa de Pós-Graduação em Ciências Fisiológicas – Fisiologia Animal Comparada da Universidade Federal do Rio Grande – FURG como requisito para obtenção do título de Mestre.

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Sumário

Lista de Abreviaturas

E	Grupo Exercício
LA	Grupo Ácido Lipóico
ELA	Grupo Exercício + Ácido Lipóico
VEGF	Fator de Crescimento Endotelial Vascular
PD-ECGF	Fator de Crescimento Celular Endotelial Derivado da Plaqueta
PIGF	Fator de Crescimento Placentário
PI3K	Fosfatidilinositol 3-Quinase
eNOS	Óxido Nítrico Sintetase endotelial
IP3	Inositol Tri-Fosfato
DAG	Diacilglicerol
PKC	Proteína Quinase C
HIF	Fator Induzível pela Hipóxia
AMPK	Proteína Quinase Ativada por AMP
AL	Ácido Lipóico

Resumo Geral

Atualmente o exercício físico vem sendo utilizado com intuito de redução de massa corporal, em especial de massa gorda. Entretanto cresce o número de indivíduos que, associado ao exercício físico, utiliza substâncias com característica lipolítica, como é o caso do ácido lipóico. Tanto o exercício físico, quanto a utilização de suplementação com ácido lipóico são responsáveis por remodelagem vascular (devido à interferência no processo de angiogênese) e modificação de fatores de risco cardiovascular (como hipertensão e lipídeos sanguíneos elevados). Desta forma, o presente estudo buscou analisar a influência do exercício físico de moderada intensidade e da suplementação com ácido lipóico sobre a frequência cardíaca, pressão arterial, bioquímica sanguínea e angiogênese no músculo cardíaco e no músculo esquelético de ratos Wistar. Foram utilizados 80 ratos Wistar, divididos em quatro grupos: controle, ácido lipóico (LA), exercício (E) e associação (ELA). Os animais foram submetidos a um programa de adaptação e treinamento de natação (9 e 17 semanas) com um aumento progressivo no tempo natação (até 1h/dia) e intensidade de carga (até 5% do peso corporal). Os animais receberam ácido lipóico 5 vezes por semana (da 10ª à 17ª semana), 60 mg / Kg / dia. O exercício crônico de intensidade moderada promoveu bradicardia, mas sua associação com a suplementação de ácido lipóico interrompeu este benefício. A suplementação com LA mostrou-se eficaz em melhorar o perfil lipídico, mas associado ao exercício não apresentou redução. A angiogênese foi aumentada no coração e gastrocnêmio dos animais exercitados, a largura da fibra de E, LA e ELA foi reduzida no coração, enquanto no gastrocnêmio apresentaram um aumento na largura das fibras apenas por LA e ELA. A espessura do ventrículo esquerdo diminuiu no grupo E, enquanto que a área da câmara do ventrículo esquerdo, e os níveis de VEGF circulantes, não mostraram nenhuma diferença significativa. Foi observada uma interação negativa entre o exercício físico e a suplementação com ácido lipóico (supressão da bradicardia do exercício de um lado, e a perda da adaptação do perfil lipídico induzido por suplementação de AL por outro lado). Este estudo mostra pela primeira vez, a interação entre o exercício crônico de intensidade moderada e a suplementação com ácido lipóico sobre a remodelação cardíaca e angiogênese, confirmando os benefícios da prática física em melhorar o fornecimento de sangue do músculo, que não foi afetado pelo consumo de ácido lipóico. O ácido lipóico em animais não treinados não foi capaz de estimular a

angiogênese cardíaca e ao contrário mostram uma tendência para a redução dos novos vasos.

1. Introdução Geral

Durante o desenvolvimento de vertebrados, novos vasos sanguíneos são recrutados por um processo que se inicia no período fetal e continua por toda vida, através de dois mecanismos: a vasculogênese e a angiogênese (CARMELIET e JAIN, 2000). Vasculogênese é o processo inicial do crescimento vascular a partir de precursores de células endoteliais, angioblastos, que migram e sofrem diferenciação *in situ* formando sólidos cordões endoteliais. Esse processo é seguido de crescimento, ampliação e remodelação desses vasos primitivos que se transformam em uma rede vascular madura, esta etapa é denominada como angiogênese (CONWAY et al, 2001). A angiogênese é um mecanismo complexo pelo qual são criados capilares a partir de vasos sangüíneos pré-existentes e é regulada por um grande número de fatores pró-angiogênicos e anti-angiogênicos (POLVERINI, 1996; NORRBY, 1997; SHINTARO et al, 2010).

Outras modificações funcionais das artérias de maior calibre ocorrem durante a arteriogênese, que é caracterizada pelo crescimento de vasos colaterais, sendo importante devido à capacidade de preservar a estrutura e função de membros e de órgãos após a oclusão de grandes vasos (CONWAY, COLLEN e CARMELIET, 2001; HEIL e SCHAPER, 2004).

A angiogênese pode ocorrer por dois processos diferentes, por brotamento ou por divisão, sendo que ambos ocorrem tanto no embrião quanto em indivíduos adultos. O processo de angiogênese por brotamento teve seu estudo iniciado no século XIX, enquanto que os trabalhos científicos relacionados a angiogênese por divisão começaram há alguns anos por BURRI e DJONOVI (2002). A angiogênese por brotamento é caracterizada por broto composto por células endoteliais, que cresce geralmente em resposta à um estímulo pró-angiogênico, sendo capaz de adicionar vasos sanguíneos para porções de tecidos anteriormente desprovidos de vasos (BURRI e DJONOVI, 2002). Na figura 1, retirada de BURRI e DJONOVI (2002), podemos observar a formação de capilares por brotamento em tecido tumoral.

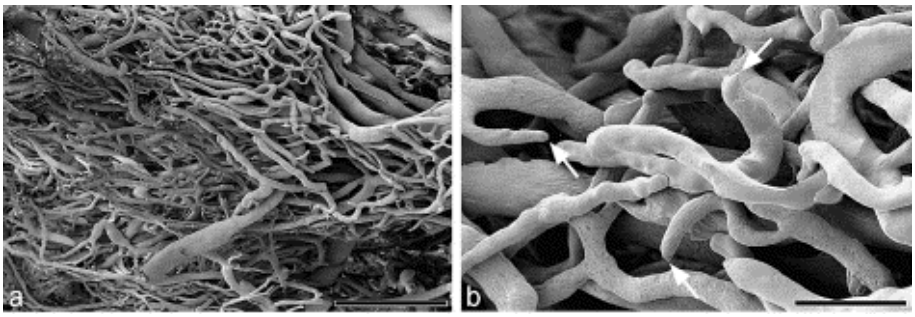

Figura 1: Formação de capilares por brotamento em tumor (retirado de BURRI e DJONOVI, 2002), a imagem b mostra com maior aproximação os novos vasos sendo formados (setas brancas).

Já na angiogênese por divisão (Figura 2) os elementos dos tecidos intersticiais invadem vasos pré-existentes, gerando pilares transversais formados de tecido que se expandem separando o vaso original em dois novos vasos (BURRI e DJONOV, 2002; BURRI, HLUSHCHUK e DJONOV, 2004). Na figura 3 observa-se o processo de angiogênese por divisão, representado pelos orifícios que se formam na parede dos vasos pré-existentes e que provocarão a divisão dos vasos.

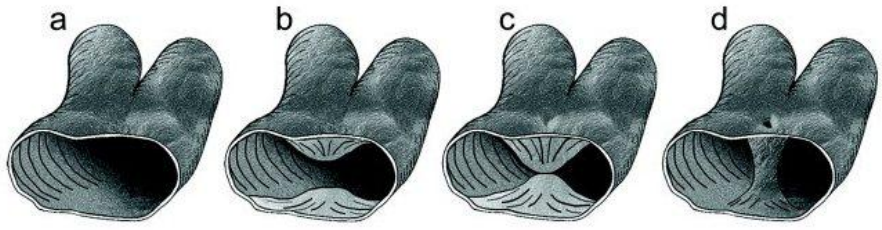

Figura 2: Representação em três dimensões do processo de angiogênese por divisão (retirado de BURRI, HLUSHCHUK e DJONOV, 2004). O processo inicia-se com a saliência de paredes opostas no lúmen do capilar (a, b). Depois de estabelecer um contato (c) a dupla camada endotelial e as membranas basais são perfuradas (d).

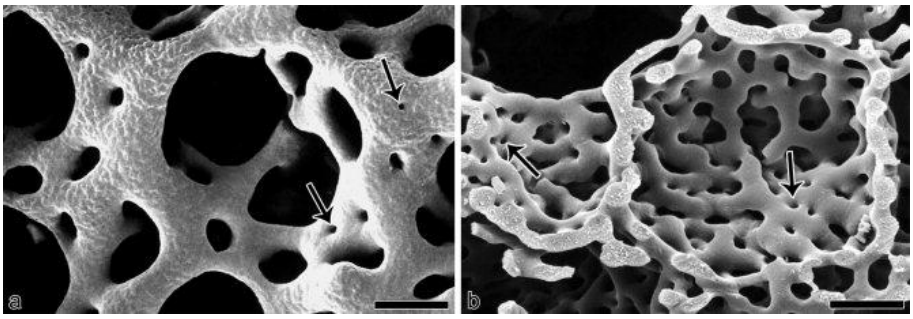

Figura 3: Orifícios representam pilares na rede capilar no pulmão de ave no período pré-natal (a) e roedor no período pós-natal (b) (retirado de BURRI, HLUSHCHUK e DJONOV, 2004). As setas indicam pequenos furos com diâmetros de cerca de 2 µM que correspondem aos pilares de tecido que se prolongam através do lúmen capilar.

As pesquisas sobre angiogênese por divisão são relativamente recentes, tendo em vista que a angiogênese por brotamento é estudada há mais de um século, e por este motivo ainda não se conhece quando ocorre cada uma destas.

No grupo de moléculas anti-angiogênicas encontram-se a angiostatina, endostatina e o inibidor endógeno de tromboplastina. Entre as moléculas pró-angiogênicas destacam-se os membros da família conhecida como fator de crescimento endotelial vascular (VEGF), a angiogenina e o fator de crescimento endotelial celular derivado da plaqueta (PD-ECGF) (FOLKMAN e SHING, 1992). O VEGF tem sido amplamente estudado (OLFERT, 2009, OLFERT, 2010). Este fator é essencial durante o crescimento e o desenvolvimento do indivíduo, tendo como função na vida adulta a regulação do número de capilares por ser um potente estimulante da angiogênese (QI et al, 2003; OLFERT, 2009; OLFERT, 2010).

Estruturalmente, o VEGF pertence a uma família genética composta por seis membros que são polipeptídeos homodiméricos: VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E e fator de crescimento placentário (PIGF). Para este grupo de proteínas, existem três receptores celulares: VEGF-R1, VEGF-R2 e VEGF-R3. Os receptores do VEGF são glicoproteínas transmembrânicas com domínio de ligação extracelular (MATSUMOTO e CLAESSON-WELSH, 2001). Cada receptor é expresso em um local específico:

- VEGF-R1: expresso nos macrófagos, células hematopoiéticas e monócitos.
- VEGF-R2: expresso no endotélio vascular.
- VEGF-R3: expresso no endotélio linfático.

A ligação do VEGF nos domínios extracelulares causa a dimerização do receptor e promove adicionalmente a interação entre os monômeros do receptor através do domínio 7 do tipo-imunoglobulina, ativando a região intracelular das enzimas tirosina-quinases (FREITAS et al, 2009).

De acordo com MATSUMOTO e CLAESSON-WELSH (2001) a ativação do receptor de VEGF fosforilará várias moléculas intracelulares, como:

- Fosfatidilinositol 3-quinase (PI3K): que é responsável por aumentar a produção de óxido nítrico (pela fosforilação da enzima óxido nítrico sintetase - eNOS);
- Inositol tri-fosfato (IP3): que se ligará a um receptor específico na membrana do retículo sarcoplasmático, liberando as reservas de cálcio intracelular; e
- Diacilglicerol (DAG): que ativará a proteína quinase C (PKC), tendo como resposta transcrição de DNA e proliferação celular.

O exercício físico é um importante fator neste processo, pois leva ao recrutamento de novos vasos, sendo caracterizado como importante fator pró-angiogênico (PRIOR et al, 2004; BENETTI et al, 2010).

O treinamento físico vem sendo utilizado como ferramenta importante em estudos das respostas cardiocirculatórias ao exercício, tanto na prevenção quanto no tratamento das cardiopatias (BENETTI et al, 2010).

Neste projeto a natação foi utilizada como exercício físico, o qual é definido como toda atividade motora com o objetivo específico de promover uma adaptação morfofuncional no organismo, estando associada com treinamento e tendo como característica fundamental a repetição. O exercício físico é diferente de atividade física, a qual engloba todos os movimentos realizados pelo animal utilizando as propriedades motoras sem objetivo pré-definido (ZILIO, 2005).

Em relação à angiogênese é sabido que o exercício físico gera um aumento do fluxo sanguíneo capaz de estimular a síntese de fatores de crescimento pró-angiogênicos e promover a proteção vascular. A presença dos receptores de VEGF é essencial para a angiogênese induzida por exercício físico, como foi comprovado no estudo de OLFERT (2010) onde o VEGF se mostrou essencial para o desenvolvimento e manutenção da capilaridade muscular esquelética em ratos, comparando ratos selvagem e ratos knockout para VEGF ambos exercitados por 6 semanas em esteira Essa relação ocorre pois o VEGF tem sua expressão iniciada pela hipóxia tecidual gerada durante o exercício aeróbico (PRIOR et al, 2004).

A hipóxia induzida pelo exercício físico ativa mecanismos de reparo, tais como angiogênese através da ativação do Fator Induzível pela Hipóxia (HIF), fator de transcrição chave na resposta a hipóxia (TONINI et al, 2003). A proteína HIF pode se ligar às regiões promotoras de genes-alvo, denominado elementos de resposta à hipóxia (ERHs) e induzir a expressão de genes, como o VEGF (gene alvo principal da transcrição) (PAGÉ et al, 2003; PUGH E RATCLIFFE, 2003;KALUZ et al, 2008; OHNO et al, 2012).

A aplicação do exercício é reconhecida para a melhora da saúde como um todo, sendo eficaz para a prevenção primária e secundária das doenças cardiovasculares, modificando perfis de risco devido à redução de diversos fatores como hipertensão, níveis de lipídios plasmáticos, insulina associado à melhora no metabolismo de glicose (HENRIKSEN, 2006; LIM, 2012; MATSUMOTO et al, 2012; MICHELSEN et al, 2012).

A hipertensão e os níveis de lipídeos sanguíneos são fatores de risco para doenças cardiovasculares, como o acidente vascular cerebral e o aneurisma arterial, sendo que qualquer elevação destes está relacionada com uma redução na expectativa de vida. (SHAY et al, 2009; LIM, 2012).

Na atualidade o exercício físico, em especial o exercício aeróbico, é empregado para a redução da massa corporal principalmente massa gorda, e naturalmente com a mudança da composição corporal é um estimulo para a remodelagem vascular nos tecidos exercitados. Também é corrente a utilização de substâncias que pretensamente acelerariam o metabolismo das gorduras, por exemplo, a suplementação com ácido lipóico (WOLLIN e JONES, 2003) que tem efeitos anti-obesidade por suprimir a ativação da proteína quinase ativada por AMP (AMPK) no hipotálamo (KIM et al, 2004). A ativação da enzima AMPK desliga as vias anabólicas de consumo de ATP, tais como a síntese dos ácidos graxos, e ativa vias catabólicas de geração de ATP por oxidação dos ácidos graxos para fornecer energia para a célula (MISRA, 2008).

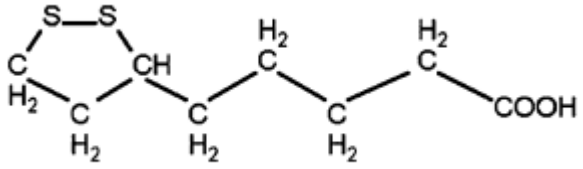

Figura 4: Estrutura química do ácido alfa-lipóico.

O ácido alfa-lipóico (ácido 6,8-ditio-octanóico) é um composto tiol com propriedades antioxidantes, sintetizado por plantas e animais (GHIBU et al, 2008), que está presente nos tecidos em pequenas quantidades tendo função antioxidante, e por ser solúvel tanto em água quanto em gordura é considerado um antioxidante universal (METZLER, 2001). A suplementação com ácido lipóico (AL) tem sido empregada cientificamente para distintos fins e tem apresentado diferentes efeitos. O estudo de Kim e colaboradores (2004) mostrou que a suplementação com ácido lipóico em ratos por duas semanas resultou na diminuição da ingestão alimentar, sugerindo um efeito anorexígeno.

No estudo de Saengsirisuwan e colaboradores (2001) o ácido lipóico associado ao exercício físico aumentou a ação da insulina em ratos obesos resistentes à insulina. Em outro estudo, com camundongos tratados com cisplatina (quimioterápico), o ácido lipóico reduziu efeitos nefrotóxicos, neurotóxicos e inflamatórios (PYO et. al, 2009).

Quanto aos efeitos anti-angiogênicos do ácido lipóico, o estudo de Larghero e colaboradores (2007) demonstrou a capacidade de limitar o crescimento tumoral, apresentando mecanismo anti-angiogênico pela inibição da migração de células endoteliais de veia umbilical humana em cultura celular. Este mecanismo anti-angiogênico se explica pela capacidade do ácido lipóico em reduzir a expressão de genes relacionados com a angiogênese, dentre eles o VEGF. Como foi comprovado no estudo de ALLEVA e colaboradores (2008), ratos suplementados com ácido lipóico tem menor expressão de VEGF em feridas experimentais prolongando o tratamento de cicatrização. A relação direta do ácido lipóico com o VEGF também foi verificada em um estudo realizado por LIN e colaboradores (2006), que observaram a redução da expressão do VEGF em 43%, em ratos diabéticos suplementados com ácido lipóico.

Os benefícios da suplementação com AL para a saúde se devem à ação antioxidante (já que a produção excessiva de espécies reativas de oxigênio associada com a inflamação crônica é um fator comum para o desenvolvimento das doenças cardiovasculares) (ROCHETTE et al, 2013). Somado a este fato o AL tem sido descrito como um agente de desintoxicação, e um medicamento para a diabetes (SHAY et al, 2009).

Apesar de diversos estudos realizados para investigar os supostos mecanismos responsáveis pelos efeitos cardioprotetores do exercício, ainda há muito a ser esclarecido, assim como o reconhecimento completo dos efeitos do LA sobre o sistema

cardiovascular e da sinergia entre a prática de exercício e a suplementação com LA sobre a função cardíaca, bioquímica e angiogênese.

Considerando a importância de estudos que avaliem a influência de exercícios aeróbicos em indivíduos saudáveis e a falta de informações sobre os efeitos do ácido lipóico em sujeitos saudáveis exercitados sobre os parâmetros da função cardiovascular, bioquímica sanguínea e angiogênese, justifica-se o presente estudo experimental.

1.1. Objetivo Geral

O objetivo do presente estudo foi analisar a influência do exercício físico moderado crônico e da suplementação contínua com ácido lipóico, sobre a angiogênese, a função cardíaca e a bioquímica sanguínea de *Rattus norvegicus* variedade Wistar.

1.2. Objetivos específicos

- Verificar a influência do exercício físico de intensidade moderada na angiogênese cardíaca e muscular esquelética.
- Investigar o papel do VEGF na angiogênese induzida pelo exercício físico de intensidade moderada.
- Averiguar a influência da suplementação com ácido lipóico no processo de angiogênese cardíaca e muscular esquelética, durante o treinamento de intensidade moderada.
- Determinar o efeito do exercício físico de intensidade moderada e da suplementação com ácido lipóico sobre a hipertrofia cardíaca.
- Analisar o efeito do exercício físico de intensidade moderada e da suplementação com ácido lipóico sobre a bioquímica sanguínea (colesterol total, fração de HDL, triglicerídeos e glicose).
- Quantificar o efeito do exercício físico de intensidade moderada e da suplementação com ácido lipóico sobre a frequência cardíaca e a pressão arterial sistêmica.

Artigo a ser submetido à Revista Journal of Applied Physyiology.

BRADYCARDIA AND LIPID PROFILE AMELIORATION INDUCED BY CHRONIC MODERATE AEROBIC EXERCISE AND LIPOIC ACID SUPPLEMENTATION IN *RATTUS NORVEGICUS*

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SHORT TITLE: BRADYCARDIA AND LIPIDEMIC ADAPTATION BY SWIM AND LIPOIC ACID

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Abstract

Cardiovascular diseases are the leading cause of death and most cardiovascular diseases are caused by high blood pressure and levels of blood lipids that can be prevented with exercise. Some substances as lipoic acid (LA) are used to supplementary feeding to stimulate lipolysis. This study examined the influence of chronic moderate aerobic exercise and supplementation with LA in healthy *Rattus norvegicus*. Eighty male Wistar rats were divided into four groups: control, lipoic acid, exercise, association. The animals were submitted to a program of adaptation (till 9^{th} week) and swimming training (from 10^{th} to 17^{th} week) of moderate intensity (5% of body load). The animals received lipoic acid 5 times a week during training period (60 mg/Kg/day). Systemic blood pressure (SBP) and heart rate (HR) (twenty rats per group) and blood biochemistry (ten rats per group) were determined. Neither systolic or diastolic blood pressure were modified by exercise or LA supplementation. Moderate chronicle exercise promoted a significantly bradycardia in both 9^{th} and 17^{th} weeks of 8,4 and 9,6%, respectively, comparing to control group, but its association with lipoic acid supplementation disrupted that benefit. Moderate chronic exercise and LA supplementation proved to be effective in ameliorate the lipid profile, rising HDL-cholesterol and diminishing triglycerides and LDL-cholesterol, during adaptation period (9^{th} week), but its association do not sustain that result. For the first time a negative interaction between moderate chronic exercise and LA supplementation was recorder to important cardiovascular parameters to the cardiac health.

Keywords: Swim, lipoic acid, heart hate, blood biochemistry.

Introduction

Cardiovascular diseases are the leading cause of death worldwide and represent high costs for public health in direct or indirect treatments (7). Most cardiovascular diseases can be prevented by reducing risk factors with a healthy eating, physical activity and control of blood pressure, blood glucose and lipids (14). High blood pressure and levels of blood lipids are risk factors for cardiovascular diseases such as stroke and arterial aneurysm and any elevation of these is related to a reduction in life expectancy (14, 29).

The exercise is recognized for improving health being effective for primary and secondary prevention of cardiovascular disease by modifying risk profiles due to reduction of various risk factors such as hypertension, plasma lipid levels, insulin associated with an improvement in glucose metabolism (10, 16, 17).

It is also common to use substances such as lipoic acid to supplement the diet. The alpha-lipoic acid (LA), 6,8-dithio octanoic acid, is a thiol compound with antioxidant properties, synthesized by plants and animals (6). Its health benefits are due to antioxidant action, because it reduces the overproduction of reactive oxygen species associated with chronicle inflammation, which is a common factor for the development of cardiovascular disease (25). Also, LA has an anti-obesity effect by suppression of the AMP-activated protein kinase (AMPK) activation in the hypothalamus (11) and due to the acceleration of fat metabolism (33). Indeed, LA has been described as a detoxifying agent, and a medicine for diabetes (29).

Although there are several studies that investigate putative mechanisms responsible for the cardioprotective effects of exercise, many remains to be clarified,

and the full recognition of the effects of LA on the cardiovascular system and the synergy between exercise training and supplementation with LA, specially to healthy subjects, is still unknown.

Thus, this study examined the influence of chronic moderate aerobic exercise and supplementation with LA, on the systemic blood pressure (SBP), heart rate (HR) and blood biochemistry (glucose, cholesterol and fractions and triglycerides) in murine model *Rattus norvegicus*.

Materials and Methods

The study was approved by the Ethical Committee for Animal Use (CEUA nº P059/2011) of the Universidade Federal do Rio Grande - FURG, Brazil and the animals were treated according to the Brazilian Guidelines of Practice for the Care and Use of Animals for Scientific Purposes and Teaching (DBPA).

1. Animal model:

Were used forty heterogenic male Wistar rats, 2 to 3 month/old, weighing 200 to 300g at baseline. The animals were obtained from the Central Animal Facility of the Universidade Federal do Rio Grande – FURG, Brazil, were housed in collective cages (five animals per cage) and fed commercial rodent food (15g/rat/day) according AIN-93 (24) and received water *"ad libitum"* diary. They were maintained in a temperature controlled room (24 ± 2° C) with a 12h light/dark cycle.

2. Swimming program:

A program of adaptation and swimming training was conducted adapted from Guerreiro (8). Rats were exercised five times a week with a progressive increase in swimming time and load intensity. In the first week the animals were adapted progressively to the swimming tank: on the first day for 15 minutes, increasing 15 minutes a day, reaching 60 minutes on the last day according to protocol of Rombaldi (26).

In the second week the adaptation period to a moderate intensity of swimming was started, by placing a load of lead (attached to the back of the animal by an elastic) progressively increased, from 1% of body weight/week until reaching 5% (maximum

load for moderate intensity according Kokubun, 1990). For each increase of overweight there was an adaptation to the swimming time, as described above, so that the animal had the ability to maintain the exercise for 60 minutes at the new load. To determine the load, the animals were weighed weekly.

The period of adaptation to the maximum swimming time (60 min/day) and the maximum load (5% of body weight) took 9 weeks, followed by another 8 weeks of training with maximum time and load. The whole experiment took place in 17 weeks. To ensure that the exercise conducted was a moderate effort, characterizing an aerobic activity, lactate levels were measured at the times 9 and 17. During exercise the rats were removed and dried of water, blood samples (25 μl) were collected cutting of the tip of the tail. Lactate levels between 5 - 5,5 mmol/L indicates an exercise of moderate intensity and an aerobic pathway utilization (32). Lactate concentrations were determined mmol/L with a lactate analyzer.

3. Lipoic acid supplementation:

The animals received lipoic acid 5 times a week during 8 weeks of training, 60 mg/Kg/day administered intraperitoneal (IP) following a dose utilized by Lin et al. (15) that is capable to reduce in 43% VEGF in the retina and in 88% in angiogenesis in diabetic rats. Lipoic acid was dissolved in a solution of NaOH 2M containing NaCl 154 mM. Then the pH was adjusted to 7.4 with concentrated HCl. Control animals received 1mL/Kg/day of a saline solution injected IP (30).

4. Experimental groups:

Eighty animals were divided into four groups (n = 20 animals): control group (C), lipoic acid group (LA), exercise group (E) and association group (ELA). In the Control group (C) the rats were placed in a swimming tank to mimic water stress, for 1 minute, 5 times a week, during 17 weeks, and from week 10 to 17 received 1mL/Kg/day of saline injection IP to simulate injection stress). In lipoic acid group (LA) the animals were submitted to simulation water stress like control group and were supplemented with lipoic acid solution, 5 times a week, 60mg/Kg/day from the 10 th to 17th week. Exercise group (E) the rats were submitted to the program of adaptation and swimming training, 5 times a week, with a 5% of body weight load. In the association group (ELA) the animals were submitted to the program of adaptation and swimming training, 5 times a week and supplemented with lipoic acid, 5 times a week, 60mg/Kg/day from the 10 th week to 17th week.

5. Tail-cuff plethysmography:

Measurements of blood pressure (systolic and diastolic) and heart rate were made through a non-invasive method with a tail plethysmograph before the adaptation period (time 0), after the adaptation period (time 9) and after the training period (time 17). The animals were habituated to this technique for two weeks before the experiments.

The room temperature was placed at 34°C and the animal was left in this place for 10 minutes. After the animal was contained in a acrylic cylinder. The temperature was kept constant. On the tail was placed a cuff with a frequency cardiac counter, ten measurements were made for each animal each time (0, 9, 17) to all groups.

6. Blood biochemical analysis:

To monitor the biochemical profile of trained animals, and the influence of lipoic acid, 5 animals from each experimental group were anesthetized with ketamine (90mg/kg) and xylazine (10mg/kg) (33) and euthanized by decapitation with guillotine after 9th (n=5) and 17th week (n=5). Blood samples (4 mL) were collected from decapitated animal body in a tube for vacuum blood collection and aliquots were separated for determination of glucose (glucose oxidase assay, 450 nm), total cholesterol (cholesterol liquiform, 500 nm), HDL (cholesterol HDL, 500 nm), and triglycerides (triglycerides liquiform, 505 nm) using commercial enzymatic assays and determined by spectrophotometry. LDL was calculated indirectly, by the Friedewald equation. Values were calculated in mg/dL.

7. Statistical Analysis:

Values were expressed as mean ± S.D. The Shapiro-Wilks test was used to verify normal distribution, and Levene test to homocedasticity. Parametric data were analyzed by one-way ANOVA, while nonasymmetric data were analyzed using the Kruskal-Wallis test. *Post hoc* tests was conducted by Tukey HSD. The alpha error rate was 5% (P = 0,05).

Results

Blood pressure and heart rate

Table 1 presents the effects of moderate physical exercise through the time. A significantly bradycardia in both 9 and 17 weeks was observed in exercised groups (reduction of 8,4 and 9,6%, respectively, $p < 0,05$) comparing to control groups at time 0 and 17. Neither systolic or diastolic blood pressure were affected by exercise through the time ($p = 0,06$).

Table 3 shows the effects of supplementation with lipoic acid at the end of the experimental period (17^{th} week). The exercise continues to promote heart rate reduction comparing to controls (decrease of 10,8%, $p < 0,05$), but lipoic acid supplementation abolished the exercise bradycardia effect. Neither systolic or diastolic blood pressure were modified by LA supplementation.

Blood biochemical analyses

The lactate varied through the time (9, 17), the highest levels were observed at 9 weeks ($p > 0,05$) (Table 2). When comparing groups at the 17^{th} week, animals exercised (E and EAL) had higher lactate levels (5,5 and 5,7, respectively) than controls and AL group, as expected, and these values indicate a performance of a moderate-intensity exercise ($p = 0,013$).

In table 2 we can also observe the effect of exercise through the time on blood biochemistry. Control group showed a significantly HDL elevation at time 17 comparing to controls at time 9 week. Exercised animals showed a significant reduction in LDL (- 54%), tryglicerides (- 28%) and increase in HDL (+ 485%) at time 9, compared to control group. At time 17 this benefits of exercise were lost and values returned to levels of control animals in the same time (C at 9^{th} week). Glucose and total

cholesterol do not show any significant alteration in relation to exercise at any time experimented. Table 3 presents the effect of supplementation with LA on blood biochemistry at the end of the experimental period (17^{th} week). Lipoic acid lowered triglycerides levels by 17,6% when compared to the exercise group ($p = 0,004$). Rats exercised and with LA lost the beneficial reduction to tryglicerides promoted by LA isolated. Supplementation with lipoic acid (both isolated and associated with exercise) shows an increase in HDL compared to the exercise group (217% and 165%, respectively, $p < 0,001$. All other parameters (LDL, glucose and total cholesterol) were not affected by treatments.

Discussion

There is evidence that moderate exercise is better than excessive exercise in terms of improving cardiovascular function, up regulation of the immune system and modulation of redox homeostasis (22). Moreover, the LA is present in several multivitamins and causes biochemical activities with therapeutic potential against a number of pathophysiological insults (29). Some of these benefits of moderate exercise and LA supplementation were confirmed in this study but an unusual negative interaction of them was described for the first time.

In this study the chronic exercise (seventeen weeks) of moderate intensity caused a reduction in HR in a time-dependent manner dependent on the treatment time, since the week 9 reduced HR in 14,3% and week 17 lowered HR in 28,6%. The effect of bradycardia was also observed by Barretti (1), which achieved a 12% reduction in the HR of exercised rats compared with untrained rats at 10 weeks of moderate swimming intensity. The HR is generally used as an indicator of cardiac physiologic function, considered a consequence rather than a cause of a condition (23). There is evidence that physical exercise causes changes in HR that depend on time and intensity. Acute exercise causes an increase in HR by decreasing vagal modulation and increased Central Nervous System (CNS) activity, while chronic exercise leads to a reduction in HR by increasing vagal modulation and decreased central nervous system (CNS) activity (parasympathetic predominance) (9). This study also showed that after a period of adaptation (9 weeks) and during the period of training (until 17^{th} week) a time dependent bradycardia was induced by exercise. Another important found was that for the first time was observed that supplementation with lipoic acid abolished the bradycardia induced by exercise in healthy animals. This negative interaction of LA and

exercise induced bradycardia is an important theme for future studies that could elucidate if its supplementation might or not be indicated to human beings.

Systolic and diastolic blood pressure wasn't affected by exercise, wich is not uncommon since the study was performed in healthy rats. Midaoui et al (19) and Midaoui and Champlain (18) found that supplementation with LA (500 mg/Kg) prevented the increase in systolic blood pressure in rats treated with glucose (10%), maintaining the pressure values of this group statistically equal to control rats. Further, Queiroz, et al (21) showed that LA (60mg/kg) reduced mean arterial pressure in rats with renovascular hypertension. As far as we known no data were presented observing the LA influence in healthy subjects until this moment.

In the present study there was no significant difference in blood pressure between treatments, and we could suggest that chronicle moderate exercise and supplementation with 60mg/Kg/day of LA or its association are not capable to promote any alteration at blood pressure of healthy rats.

The highest levels of lactate were observed in the adaptation period (9^{th} week). At the end of the experiment (17^{th} week) exercised rats (E and ELA) had higher lactate levels ($p = 0,013$) than control ones. Lactate levels indicate the use of aerobic or anaerobic metabolism in the supply of adenosine triphosphate to muscle activity, setting the exercise intensity (3), and in this study confirming that moderate intensity and aerobic metabolism were performed. Lactate levels were higher in 9 weeks, possibly because the load adaptation that occurred weekly in this period required more effort than the training period, when the load was constant, and when habituation could be occurring.

Saengsirisuwan et al (27) shows that exercise of moderate to high intensity increases glucose tolerance and elimination in obese Zucker rats resistant to insulin,

mainly due to adjustments in the activity of glucose transport and translocation of GLUT4 protein. Barretti et al (1) observed that glucose levels in rats trained for 36 weeks were significantly lower than the sedentary and control group, beyond reduce triglyceride levels and a tendency to reduction in cholesterol of trained group. Our results did not showed any significant alteration in glycemia or total cholesterol levels respect to control group, probably because our rats are not insulin resistants or due to our training time be minor (17 weeks) than that conducted by Barretti et al (1).

At 9 week, exercised animals showed a significant amelioration of lipid profile, with reduction in LDL-cholesterol and triglycerides and elevation of HDL cholesterol. Similarly to this study, Tolfrey et al (31) demonstrated that exercise is capable of modifying the lipid profile, indicating an increase in HDL and decrease LDL and triglycerides that had no significant changes in total cholesterol. The increase in HDL levels observed in this study indicates cardiac benefits, since HDL increases reverse cholesterol transport (5). No significant decrease in cholesterol and glucose herein observed could be because these values were within normal limits. These lipid profile amelioration probably were not sustained during the training period because the workload was not modified (5% body weight whole period) and in this situation it would be possible an habituation to the load and the loss of physiological adaptation.

After 17 weeks of experiment (table 3) LA supplementation lowered triglycerids levels by 17,6% when compared to the exercise group, a benefit that was not sustained at this time in the association with exercise and LA. Again a negative interaction of the association of exercise and LA, that in this time reduce the lipoic acid beneficial influence to lipid profile. Supplementation with lipoic acid (both isolated and associated with exercise) also showed an increase in HDL compared to the exercise group (217% and 165%, respectively). Some studies have observed effects of LA in

improving the lipid profile, for example, Seo et al (28) observed significant reduction in plasma lipid profile of Sprague-Dawley rats fed with a high-fat diet who received LA (0,25 and 0,50% of LA in the diet). In the study by Park et al (20) LA reduced hepatic lipogenesis through AMPK-dependent pathways. Kuo et al (13) showed that LA decreases intracellular lipid accumulation by activating AMPK.

Moderate chronicle exercise promotes bradycardia, that is an important adaptation to cardiovascular system health, but its association with lipoic acid supplementation disrupted that beneficial. Moderate exercise at 9^{th} week or LA supplementation at 17^{th} week proved to be effective in ameliorating the lipid profile, which means that both are important cardioprotective agents, but again it was not observed when in association, when exercise abolishes LA tryglicerides reduction. This study with an experimental chronic protocol of moderate intensity of swimming training could demonstrate some of that expected cardiovascular benefits induced by exercise and lipoic acid to healthy subjects (like bradycardia and lipid profile improvement), and a negative interaction with lipoic acid supplementation (exercise bradycardia abolishment in one side, and a lost of lipid amelioration induced by LA supplementation on the other side). Considering that LA consumption are so widespread due to lipolytic effects, specially by subjects with obesity and metabolic syndrome (diabetes, hypertension, renopathologies, more ever), and the experimental observation that exercise and LA have a negative interaction in such cardiovascular parameters, future studies must focus on proving if that negative interaction could be occuring also to other experimental models such as human beings to orientate the lipoic acid consumption without risks to human health.

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Table 1: Temporal analysis of the effect of moderate exercise on blood pressure and heart rate for the control group (C) and exercise (E) at 9 weeks and 17 weeks in *Rattus norvegicus*.

Parameters	C (n= 40)	E9 (n= 20)	E17 (n= 20)	P value
DBP (mmHg)	153±26,8	139±27,2	157±35,0	0,195
SBP (mmHg)	200±34,4	178±38,6	197,93±39,1	0,082
HR(mmHg)	434±36,1	403±24,2 *	398±25,1*	0,000

Values are presented as mean and standard deviation. Diastolic blood pressure (DBP), systolic blood pressure (SBP) and heart rate (HR). * P <0:05 vs. control.

Table 2: Temporal analysis of the effect of moderate exercise on blood biochemistry for the control group (C) and exercise (E) at week 9 and week 17 in *Rattus norvegicus*.

Parameters	C (n= 20)	E9 (n= 10)	E17 (n= 10)	P value
Glucose	109,6±12,6	121,5±14,4	103,0±6,1§	0,026
Cholesterol	129,6±9,1	122,9±9,7	133,6±16,4	0,198
TGs	135,7±31,1	101,2±5,5*	147,4±35,0§	0,005
HDLc	13,3±3,7	68,5±0,9*	10,9±2,5§	0,000
LDLc	116,4±11,6	54,5±9,4*	122,2±15,3§	0,000
Lactate	2,9±0,8	5,8±0,8*	4,4 ± 0,9§	0,000

Values are presented as mean and standard deviation. Diastolic blood pressure (DBP), systolic blood pressure (SBP) and heart rate (HR). * P <0:05 vs. control, § P <0:05 vs. exercise at week 9.

Table 3: Values of blood pressure and heart rate (n=10 rats/group) and blood biochemistry (n=5 rats/group) at 17th week.

Parameters	Control	Exercise	LA	Exercise + LA	pvalue
DBP (mmHg)	147 ± 9,6	157 ± 17,9	159,1 ± 7,8	130,2 ± 3,0	0,708
SDP (mmHg)	180,7 ± 10,1	206,3 ± 21,8	200,3 ± 10,2	170,1 ± 4,1	0,528
HR (mmHg)	445 ± 6,9	396,8 ± 11,5*	419,2 ± 6,9	409,2 ± 16,6	<0,001
Glucose (mg/dL)	106,4 ± 3,6	103 ± 2,7	101,6 ± 8,2	113,1 ± 5,1	0,450
Cholesterol (mg/dL)	127,6 ± 5,7	133,1 ± 7,3	140,9 ± 3,8	124,7 ± 9,4	0,390
TGs (mg/dL)	125 ± 4,6	147,4 ± 15,6	102,9 ± 6,3†	153,4 ± 4,6‡	0,004
HDLc (mg/dL)	16,5 ± 0,8	11 ± 1,1	23,9 ± 3,2†	18,2 ± 0,7†	<0,001
LDLc (mg/dL)	111,1 ± 6,3	122,1 ± 6,8	117 ± 4,7	106,4 ± 8,8	0,415
Lactate (mmol/L)	3,3 ± 0,5	5.5 ± 0,4*	3.2 ± 0.4	5,7 ± 0,2*	<0,001

Values are presented as mean and standard deviation. Diastolic blood pressure (DBP), systolic blood pressure (SBP), heart rate (HR), glucose, total cholesterol and fractions (HDLc and LDLc), triglycerides (TGs) and lactate. * P <0:05 vs. control, † P <0:05 vs. exercise; ‡ P <0:05 vs. lipoic Acid.

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MODERATE EXERCISE AND LIPOIC ACID SUPPLEMENTATION ON CARDIAC REMODELLING AND ANGIOGENESIS IN *RATTUS NORVEGICUS*.

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SHORT TITLE: MODERATE EXERCISE AND LIPOIC ACID ON CARDIAC REMODELLING AND ANGIOGENESIS

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Abstract

Angiogenesis is a complex mechanism that creates new vessels and that is regulated by a large number of pro-angiogenic and anti-angiogenic factors vascular endothelial growth factor (VEGF) is essential for angiogenesis and its expression is increased with exercise and decreased with lipoic acid (LA). This study examined in a murine model the influence of moderate aerobic chronicle exercise and LA supplementation on angiogenesis, skeletal and cardiac width muscle fiber, wall thickness and chamber area of the left ventricle. Forty male Wistar rats were divided into: control (C), lipoic acid (LA), exercise (E) and association (ELA). A program of swimming training was conducted for 17^{th} weeks, 5 times/week with a progressive time and load intensity (5% of body load). The animals received LA 5 times/week (10^{th} to 17^{th} week), 60 mg/Kg/day. Angiogenesis was increased in the heart and gastrocnemius of exercised animals, the fiber width of E, LA and ELA reduced in the heart while in gastrocnemius had an increase in fibers width only in LA and ELA. The left ventricle thickness decreased in E group. Area of the left ventricle chamber and VEGF circulation levels shows no significant differences. This study for the first time focus the interaction between chronic moderate exercise and lipoic acid supplementation, confirming the benefits of the physical practice in improving muscle blood supply, that was not affected by lipoic acid consumption. However lipoic acid in untrained animals do not stimulate cardiac angiogenesis and in an opposite way showed a tendency to reduce the new vessels.

Keywords: Exercise, lipoic acid, angiogenesis.

Introduction

Angiogenesis is a complex mechanism by which capillaries are created from preexisting blood vessels and can occur by two different processes that happen throughout life. The first one, sprouting angiogenesis**,** is characterized by a sprout composed of endothelial cells which grow in response to pro-angiogenic stimulus (4). The other one, intussusceptive angiogenesis, is a process where the elements of existing vessels invade the interstitial tissues, leading transverse columns formation that expands separating the original vessel into two new vessels (4, 5).

These mechanism is regulated by a large number of pro-angiogenic and anti-angiogenic factors (20, 24, 31). Among the molecules able to stimulate this process, angiogenin, platelet-derived endothelial cell growth factor and the vascular endothelial growth factor (VEGF) are highlighted (6). VEGF has been widely studied. It is essential for the growth and development of the individual, and functions in adult life regulating the number of capillaries. (21, 22, 26).

There is evidence of stimuli that enhance or block the expression of VEGF. Among them is the exercise, acting in this process by increasing the recruitment of new blood vessels by stimulating the production of VEGF (3, 25, 36). Unlike, there is the lipoic acid (LA), a common ingredient in multivitamin supplements, anti-aging, and even feed (31) that is present in small amounts in tissues with antioxidant function (18). It has the capacity to reduce the expression of genes related to angiogenesis, including VEGF (2, 12, 17).

Furthermore chronicle exercise is able to generate some adaptations such as hypertrophy and increase in the width of cardiomyocytes (9, 15).

Nowadays it's common the LA supplementation in association with the practical of exercises due to its fatty burner effects. However, the influence of these antagonic stimuli to angiogenesis was not known at all. Also the effects of LA consumption to the heart morphology still remains unknown. Thus, this study examined in a murine model the influence of moderate aerobic chronicle exercise and LA supplementation on angiogenesis, skeletic and cardiac width muscle fiber, wall thickness and chamber area of the left ventricle.

Material and Methods

The study was approved by the Ethics Committee for Animal Use (CEUA nº P059/2011) of the Universidade Federal do Rio Grande – FURG, Brazil and the animals were treated according to the Brazilian Guidelines of Practice for the Care and Use of Animals for Scientific Purposes and Teaching (DBPA).

1. Animal model:

Forty heterogenic male Wistar rats, 2 and 3 month/old, weighing 200 to 300g at baseline were used. The animals were obtained from the Central Animal Facility of the Universidade Federal do Rio Grande – FURG, Brazil, were housed in collective cages (five animals per cage) and fed commercial rodent food (15g/rat/day) according AIN-93 (27) and received water *ad libitum* diary. They were maintained in a temperature controlled room (24 ± 2° C) with a 12h light/dark cycle.

2. Swimming program:

A program of swimming training was conducted adapted from Guerreiro (7). Rats were exercised five times a week with a progressive increase in swimming time and load intensity. In the first week the animals were adapted progressively to the swimming tank: on the first day for 15 minutes, increasing 15 minutes a day, reaching 60 minutes on the last day according to protocol of Rombaldi (28).

In the second week was started the adaptation period to a moderate intensity of swimming, by placing a load of lead (attached to the back of the animal by an elastic) progressively increased, from 1% of body weight/week until reach 5% (maximum load for moderate intensity according Kokubun, 1990). For each increase of overweight there

was an adaptation to the swimming time, as described above, so that the animal had the ability to maintain the exercise for 60 minutes at the new load. To determine the load, the animals were weighed weekly.

The period of adaptation to the maximum swimming time (60 min/day) and the maximum load (5% of body weight) took 9 weeks, followed by another 8 weeks of training with maximum time and load. The whole experiment took place in 17 weeks.

3. Lipoic acid supplementation:

The animals received lipoic acid 5 times a week, (10^{th} to 17^{th} week), 60 mg/Kg/day administered intraperitoneal (IP) following a dose utilized by Lin et al. (2006) that is capable to reduce 43% VEGF in the retina and 88% in angiogenesis in diabetic rats. Lipoic acid was dissolved in a solution of NaOH 2M containing NaCl 154 mM. Then the pH was adjusted to 7.4 with concentrated HCl. Control animals received 1mL/Kg/day of a saline solution injected IP. (33).

4. Experimental groups:

Animals were divided into four groups (n= 10 animals): control group (C): rats were placed in the swimming tank to mimic water stress, for 1 minute, 5 times a week, during 17 weeks, and from week 10 to 17 received 1mL/Kg/day of saline injection IP (to simulate injection stress); lipoic acid group (LA): were submitted to simulation water stress like control group and were supplemented with lipoic acid solution, 5 times a week, 60mg/Kg/day from the 10^{th} to 17^{th} week; exercise group (E): rats were submitted to the program of adaptation and swimming training, 5 times a week, with a 5% of body weight load; association group (ELA): animals were submitted to the

program of adaptation and swimming training, 5 times a week and supplemented with lipoic acid, 5 times a week, 60mg/Kg/day from the 10 th week to 17th week.

5. Heart morphometry and the Cardiac and Skeletal Angiogenesis:

At the end of the experiment (17 weeks of swimming training) five animals from each group were kept 72 hours without supplementation or physical training and were euthanized by decaptation, heart and left gastrocnemius muscle were dissected, and left ventricule was separated from the rest of the heart. Tissues were fixed with paraformol 4% and processed by standard histological techniques, stained by Hematoxilin and Eosin to determine myocardial morphometry or processed according Lodja (16) by the method of alkaline phosphatase to determine the new vessels (angiogenesis). In summary, the tissues (heart and gastrocnemius) were collected and kept at -80° C, then were cut in a cryostat and stained immediately (N,N-dimethylformamide mixed with Fast Blue B salt was dissolved in phosphate buffer, it was filtered and incubated at room temperature, rinsed in distilled water and incubated into alkaline phosphatase). Slides were digitalized by a camera coupled to a microscope and the following measurements were analyzed: skeletal and myocardial fiber width (μm), wall thickness (transformed values, Kruskal-Wallis test) chamber area (mm^2) of the left ventricule (figure 1) and number of new vessels (expressed as a percentage of the tissue area) as shown in the figure 2. Images were recorder by a computer and measured with the Software Image J.

6. Quantitative determination of VEGF:

Body blood samples were colected and were centrifuged to plasma separation. Assay was conducted according to manufactory instruction (Multiplex Map Kit,

Multipore). VEGF present in the samples were detected by total antibodies. Plate was read on Luminex 100 IS and the median fluorescent intensity was analyzed using a weighted 5-parameter logistic or spline curve-fitting method for calculating analyte concentration in samples.

7. Statistical Analysis:

Values were expressed as mean ± S.D. The Shapiro-Wilks test was used to verify that the data had normal distribution. Variables with normal distribution were analyzed by one-way ANOVA, since the asymmetric distribution were analyzed using the Kruskal-Wallis test. The alpha error rate was 5% (P = 0.05).

Results

Angiogenesis at heart increased significantly by 58% and 68,1% in the groups ELA and E respectively, compared to LA group ($p < 0,05$). Also at gastrocnemius angiogenesis was augmented, ELA and E groups showed 58% and 47,9%, increase of new vessels, respectively, compared to the LA group, ($p = 0,001$). In both, heart and gastrocnemius, LA group has a tendency to reduction of new vessels compared to the C group ($p= 0,07$) as can be seen in Figure 3.

The fiber width (Figure 4) was analyzed and results demonstrated that in the heart, a reduction in E, LA and ELA (30,2±0,8; 27,7±0,7 and 29,2±0,9 µm, respectively) compared to the group C (47,5±2,9 µm), $p <0,001$. Gastrocnemius inversely showed an increased in fibers width in LA (107,3±2,1 µm) group compared to the C group (96,6±3,4µm), while LA and ELA (104,9±2,5 µm) groups showed higher muscle fibers widths than E group (88,3±2,6 µm), $p < 0,001$. E group shows a tendency to decrease gastrocnemius fibers compared to the C group.

The left ventricle (LV) thickness values, shown in Figure 5, demonstrate decreased of LV wall in E (47,7±1,7) group compared to LA and ELA (52,8±0,5 and 54,0±0,8, respectively), $p = 0,0124$. When comparing the E with the C group noticed a tendency to reduce the thickness in the E group ($p =0,07$).

The area of the LV chamber is shown in Figure 6, and presented no significant difference between the groups ($p = 0,1475$).

The values of VEGF (tabela 1) in circulation showed no significant difference in the experimental groups ($p=0,8226$).

Discussion

The present study demonstrated that exercise, either alone or associated with LA, increased angiogenesis in myocardial and in skeletal muscle compared to the LA group. Another study, Silva Junior et al (32), found that trained rats (swimming of moderate and high intensity) showed significantly greater angiogenesis than sedentary. They also observed that response was positively swimming intensity dependent.

Lin et al (15) got similar results to LA with diabetic Wistar rats, checking 88% reduction in the number of capillaries in animals treated with LA (ip 60mg/Kg/day for 30 weeks) compared to diabetic rats untreated.

Angiogenesis is the expected result of chronic exercise, because exercise induces multiple cellular signals, including mechanical stimuli resulting from increase of blood flow and muscle contraction and a decrease in intracellular oxygen levels (34). Hypoxia increases the levels of HIF (hypoxia-inducible factor) which increases VEGF synthesis (9). Whereas supplementation with LA has the opposite effect, since LA is able to reduce the expression of genes related to angiogenesis, including VEGF (11).

We did not obtain significant reduction of angiogenesis in animals supplemented with LA possibly, but a trend to reduction could be pointed out.

In this study we observed a reduction of heart fiber width in rats exercised and LA supplemented whereas in gastrocnemius an increase in the fiber width to groups LA supplemented was verified. Similar results were obtained by Pereira et al (22) found a reduction in width of cardiomyocytes in exercised mice with cardiac failure (60 minutes, 5 days/week for 8 weeks), improving ventricular function. Ahtiainena (1) observed an increase in the width of skeletal muscle fibers in humans undergoing resistance training for 21 weeks.

Adjustment of the force is essentially a matter of regular fiber size and the size is controlled by altering the balance between synthesis and degradation of proteins in each muscle fiber (8). Herein a reduction of fiber width in the heart as result of chronicle exercise was not accompanied by an increased of LV chamber, a classic eccentric hypertrophy exercise adaptation. Also the expected skeletal muscle hypertrophy was not observed probably because a habituation to training load. For the first time we could demonstrate that LA acted reducing cardiac fiber and increasing skeletal muscle fiber. These antagonical results could be related to diverse cellular mechanisms elicited in each tissue, and point out to a possible risk of LA in remodeling cardiomyocites reducing angiogenesis and fiber width.

Also the wall thickness of LV decreased significantly in the EX group compared to LA and ELA, and a tendency to EX group show a minor thickness of the LV compared to C group. Libonati et al (11) reported that aerobic exercise (60 minutes, 5 days/week for 12 weeks) increases cardiac remodeling in spontaneously hypertensive rats by increasing the proliferation and hypertrophy of cardiomyocytes. While, Lee et al (2012) did not observe significant differences in the thickness of LV in diabetes-prone mice treated with LA (200 mg/Kg/day for 16 weeks) compared with untreated animals. Guerreiro (7) observed with the same training protocol a significant augment of left ventricular chamber area without alteration on wall thickness.

The chronic physical training results in a phenotype of eccentric hypertrophy, characterized by dilation of the LV chamber with an increase in the size of cardiomyocytes (18). In this study, the area of the LV chamber showed no significant difference between the experimental groups. Libonati (14) noted eccentric cardiac hypertrophy induced by exercise (treadmill running 60 min, 5 days/week for 6 weeks), with an increase in the width of cardiomyocytes (7%). This increase induces

enlargement of the LV chamber, adaptive pattern that results in an improvement in the blood volume ejected at rest and during exercise. Supplementation with LA showed no difference from the control group and for all we know there are no reports of the influence of the LA in heart chamber area in healthy rats.

The values of VEGF showed no significant difference in the experimental groups. Silva Junior et al (32) studied the expression of VEGF in the heart after exercise and found increased of 37% in the moderate-intensity swimming (for 10 weeks) and 108% in swimming high intensity when compared to the sedentary group. In contrast, the study of Alleva et al (2) LA supplementation in patients with chronic wounds led to decreased expression of VEGF. Ours finds could be inconclusive probably because VEGF was determined in circulation, a technical limitation of the study that do not aloud known LA effects to cardiac and skeletal muscles.

One of the known mechanisms that explain how exercise could stimulates VEGF synthesis is that exercise hypoxia rise hypoxia inducible factor (HIF) that increases VEGF gene transcription (35, 9). Our study did not obtain significant differences between experimental groups, but exercise improved angiogenesis, and we cannot exclude that VEGF or other pro-angiogenic factors were influenced by moderate exercise.

This study focus for the first time the interaction between chronicle moderate exercise and lipoic acid supplementation upon cardiac remodeling and angiogenesis, confirming the benefits of the physical practice in improve muscle blood supply, that was not affected by lipoic acid consumption. Lipoic acid in untrained animals failed to stimulate cardiac angiogenesis and in opposite way show a tendency to reduction the new vessels. Indeed, our exercise protocol promotes a reduction in fiber width and ventricular wall thickness, with a tendency to enlargement of the left ventricular area, a

morphological adaptation that may be improving cardiac function, and that was abolished when submitted to lipoic acid supplementation. Further studies, with highest sample number and longest training periods, could corroborate this negative interaction of exercise and lipoic acid consumption, proving the possible antiangiogenic effect and a prejudice to cardiac function upon an exercised heart that could not elicited the expected physiological hypertrophy.

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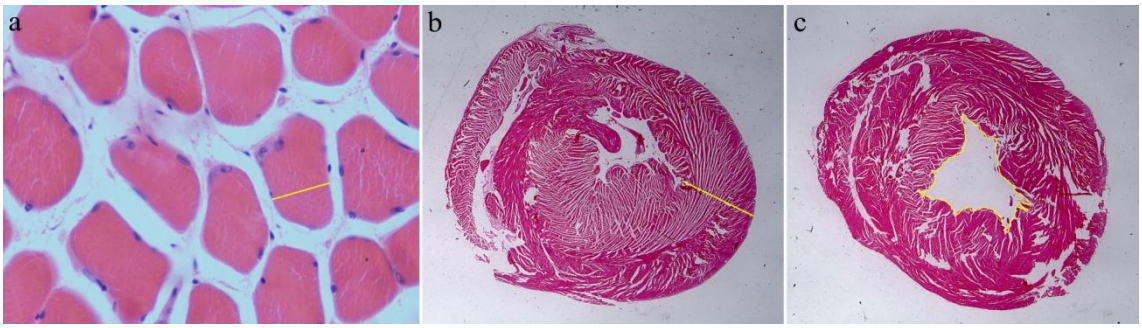

Figure 1: Pattern of measurements of the gastrocnemius and heart of *Rattus norvegicus.* (a) Minor width muscle fiber; (b) thickness of the wall of left ventricule (LV); (c) Chamber area of the LV (c). HE stain, augment 20x (a) and 10x (b, c).

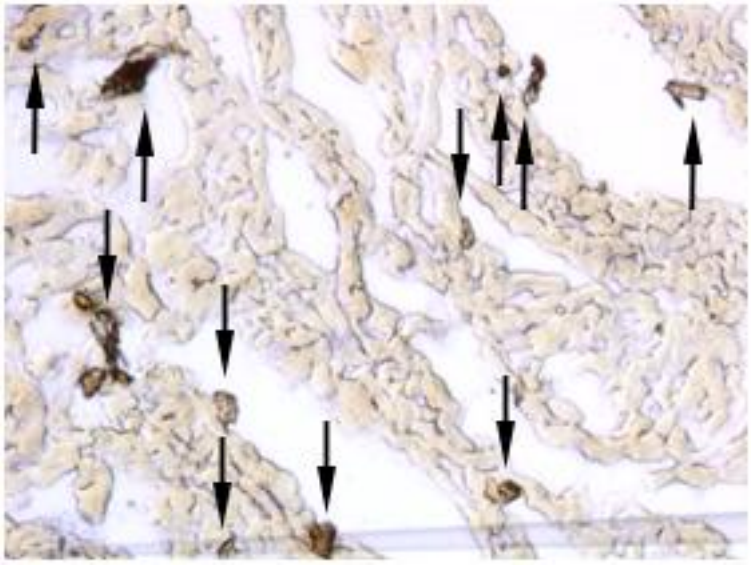

Figure 2: The figure shows the histological section of the gastrocnemius muscle with marking of new vessels (arrow head) (staining according to Lodja (16) by the method of alkaline phosphatase to determine angiogenesis, augement 10x).

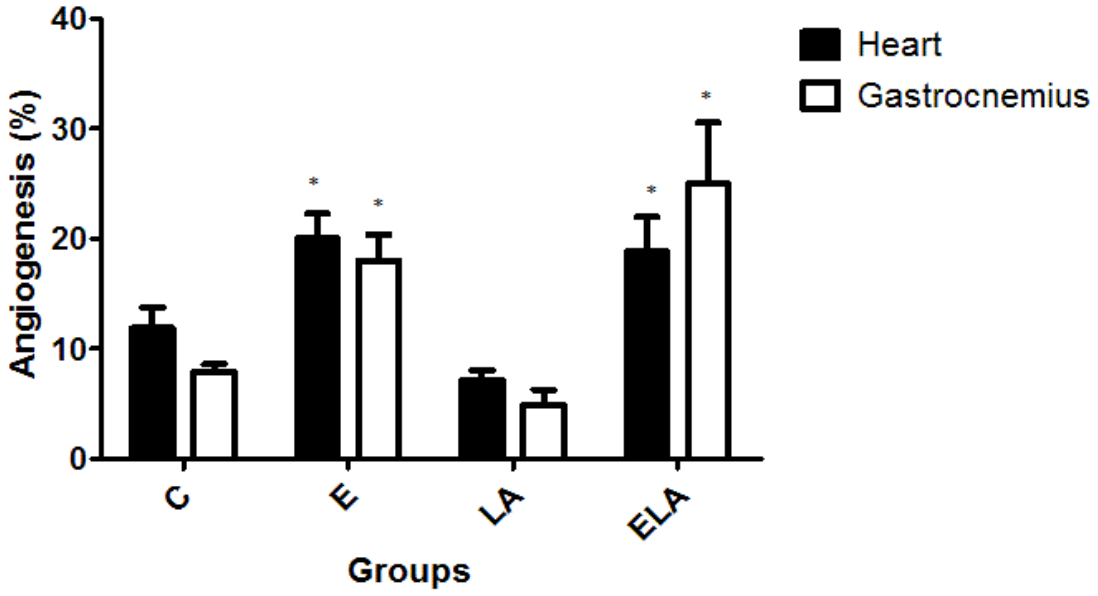

Figure 3: Angiogenesis (% of new vessels) in heart (black bars) and gastrocnemius (white bars) of *Rattus norvegicus*. C= controls; E= exercised rats submitted to a swimming training protocol of seventeen weeks; LA= lipoic acid supplementation (60mg/rat/day); ELA = exercised rats submitted to a swimming training protocol of seventeen weeks and supplemented with LA. * significant differences respect to LA group (P <0:05).

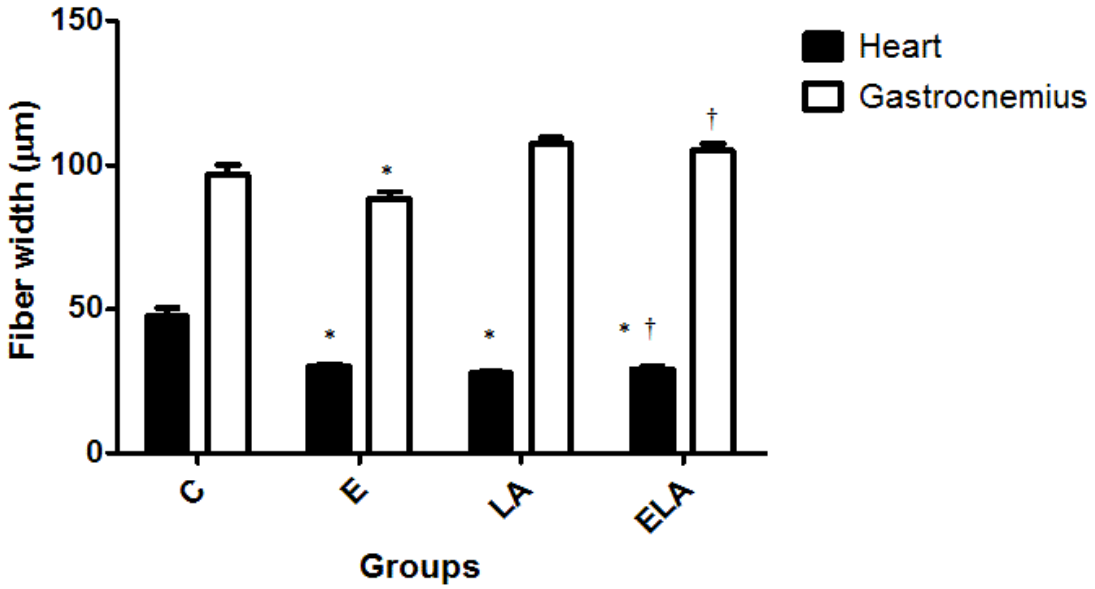

Figure 4: Heart (black bars) and the gastrocnemius (white bars) fiber width of *Rattus norvegicus*. C= controls; E= exercised rats submitted to a swimming training protocol of seventeen weeks; LA= lipoic acid supplementation (60mg/rat/day); ELA = exercised rats submitted to a swimming training protocol of seventeen weeks and supplemented with LA. * significant differences respect to control group and † significant differences respect to exercise (P <0,05).

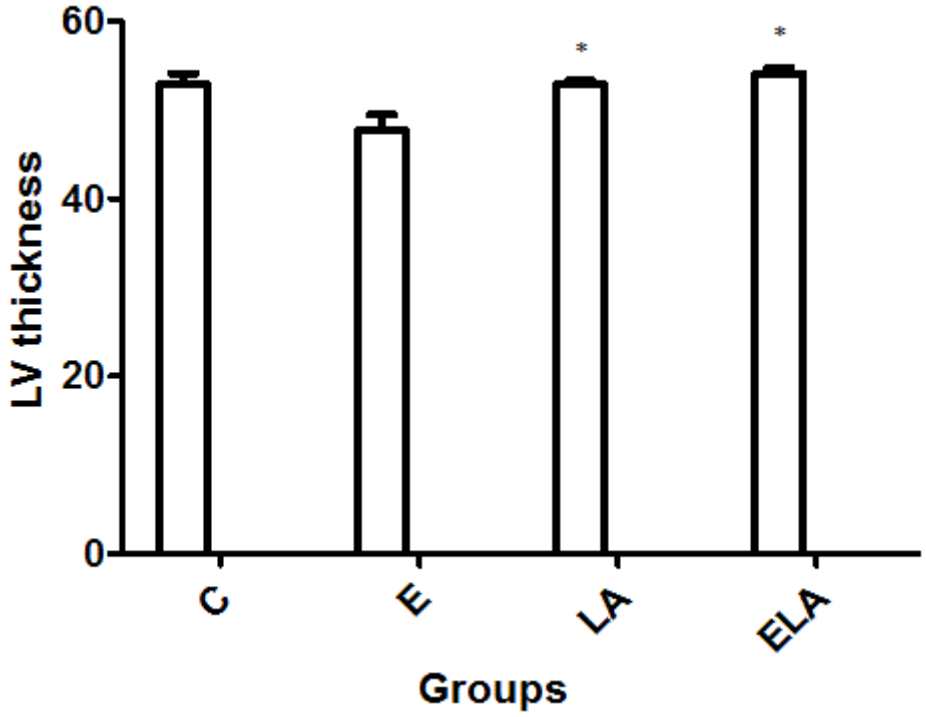

Figure 5: Left ventricle thickness of *Rattus norvegicus*, transformed values (Kruskal-Wallis test). C= controls; E= exercised rats submitted to a swimming training protocol of seventeen weeks; LA= lipoic acid supplementation (60mg/rat/day); ELA = exercised rats submitted to a swimming training protocol of seventeen weeks and supplemented with LA. * significant differences respect to E group (P <0:05).

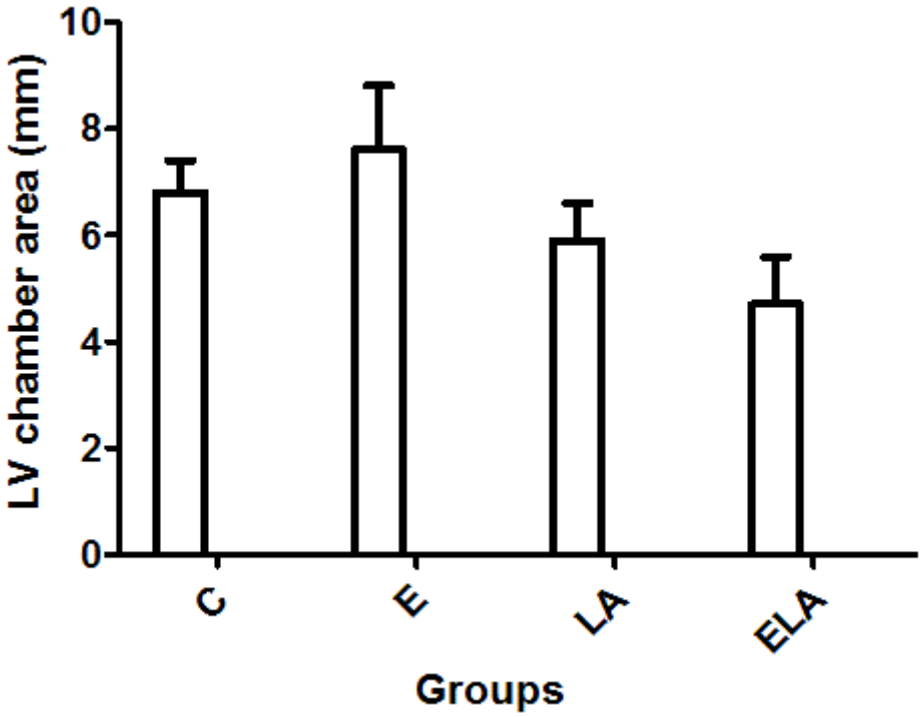

Figure 6: Area of th left ventricle chamber (mm^2) of *Rattus norvegicus*. C= controls; E= exercised rats submitted to a swimming training protocol of seventeen weeks; LA= lipoic acid supplementation (60mg/rat/day); ELA = exercised rats submitted to a swimming training protocol of seventeen weeks and supplemented with LA.

Table 1: Morphological adaptations and levels of circulating vascular endothelial growth factor (VEGF) of *Rattus norvegicus* after seventeen weeks of swimming training.

Variables	Control	Exercise	LA	Exercise + LA	p value
VEGF	8748,7 ± 1437,5	10172,1±2974,2	6623,7±773,2	13339,2±5237,2	0,8226
Angiogenesis-H	11,9±1,9	20,0±2,3*	7,2±0,8	18,8±3,2*	0,038
Angiogenesis-G	7,9±0,7	18,0±2,4*	4,9±1,4	25,0±5,6*	0,002
Fiber width-H	47,5±2,9	30,2±0,8†	27,7±0,7†	29,2±0,9†‡	<0,001
Fiber width-G	96,6±3,4	88,3±2,6	107,3±2,1†	104,9±2,5‡	<0,001
LV thickness	52,8±1,3	47,7±1,7	52,8±0,5‡	54,0±0,8‡	0,012
LV chamber area	6,8±0,6	7,6±1,2	5,9±0,7	4,7±0,9	0,147

Values are presented as mean and standard error. Vascular endothelial growth factor (pmol/L), angiogenesis in the heart (%), angiogenesis in the gastrocnemius (%), fiber width in the heart (µm), fiber width in the gastrocnemius (µm), left ventricle thickness (transformed values, Kruskal-Wallis test) a left ventricle chamber area (mm). * P <0:05 vs. lipoic acid, † P <0:05 vs. control; ‡ P <0:05 vs. exercise.

Conclusão Geral

Este estudo mostrou pela primeira vez a interação entre o exercício crônico de intensidade moderada e a suplementação com ácido lipóico sobre a frequência cardíaca, bioquímica sanguínea, remodelagem cardíaca e angiogênese.

Comprovamos que alguns benefícios cardiovasculares são induzidos pelo exercício e pelo ácido lipóico em indivíduos saudáveis (como bradicardia e melhora o perfil lipídico). Porém também identificamos uma interação negativa do exercício com o ácido lipóico (supressão da bradicardia do exercício pela suplementação com AL, e perda da adaptação no perfil lipídico induzida pelo AL quando em associação com exercício).

O estímulo da angiogênese foi confirmado como benefício da prática de atividade física moderada, por melhorar o fornecimento de sangue ao músculo cardíaco, efeito que não foi afetado pelo consumo de ácido lipóico. O ácido lipóico em animais não treinados não foi capaz de estimular a angiogênese cardíaca e, ao contrário, mostra uma tendência para a redução dos novos vasos.

Nosso protocolo de exercício promoveu uma redução na largura da fibra e espessura da parede ventricular esquerda, com uma tendência de aumento da área do ventrículo esquerdo, uma adaptação morfológica que pode estar melhorando a função cardíaca e que foi abolida quando adicionada a suplementação com ácido lipóico.

Considerando-se que o consumo de AL é tão difundido devido ao seu efeito lipolítico, especialmente por sujeitos com obesidade e síndrome metabólica (diabetes, hipertensão), e que a observação experimental de que o exercício e AL têm uma interação negativa em tais parâmetros, estudos futuros devem se concentrar em provar se essa interação negativa poderia ocorrer também com outros modelos experimentais, incluindo estudos com seres humanos, para conduzir uma orientação do consumo de ácido lipóico, sem riscos para a saúde humana.

Referências da Introdução Geral

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