PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
MESTRADO EM CIÊNCIA ANIMAL

ASSOCIAÇÃO DO HORMÔNIO DO CRESCIMENTO COM EXERCÍCIO RESISTIDO SOBRE A MORFOLOGIA, EXPRESSÃO GÊNICA E MARCADOR CARDÍACO.

ADRIANA JUNQUEIRA

Presidente Prudente – SP
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Exame geral de Defesa de Dissertação apresentada a Pró-Reitoria de Pesquisa e Pós-Graduação, Universidade do Oeste Paulista, como parte dos requisitos para obtenção do título de Mestre em Ciência Animal.

Área de Concentração: Fisiopatologia Animal

Orientadora: Profª. Drª. Francis Lopes Pacagnelli

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ADRIANA JUNQUEIRA

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Presidente Prudente, 29 de Setembro de 2014.

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DEDICATÓRIA

Aos meus pais: José Garcia Junqueira Sobrinho e Maria Helena Matos Junqueira, que sempre souberam me ensinar os melhores valores da vida, e incentivar a buscar o saber e o aprender sem nunca desistir diante dos obstáculos e dificuldades.

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“A medida do amor é amar sem medidas”.
(Santo Agostinho)

RESUMO

ASSOCIAÇÃO DO HORMÔNIO DO CRESCIMENTO COM EXERCÍCIO RESISTIDO SOBRE A MORFOLOGIA, EXPRESSÃO GÊNICA E MARCADOR CARDÍACO

Objetivo- Este estudo teve como objetivo avaliar os efeitos do Hormônio do Crescimento (GH), em ratos submetidos ao Treinamento Resistido (TR) sobre remodelação cardíaca nos aspectos morfológicos, genes do Ca2+ e marcador cardíaco.
Desenho- Ratos Wistar machos foram distribuídos em 4 grupos: controle (CT, n=7), Hormônio do Crescimento (GH, n=7), Treinamento Resistido (TR, n=7) e Treinamento Resistido com Hormônio do Crescimento (TRGH, n=7). A dose do GH foi de 0,2 UI/Kg, via subcutânea, a cada dois dias e o modelo de TR utilizado foi o salto vertical na água (4 séries de 10 saltos/dia, 3 sessões/semana) ambos por 30 dias consecutivos. Foram avaliadas as variáveis anatômicas, peso corporal final (PCF), peso do Ventrículo Esquerdo (VE) e a relação VE/PCF. A análise morfológica constou da avaliação da área dos cardiomiócitos (Hematoxilina e Eosina-HE) e da fração de colágeno (Picrosirius-red). O nível de expressão do RNAm das proteínas da bomba de cálcio (Ca2+) do retículo sarcoplasmático Ca2+ ATPase (SERCA2a), fosfolambam (PLB) e rianodina (RyR) do miocárdio foi avaliado por PCR em tempo real (qPCR). A dosagem da creatina quinase fração músculo-cérebro (CK-MB), foi avaliada por meio de análise sérica.
Resultados- O PCF, peso do VE, a relação VE/PCF, a área dos cardiomiócitos, e a expressão gênica SERCA2a, PLB e RyR, não mostrou diferença estatística entre os grupos. Para o colágeno, houve aumento do grupo TR ($p < 0,05$), quando contrastado com os demais grupos (CT, GH e TRGH). Na análise bioquímica da CK-MB houve diferença estatística dos grupos treinados em relação aos grupos não treinados.
Conclusão- O TR interfere na remodelação cardíaca aumentando o colágeno intersticial, que pode ser decorrente da lesão miocárdica caracterizada pelo aumento da CK-MB, porém, quando associado ao GH o colágeno não se alterou.

Palavras-chave: Expressão gênica, miocárdio, cálcio, hormônio do crescimento, treinamento.

ABSTRACT

ASSOCIATION OF GROWTH HORMONE WITH RESISTANCE EXERCISE ON MORPHOLOGY, GENE EXPRESSION AND CARDIAC MARKER

Objective-This study aimed to evaluate the effects of growth hormone (GH), in rats submitted to resistance training (RT) on cardiac remodeling in morphological aspects, Ca^{2+} genes and cardiac marker.
Design- Wistar male rats were divided into 4 groups: control (CT, n = 7), Growth Hormone (GH, n = 7), Resistance Training (RT, n = 7) and Resistance Training with Growth Hormone administration (RTGH, n = 7). The GH dose was 0,2 IU/kg through subcutaneous administration every two days, and the RT model used was the vertical jump in water (4 sets of 10 jumps/day, 3 sessions/week) both for 30 consecutive days. Anatomical variables such as final body weight (FBW), left ventricle weight (LV) and the LV/FBW ratio of rats were evaluated. The morphology analysis consisted of the cardiomyocytes cross-sectional area (HE) and collagen fraction (picrosirius). The mRNA of the proteins of the calcium pump of the sarcoplasmic reticulum Ca^{2+} ATPase (SERCA2a), phospholamban (PLB) and ryanodine (RyR) infarction were evaluated by real-time PCR (qPCR). The cardiac marker dosed was creatine kinase muscle-brain fraction (CK-MB), through serum analysis
Results- FBW, LV weight, LV/FBW ratio, cardiomyocytes cross-sectional area, as and SERCA2a, PLB and RyR gene expression, showed no statistical difference between the groups, whereas for the collagen assessment , an increase in RT group ($p < 0,05$) was observed when compared to other groups (CT, GH and RTGH). In biochemical analysis of CK-MB there was statistical difference between trained groups and untrained groups.
Conclusion- The RT interferes with cardiac remodeling by increasing interstitial collagen, which may result from the myocardial injury characterized by increased CK-MB, however, when combined with GH collagen remained unchanged.

Key words: Gene expression, myocardium, calcium, growth hormone, training.

LISTA DE ABREVIAÇÕES

ACTB – Beta actina

AngII – Angiotensina II

ANOVA – Análise de variância

ºC – Grau Celsius

Ca^{2+} – Cálcio

cDNA - Ácido desoxirribonucleico complementar

CK-MB – Creatina quinase fração músculo-cérebro

CT – Controle

DNA – Ácido desoxirribonucleico

GH – Hormônio do crescimento

HE – Hematoxilina eosina

IC – Insuficiência cardíaca

IGF-1 – Fator de crescimento semelhante a insulina 1

MHC – Miosina de cadeia pesada

NO – Óxido Nítrico

PCF – Peso corporal final

PLB – Fosfolambam

rhGH – Hormônio do crescimento recombinante humano

RNA – Ácido ribonucleico

RNAm – Ácido ribonucleico mensageiro

RS – Retículo sarcoplasmático

RT-qPCR – Transcrição reversa da reação em cadeia da polimerase em tempo real

RyR – Rianodina

SERCA2a – Retículo sarcoplasmático cálcio ATPase

TNF-α – Fator de necrose tumoral alfa

TR – Treinamento resistido

TRGH – Treinamento resistido com hormônio do crescimento

VE – Ventrículo esquerdo

VE/PCF – Relação ventrículo esquerdo e peso corporal final

SUMÁRIO

1 ARTIGO CIENTÍFICO[1]

ASSOCIAÇÃO DO HORMÔNIO DO CRESCIMENTO COM EXERCÍCIO RESISTIDO SOBRE A MORFOLOGIA, EXPRESSÃO GÊNICA E MARCADOR CARDÍACO

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GH e treinamento de resistência na insuficiência cardíaca

Destaques:

Treinamento de Resistencia training induced collagen synthesis

rhGH combined with training did not alter cardiac calcium genes

rhGH associated with exercise prevented increased collagen

[1] Normas da Revista Growth Hormone and IGF Research

RESUMO

Objetivo- Este estudo teve como objetivo avaliar os efeitos do Hormônio do Crescimento (GH), em ratos submetidos ao Treinamento Resistido (TR) sobre remodelação cardíaca nos aspectos morfológicos, genes do Ca^{2+} e marcador cardíaco.

Desenho- Ratos Wistar machos foram distribuídos em 4 grupos: controle (CT, n=7), Hormônio do Crescimento (GH, n=7), Treinamento Resistido (TR, n=7) e Treinamento Resistido com Hormônio do Crescimento (TRGH, n=7). A dose do GH foi de 0,2 UI/Kg, via subcutânea, a cada dois dias e o modelo de TR utilizado foi o salto vertical na água (4 séries de 10 saltos/dia, 3 sessões/semana) ambos por 30 dias consecutivos. Foram avaliadas as variáveis anatômicas, peso corporal final (PCF), peso do Ventrículo Esquerdo (VE) e a relação VE/PCF. A análise morfológica constou da avaliação da área dos cardiomiócitos (Hematoxilina e Eosina-HE) e da fração de colágeno (Picrosirius-red). O nível de expressão do RNAm das proteínas da bomba de cálcio (Ca^{2+}) do retículo sarcoplasmático Ca^{2+} ATPase (SERCA2a), fosfolambam (PLB) e rianodina (RyR) do miocárdio foi avaliado por PCR em tempo real (qPCR). A dosagem da creatina quinase fração músculo-cérebro (CK-MB), foi avaliada por meio de análise sérica.

Resultados- O PCF, peso do VE, a relação VE/PCF, a área dos cardiomiócitos, e a expressão gênica SERCA2a, PLB e RyR, não mostrou diferença estatística entre os grupos. Para o colágeno, houve aumento do grupo TR ($p < 0,05$), quando contrastado com os demais grupos (CT, GH e TRGH). Na análise bioquímica da CK-MB houve diferença estatística dos grupos treinados em relação aos grupos não treinados.

Conclusão- O TR interfere na remodelação cardíaca aumentando o colágeno intersticial, que pode ser decorrente da lesão miocárdica caracterizada pelo aumento da CK-MB, porém, quando associado ao GH o colágeno não se alterou.

Palavras-chave: Expressão gênica, miocárdio, cálcio, hormônio do crescimento, treinamento.

INTRODUÇÃO

Intervenções como o Treinamento Resistido (TR) e recursos ergogênicos tem sido frequentemente utilizados na manutenção da saúde e estética corporal. Dentre os recursos ergogênicos destaca-se o hormônio do crescimento (GH), que embora as agências mundiais Anti-Doping proíbam seu uso em atletas competitivos, a administração desta droga é amplamente difundida [1–3].

O GH afeta o coração e ocasiona hipertrofia cardíaca sem aumento de fibrose, resposta acompanhada por aumento da contratilidade, alterações na gênese dos potenciais de ação cardíacos e vasodilatação periférica [4–5]. Algumas pesquisas mostram o efeito cardioprotetor do GH após infarto do miocárdio amenizando a remodelação cardíaca patológica [4]. Há outros estudos que relatam os malefícios do GH em indivíduos com hipersecreção crônica deste hormônio (acromegalia) conduzindo ao desenvolvimento de hipertrofia cardíaca concêntrica com fibrose intersticial e infiltrado linfomononuclear e, se a sobrecarga do hormônio não for controlada, pode evoluir para insuficiência cardíaca (IC) [5-7]. Embora outros fatores de risco acompanhem a acromegalia, acredita-se por si só que o excesso de GH e seu mediador, o fator de crescimento semelhante a insulina 1 (IGF-1), possam ser os principais contribuintes para a doença cardiovascular [7,8].

Em relação ao TR, as pesquisas são controversas a respeito dos efeitos cardíacos. Esses exercícios promovem a remodelação miocárdica com hipertrofia ventricular esquerda, aumento da densidade capilar e alteração no tecido conjuntivo e interferem beneficamente na função do coração [9,10]. Alguns estudos têm demonstrado a influência da disfunção miocárdica seguida à prática de exercícios de endurance. Entretanto, o TR por ocasionar sobrecarga ao sistema cardiovascular também pode interferir nos marcadores de lesão miocárdica [11,12]. Em uma meta-análise, foi observado que o TR não demonstra efeitos favoráveis sobre a remodelação miocárdica na IC, como os exercícios aeróbicos; a elevação das cargas pressóricas, sistólica e diastólica, que ocorre no treinamento resistido, impede os efeitos benéficos que o exercício poderia trazer, podendo até agravar o remodelamento ventricular [13].

Assim, dependendo da dose do GH, bem como a intensidade e duração do TR podem ocorrer prejuízos ao organismo e na remodelação ventricular [5,14].

Um dos mecanismos envolvidos na função contrátil da remodelação cardíaca é a expressão dos genes relacionados com a homeostase do Ca^{2+} intracelular [15]. Entretanto, ainda não está clara a função na remodelação cardíaca quando associada à administração do GH e/ou TR. O retículo sarcoplasmático Ca^{2+} ATPase (SERCA2a), fosfolambam (PLB) e rianodina (RyR) são algumas das proteínas envolvidas com a ativação e regulação de diversos processos metabólicos como o equilíbrio entre a captação, liberação do Ca^{+2} sarcoplasmático e a saída do sarcolema e, pela força de contração cardíaca [16,17]. A RyR se liga ao Ca^{+2} no início do potencial de ação liberando-o do retículo sarcoplasmático (RS), promovendo e regulando a contração muscular [18]. A SERCA2a cataliza e ativa o transporte do Ca^{+2} para o RS por meio das vesículas do citoplasma e desempenha seu papel no relaxamento dos músculos. Este processo é regulado pela PLB, que permanece desfosforilada inibindo a ação da SERCA2a, com a sua fosforilação ela permite que ocorra a recaptação do Ca^{2+} pela SERCA2a [19].

Tendo o GH uma importante função na remodelação miocárdica, a associação exógena deste aliado ao TR pode interferir na remodelação cardíaca. A hipótese desse estudo é que o TR associado ao GH modula a morfologia da expressão de proteínas do trânsito de Ca^{2+} e do marcador de lesão miocárdica.

Não há pesquisas que avaliem a relação entre o GH, TR, marcador de lesão miocárdica e transcrição gênica de estruturas moleculares no trânsito de Ca^{2+} miocárdico. Sendo assim, este estudo teve como objetivo avaliar os efeitos da administração do GH, do TR e sua associação sobre remodelação cardíaca em relação a morfologia, níveis de expressão do RNAm de proteínas envolvidas na homeostase do Ca^{2+} e marcador de lesão miocárdica.

MATERIAL E MÉTODOS

Animais e Procedimentos

Foram utilizados 28 ratos Wistar machos, com peso médio de 235 ± 15,2 gramas, com 9 semanas de idade, provenientes do Biotério Central da UNOESTE-Universidade do Oeste Paulista, SP, Brasil. Os animais foram alojados em 7 caixas com 4 animais cada, com marcação individual, com acesso livre a àgua e ração (SupraLab®) e foram mantidas as condições ambientais padrão, com controle de luz

(ciclos claro/escuro de 12 horas, luz a partir das 7 horas), de temperatura ambiente (21 ± 5°C) e de umidade relativa de ar (55 ± 5%) .

Este estudo foi aprovado pelo Comitê de Ética em Experimentação Animal (CEUA) da Unoeste com os protocolos n° 1688 e 1689 e realizados conforme o Guide for the Care and Use of Laboratory Animals publicado pelo National Research Council [20].

Desenho do estudo

Os ratos foram direcionados ao Biotério de Experimentação Animal, UNOESTE- Universidade do Oeste Paulista, SP, Brasil e após 7 dias de aclimatação foram distribuídos em quatro grupos: Controle (CT, n=7), com administração de GH (GH, n=7), com TR (TR, n=7) e associando TR com administração de GH (TRGH, n=7).

Administração do Hormônio do Crescimento

Os animais submetidos ao uso de GH receberam 0,2 UI/Kg de GH recombinante humano (rhGH, Saizen® - Merck) via subcutâneo, a cada dois dias por 30 dias consecutivos [21]. Nos demais ratos foram administrados soluções fisiológicas em volume similar.

Treinamento Resistido (TR)

O treinamento físico foi realizado por meio de um protocolo de saltos verticais na água, três vezes por semana por 30 dias consecutivos. Uma semana antes de iniciar o experimento, os ratos foram adaptados ao exercício na água, aumentando o número de séries a cada dia de adaptação e com sobrecarga de 50% do seu peso corporal total. O treinamento ocorreu dentro de um tubo de PVC de 25 cm de diâmetro com 38 cm de profundidade de água aquecida (30°C) no seu interior, conforme descrito por De Mello Malheiro [22]. Após esse período de adaptação os animais iniciaram o protocolo de treinamento e cada sessão consistia de quatro series de 10 saltos com intervalo de 1 minuto para descanso. Os ratos foram pesados antes de cada sessão, a fim de recalcular a carga acrescentada (50% de sobrecarga, do seu peso corporal total). A sobrecarga foi feita por meio de pesos

fixos com um colete com velcro posicionado na região anterior do tórax. Ao final de cada treino os animais foram secados para retornarem às suas caixas.

Análise dos Parâmetros Anatômicos

Ao final das 4 semanas, após 72 horas da última sessão de treinamento os animais foram pesados, anestesiados com éter etílico, e mortos por exsanguinação. Os corações destes animais foram retirados, pesados e posteriormente, dissecado o ventrículo esquerdo (VE) e novamente pesados. Foi congelado o ápice do VE em nitrogênio líquido e a porção superior em formol tamponado a 10% para as análises de expressão gênica e morfológica, respectivamente.

O peso úmido do VE, normalizado para peso corpóreo final do rato (PCF), foi utilizado como índice de hipertrofia ventricular. As variáveis anatômicas utilizadas para caracterizar a remodelação cardíaca foram PCF, o peso do VE e a relação VE/PCF.

Estudo Morfológico

Amostras de tecido cardíaco foram fixadas em solução de formol tamponado a 10% por um período de 48 horas. Após fixação, o tecido foi incluso em blocos de parafina, obtendo-se a seguir cortes histológicos coronais de 4 micrômetros. Os cortes histológicos foram corados em lâmina com solução Hematoxilina-Eosina (HE) para aferição de áreas da secção transversa dos miócitos, empregando-se microscópio LEICA DM750 acoplado a câmera de vídeo, que envia imagens digitais a computador dotado de programa de análise de imagens Image Pro-plus (Media Cybernetics, Silver Spring, Maryland, USA), [23,24].

Histomorfometria - As imagens foram obtidas por meio de microscópio óptico binocular. Todas as imagens foram capturadas por câmara de vídeo no aumento de 40x. A seleção das imagens para captura e digitalização foi feita visualmente. A morfometria dessas imagens obtidas e digitalizadas foi realizada utilizando-se software apropriado para tal fim. De cada um dos quatro cortes obtidos do VE de cada animal foram realizadas capturas de campos diferentes, escolhidos de acordo com o local onde se pudessem visualizar mais células em corte transversal. Foram mensuradas cinquenta células por ventrículo analisado. Os miócitos selecionados estavam seccionados transversalmente e apresentavam forma redonda, núcleo visível no centro da célula e localizavam-se na camada subendocárdica da parede

muscular do VE. Esse cuidado visou uniformizar ao máximo o conjunto de miócitos dos diferentes grupos. As áreas seccionais médias obtidas para cada grupo foram utilizadas como indicador do tamanho celular [25].

Lâminas com cortes histológicos coronais de 6 micrômetros e corados pela técnica de Picro Sirius red, específicos para visualização de colágeno, foram feitas para avaliação do interstício do miocárdio do VE. As imagens do tecido cardíaco foram capturadas por computador LEICA DM LS acoplado a câmera de vídeo, que envia imagens digitais a computador dotado de programa de análise de imagens Image Pró-plus (Media Cyberetics, Silver Spring, Maryland, USA). Foram analisados vinte campos por ventrículo, utilizando objetiva de 40X. Os campos escolhidos estavam afastados da região perivascular [26].

Expressão gênica relativa de reguladores do Ca^{2+} intracelular

O RNA total foi extraído do tecido cardíaco (ventrículo esquerdo) utilizando-se Trizol (Invitrogen), tratado em seguida com DNAse de acordo com orientação do fabricante. A integridade do RNA foi avaliada por eletroforese. O kit High Capacity cDNA Reverse Transcription (Applied Biosystems, CA, EUA) foi usado para a síntese de DNA complementar (cDNA) a partir de 1000 ng de RNA total. Utilizou-se RT-qPCR para medir quantitativamente os níveis relativos de RNAm de SERCA2a (Rn00568762_m1), RyR (Rn01470303_m1) e PLB (Rn01434045_m1). Para tal, utilizaram-se TaqMan Universal PCR Master Mix (Applied Biosystems, CA, EUA), conforme as instruções do fabricante, e o sistema de detecção Applied Biosystems StepOne Plus. Todas as amostras foram avaliadas duas vezes. As condições de ciclagem foram as seguintes: ativação da enzima a 50°C por 2 minutos; desnaturação a 95°C por 10 minutos; amplificação dos produtos de cDNA por 40 ciclos de desnaturação a 95°C por 15 segundos; e anelamento/extensão a 60°C por 1 minuto. A expressão gênica foi quantificada em relação aos valores do grupo CT e após normalização por um controle interno β-actina (ACTB, Rn00667869_m1), sendo determinada pelo método $2^{-\Delta\Delta Ct}$, como anteriormente descrito [27,28].

Dosagem de CK-MB

Foi realizada coleta de sangue para bioquímica sérica da CK-MB em tubos (Vacutainer®) sem anticoagulante. Após a coleta o sangue total foi centrifugado a 3000 rpm (g= 1257). O soro obtido foi acondicionado em microtubos plásticos e

mantido a -20°C. A bioquímica sérica foi realizada por meio do método cinético UV automatizado (Cobas C111, Roche®) [29].

Análise dos Dados

Para comparar os parâmetros estudados entre os grupos experimentais e validação dos pressupostos de normalidade dos dados e homogeneidade de variâncias, foi realizada respectivamente os testes de Shapiro-Wilk e Levene. Para os dados com distribuição normal recorreu-se a análise de variância em uma via (ANOVA one-way) com contrastes pelo método de Tukey para os dados com distribuição normal ou ainda teste de Kruskal Wallis para os dados com distribuição não normal. Todas as análises foram realizadas segundo os métodos estatísticos descritos por Maroco [30], com o uso do software SPSS for Windows v.13.0. O nível de significância estatística adotado para todas as análises foi de 5%. Os dados estão expressos em média ± desvio padrão, mediana, valor mínimo e máximo.

RESULTADOS

Os parâmetros que indicam remodelação cardíaca, anatômicos e morfológicos estão apresentados na Tabela 1. Os valores foram expressos em média ± desvio padrão, mediana, valor mínimo e máximo. O peso corporal final, peso do VE, relação VE/PCF e área dos cardiomiócitos não mostraram diferença estatística. Já para a avaliação do colágeno (Figura 1), observou-se diferença estatística do grupo TR, quando comparado com os demais grupos (CT, GH e TRGH). A expressão do RNAm das proteínas reguladoras do trânsito de Ca^{2+}, RyR, SERCA2a e PLB, não mostrou diferença estatística. Os valores foram expressos em média ± desvio padrão, e estão representados nas figuras 2, 3 e 4, respectivamente.

Com relação a dosagem da CK-MB, houve diferença estatística significativa entre os grupos treinados, comparados aos grupos não treinados (Figura 5).

DISCUSSÃO

Este estudo foi conduzido com a finalidade de verificar a influência do TR, GH e sua associação sobre morfologia, expressão gênica das proteínas do trânsito de Ca^{2+} e do marcador de dano cardíaco, atividade da CK-MB sobre o coração. Os principais achados do estudo foram: aumento da expressão de colágeno no ventrículo esquerdo do grupo que praticou o TR isolado, diminuição da expressão de

colágeno quando o TR foi associado ao GH e do dano miocárdico ocasionado pelo TR independente do GH.

A fração de colágeno encontrada no grupo TR foi maior em comparação com os outros grupos, mas desconhecemos se esta remodelação foi um efeito fisiológico benéfico do TR, uma vez que o marcador de lesão miocárdica estava aumentado. Um dos poucos estudos que avaliou a influência do TR sobre a remodelação miocárdica encontrou aumento intersticial do colágeno e acrescenta que este aumento foi benéfico, podendo variar dependendo do tipo de estímulo físico recebido, aeróbio ou de resistência [9]. Uma possível explicação para este aumento de colágeno é que a sobrecarga de pressão cardíaca, imposta pelo estresse mecânico do TR, pode estar relacionada com a degradação do colágeno no miocárdio e como resposta ocorre a síntese com a regulação dos níveis de RNAm do colágeno [31,32].

Esse componente da remodelação cardíaca aumentado pode ser decorrente da lesão do cardiomiócito [33] evidenciada no nosso experimento, pela elevação da CK-MB dos grupos treinados. Esse resultado corrobora com dados encontrados na literatura com exercícios de alta intensidade como em atletas de meia maratona [34], maratona [35] e ultramaratona [36].

A CK-MB é um marcador de lesão miocárdica e está relacionada com a manutenção da integridade da membrana plasmática. Essa elevação da CK-MB, evidenciada em nosso experimento, pode estar relacionada com a alta intensidade do exercício, que leva a injúria e morte celular [37]. Neste processo ocorre reação inflamatória e expressão de mediadores inflamatórios como o fator de necrose tumoral alfa (TNF-α) e a Interleucina-6, que em resposta a este excesso de treinamento, pode levar a um predomínio da via de degradação de proteínas sobre a via de síntese de proteínas prejudicando a reestruturação cardíaca [38].

No nosso estudo, o GH amenizou a deposição do colágeno fato que se assemelha a outras pesquisas, que enfocam o seu efeito cardioprotetor na remodelação cardíaca patológica amenizando a formação de colágeno tipo I e III [39,40]. Entretanto não avaliamos qual tipo de colágeno (Iαl, Iαll e lll) foi modulado, sendo que estes podem variar de acordo com o estímulo, fisiológico ou patológico, assim como pela suplementação de substâncias e a intensidade de exercícios. Assim, mais pesquisas são necessárias para determinar qual o tipo de colágeno e qual via molecular foi ativada nesta resposta.

Não houve hipertrofia cardíaca quando avaliados a relação VE/PCF e diâmetro dos cardiomiócitos tanto no uso do GH, como no TR isolado e de forma combinada, e esses resultados são controversos [40-45]. Moreira et al., [40] não observaram alterações na relação VE/PCF de ratos submetidos a sobrecarga crônica pressórica no modelo de estenose aórtica após curto período de tratamento com GH (1 mg/kg por 14 dias). Estes autores mostraram como efeito cardioprotetor, apenas a atenuação da fibrose miocárdica, o que denota um efeito específico deste hormônio. Sugizaki et al., [41] também não evidenciaram diferenças na relação VE/PCF em ratos submetidos a natação com sobrecarga de peso (5% do peso corporal). Estes animais foram submetidos a cinco sessões de natação semanais, por 12 semanas consecutivas. Entretanto, Barauna et al., [42] observaram aumento no diâmetro dos cardiomiócitos nos ratos submetidos a um protocolo de treinamento de resistência no qual consistia na extensão de tronco com sobrecarga do equipamento por 4 séries de 12 repetições com 65% a 75% de uma repetição máxima por 4 ou 12 semanas. Este tipo de treino induziu hipertrofia cardíaca concêntrica sem disfunção ventricular e diminuição das câmaras.

Embora, a hipertrofia ventricular com o aumento do diâmetro dos cardiomiócitos tenha sido ainda observada em outros estudos que envolveram o TR e o GH [40,41], ressalta-se a ideia que um período maior de treinamento, carga e forma de realização e diferentes doses de GH possam influenciar [42,45].

Os mecanismos celulares e moleculares envolvidos com a ação do GH, do TR e sua associação no coração ainda não foram totalmente esclarecidos [42,43,45]. Na presente pesquisa não houve alterações nos genes relacionados ao trânsito de Ca^{2+} cardíaco. Os cardiomiócitos expressam receptores tanto para o GH como para o IGF-1 e esses receptores são influenciados por alterações hemodinâmicas e acredita-se que ambos os hormônios exercem efeitos estimulantes na contratilidade miocárdica [45]. Além disso, esses hormônios e os peptídeos liberadores de GH como a grelina, possuem efeitos benéficos cardíacos [45-47]. O estudo de Ma et al., [15] mostrou que a ativação do receptor GHS-R1a grelina produziu um efeito inotrópico em cardiomiócitos isquêmicos por lesões de isquemia/reperfusão provavelmente por proteger ou recuperar as proteínas do trânsito de Ca^{2+} como a SERCA2a e a PLB. Esperava-se no nosso estudo que este efeito na remodelação cardíaca pudesse ser mediado pela elevação na expressão

dos genes que foram avaliados, devido à administração do GH, pois ele estimula a síntese proteica da célula e a formação de RNAm, porém, isso não ocorreu. Algumas hipóteses para explicar tal fato seria a menor sensibilidade do tecido cardiovascular à ação direta do GH, como observado por Volterrani et al. [45], e ainda, a retroalimentação negativa do GH e dos IGFs, pela sua administração exógena que pode ter interferido na regulação da síntese e da secreção do GH [48].

O TR promove o aumento da concentração de Ca^{2+} no interior das células aumentando o grau de contratilidade dos cardiomiócitos; fato que induz a superexpressão de SERCA2a e consequentemente do seu regulador, a PLB [49,50]. No entanto, no nosso estudo, os genes do trânsito de Ca^{2+} não sofreram influências do TR isolado ou associado ao GH. Sugere-se que o período de quatro semanas não foi suficiente para propiciar remodelação cardíaca com alteração da expressão destes genes nos animais saudáveis, entretanto, em outro experimento que avaliou animais com disfunção cardíaca, porém com protocolo de treinamento de 8 semanas, houve diferença estatística na expressão dos genes do trânsito de Ca^{2+} cardíaco [51].

Outros mecanismos podem estar envolvidos na modulação da contratilidade cardíaca em resposta ao TR, como por exemplo os fatores neuro-humorais, o sistema nervoso simpático por meio dos receptores adrenérgicos (α1a, β1 e β2) e o sistema endócrino via angiotensina II (Ang II) [52,53], assim como, os mecanismos neuromoduladores circulatórios pela ação do óxido nítrico (NO) [54]. Ainda destaca-se a expressão da α- e β-MHC (miosina de cadeia pesada), uma vez que a MHC é a principal proteína contráctil do coração, e é essencial para a eficiência do desempenho cardíaco [55].

Avaliar a expressão destes mecanismos é essencial para uma maior compreensão do efeito inotrópico positivo na remodelação cardíaca. Por isso, é necessário mais pesquisas que analisem esta mesma metodologia mas com outro tipo de abordagem no que se refere aos mecanismos envolvidos na resposta morfológica, contrátil e bioquímica cardíaca.

Esta pesquisa não analisou os mecanismos que levaram ao dano cardíaco, por isso novos estudos são necessários.

CONCLUSÃO

O TR promoveu remodelamento intersticial do colágeno cardíaco e necrose tecidual, caracterizada pelo aumento da CK-MB, porém, quando o TR foi associado ao GH o colágeno não se alterou. Os demais resultados deste estudo mostraram que o TR associado à administração do GH não promoveu alteração nas estruturas cardíacas e nem aumento do RNAm dos principais genes do Ca^{2+} cardíaco, RyR, SERCA2a e PLB.

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Tabela 1. Parâmetros anatômicos, área seccional do cardiomiócito e da fração de colágeno intersticial expressos em média ± desvio padrão, mediana, valor mínimo e máximo.

Variáveis	Grupos			
	CT	**GH**	**TR**	**TRGH**
PCF (g)	297,27 ± 22,14 305,10 [258,80 – 317,70]	313,81 ± 14,02 309 [295,70 – 334,20]	304,30 ± 29,08 307,10 [255,70 – 343,30]	292,05 ± 15,96 285,30 [273,40 – 314,20]
VE (g)	0,74 ± 0,31 0,61 [0,47 – 1,23]	0,87 ± 0,25 0,74 [0,58 – 1,21]	0,75 ± 0,26 0,67 [0,43 – 1,22]	0,77 ± 0,23 0,68 [0,55 – 1,14]
VE/PCF (mg/g)	2,48 ± 0,95 2,04 [1,74 – 4,03]	2,76 ± 0,79 2,35 [1,92 – 3,73]	2,55 ± 1,04 2,12 [1,26 – 4,13]	2,64 ± 0,81 2,38 [1,91 – 3,93]
AS (px^2)	8834 ± 1457 9458 [6979 - 10460]	9359 ± 1242 9118 [7537 - 10800]	10613 ± 2018 9550 [9082 - 13712]	8847 ± 910,8 8820 [7842 - 10234]
IC (%)	0.099 ± 0.043 0.106 [0.026 – 0.169]	0.087 ± 0.028 0.077 [0.056 – 0.135]	0.223 ± 0.102 ***** 0.221 [0.064 – 0.409]	0.112 ± 0.029 0.099 [0.082 – 0.147]

CT: sem exercício e sem hormônio do crescimento (controle); GH: grupo sedentário e hormônio do crescimento; TR: treinamento resistido; TRGH: treinamento resistido e hormônio do crescimento; PCF: peso corpóreo final; VE: peso o ventrículo esquerdo; AS: área seccional do cardiomiócito; IC: fração de colágeno intersticial; * $p<0,05$ vs. Grupo CT, GH, TRGH.

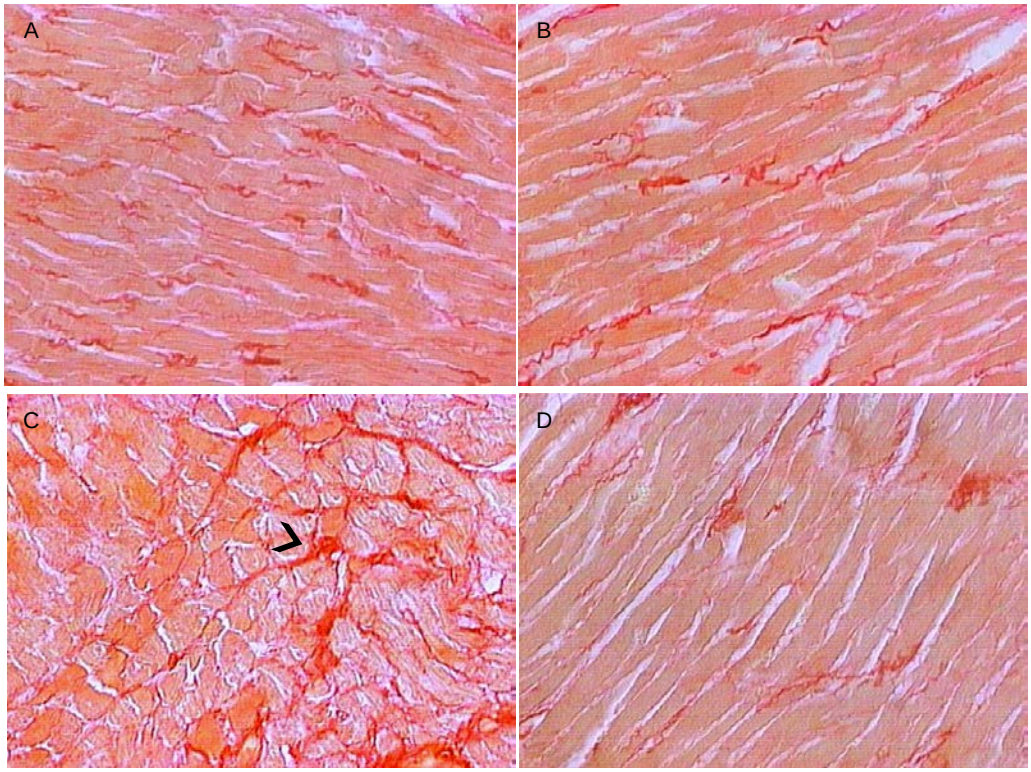

Figura 1. Picrosirius red do colágeno miocárdio. Microscópio óptico com objetiva de 40X. O colágeno é corado em vermelho. A - grupo controle (CT); B - grupo hormônio do crescimento (GH); C - grupo treinamento resistido (TR); D – grupo treinamento resistido e hormônio do crescimento.

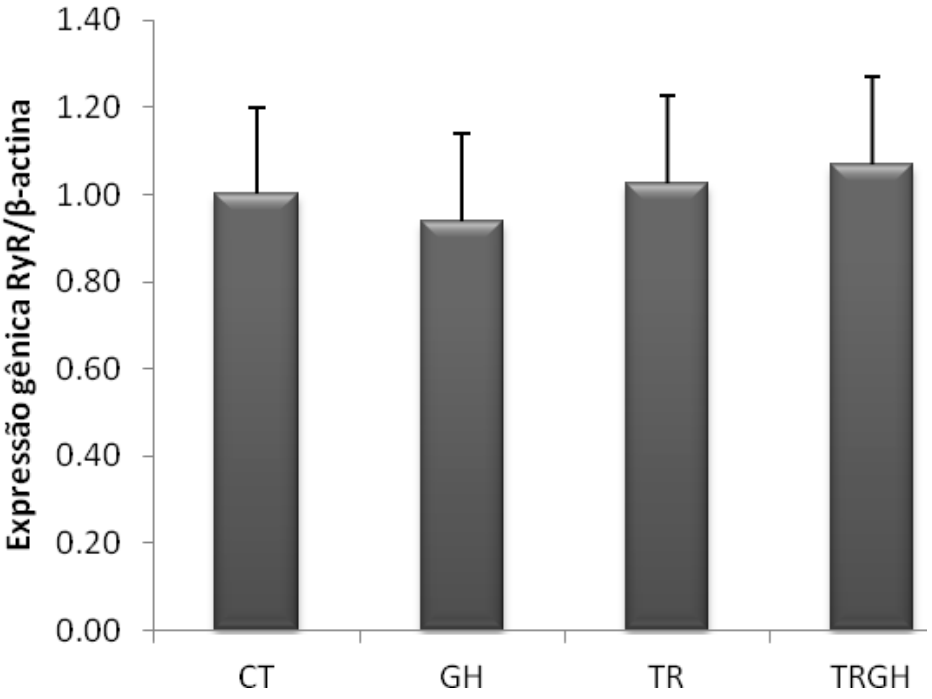

Figura 2. Níveis relativos de RNAm determinados por qPCR da Rianodina (RyR), expressos em média ± desvio padrão. CT=grupo controle; GH=grupo hormônio do crescimento; TR=grupo treinamento resistido; TRGH=grupo treinamento resistido e hormônio do crescimento.

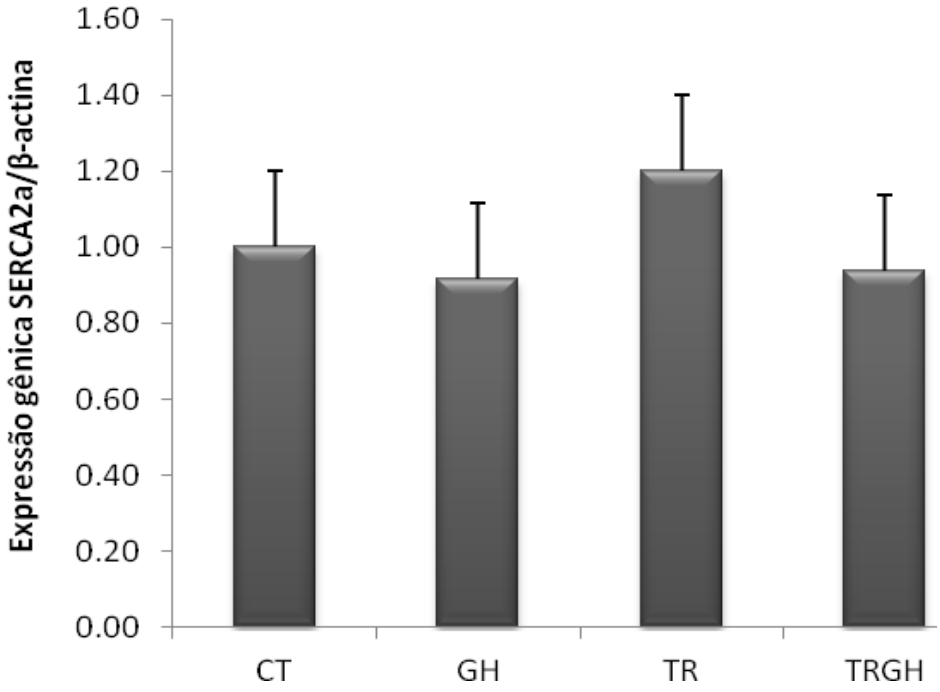

Figura 3. Níveis relativos de RNAm determinados por RT-qPCR do Retículo Sarcoplasmático Ca^{+2} ATPase (SERCA2a), expressos em média ± desvio padrão. CT=grupo controle; GH=grupo hormônio do crescimento; TR=grupo treinamento resistido; TRGH=grupo treinamento resistido e hormônio do crescimento.

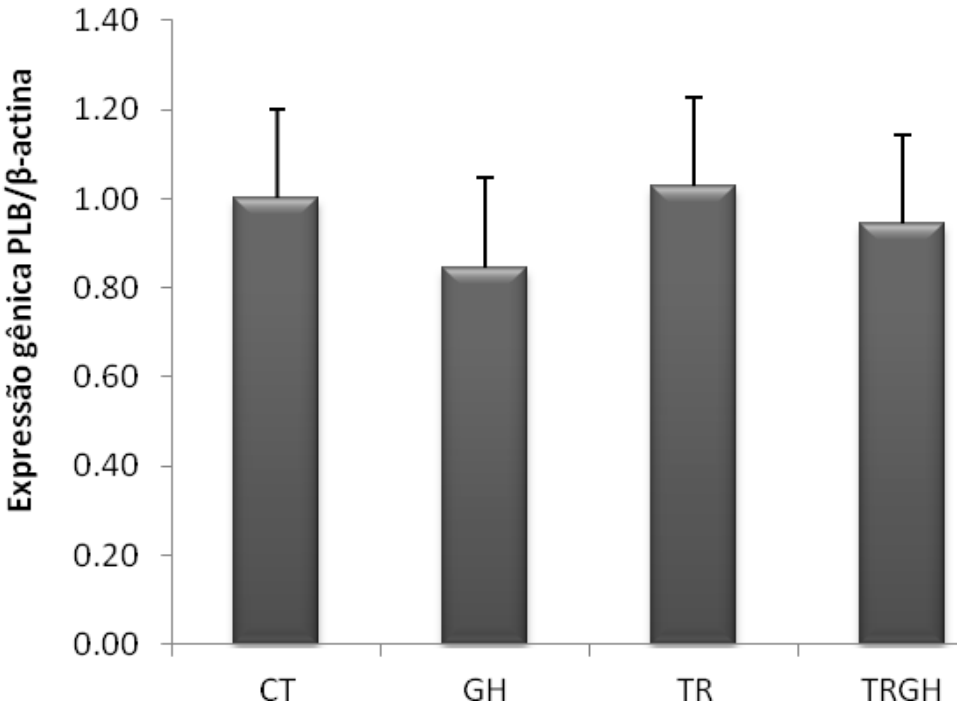

Figura 4. Níveis relativos de RNAm determinados por RT-qPCR da Fosfolambam (PLB), expressos em média ± desvio padrão. CT=grupo controle; GH=grupo hormônio do crescimento; TR=grupo treinamento resistido; TRGH=grupo treinamento resistido e hormônio do crescimento.

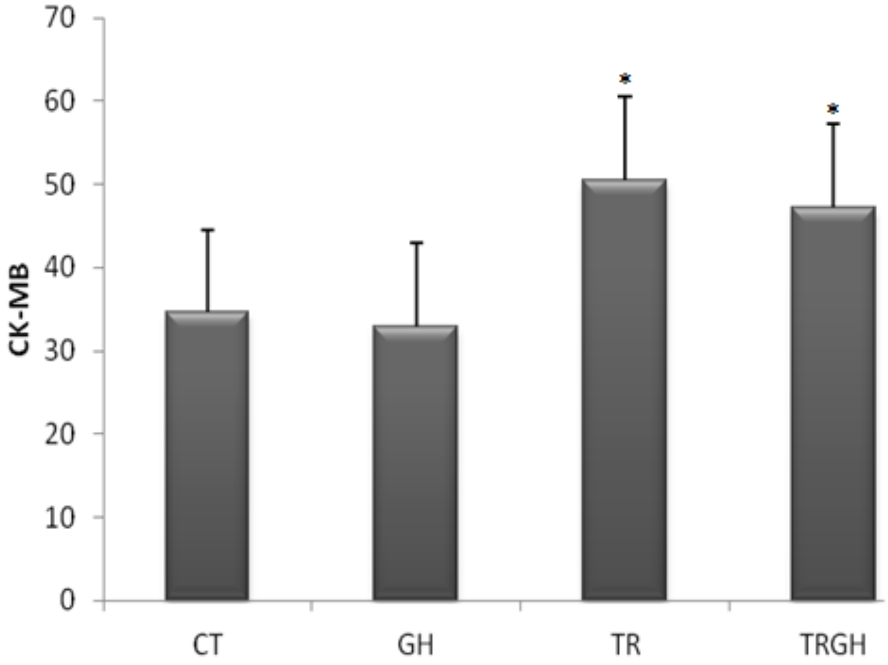

Figura 5. Dosagem da Creatina Fosfoquinase-Músculo Cérebro (CK-MB) por análise bioquímica, expresso em média ± desvio padrão. CT=grupo controle; GH=grupo hormônio do crescimento; TR=grupo treinamento resistido; TRGH=grupo treinamento resistido e hormônio do crescimento. *p<0,05 TR, TRGH vs. CT, GH.

ANEXO 1 - Artigo Enviado para Revista Growth Hormone and IGF Research

ASSOCIATION OF GROWTH HORMONE WITH RESISTANCE EXERCISE ON MORPHOLOGY, GENE EXPRESSION AND CARDIAC MARKER

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GH and resistance training in the heart

Hightlights:
Resistance training induced collagen synthesis
rhGH combined with training did not alter cardiac calcium genes
rhGH associated with exercise prevented increased collagen

ABSTRACT

Objective-This study aimed to evaluate the effects of growth hormone (GH), in rats submitted to resistance training (RT) on cardiac remodeling in morphological aspects, Ca^{2+} genes and cardiac marker.

Design- Wistar male rats were divided into 4 groups: control (CT, n = 7), Growth Hormone (GH, n = 7), Resistance Training (RT, n = 7) and Resistance Training with Growth Hormone administration (RTGH, n = 7). The GH dose was 0,2 IU/kg through subcutaneous administration every two days, and the RT model used was the vertical jump in water (4 sets of 10 jumps/day, 3 sessions/week) both for 30 consecutive days. Anatomical variables such as final body weight (FBW), left ventricle weight (LV) and the LV/FBW ratio of rats were evaluated. The morphology analysis consisted of the cardiomyocytes cross-sectional area (HE) and collagen fraction (picrosirius). The mRNA of the proteins of the calcium pump of the sarcoplasmic reticulum Ca^{2+} ATPase (SERCA2a), phospholamban (PLB) and ryanodine (RyR) infarction were evaluated by real-time PCR (qPCR). The cardiac marker dosed was creatine kinase muscle-brain fraction (CK-MB), through serum analysis

Results- FBW, LV weight, LV/FBW ratio, cardiomyocytes cross-sectional area, as and SERCA2a, PLB and RyR gene expression, showed no statistical difference between the groups, whereas for the collagen assessment , an increase in RT group ($p < 0,05$) was observed when compared to other groups (CT, GH and RTGH). In biochemical analysis of CK-MB there was statistical difference between trained groups and untrained groups.

Conclusion- The RT interferes with cardiac remodeling by increasing interstitial collagen, which may result from the myocardial injury characterized by increased CK-MB, however, when combined with GH collagen remained unchanged.

Key words: Gene expression, myocardium, calcium, growth hormone, training.

RESUMO

Objetivo- Este estudo teve como objetivo avaliar os efeitos do Hormônio do Crescimento (GH), do Treinamento Resistido (TR) e sua associação sobre remodelação cardíaca nos aspectos morfológicos, genes do Ca^{2+} e marcador cardíaco.

Desenho- Ratos Wistar machos foram distribuídos em 4 grupos: controle (CT, n=7), Hormônio do Crescimento (GH, n=7), Treinamento Resistido (TR, n=7), e Treinamento Resistido com Hormônio do Crescimento (TRGH, n=7). A dose do GH foi de 0,2 UI/Kg, via subcutâneo, a cada dois dias e o TR foram saltos verticais na água (4 séries de 10 saltos/dia, 3 sessões/semana) ambos por 30 dias consecutivos. Avaliaram-se as variáveis anatômicas como o peso corporal final (PCF), o peso do Ventrículo Esquerdo (VE) e a relação VE/PCF dos ratos. A morfologia constou da análise da área dos cardiomiócitos (Hematoxilina e Eosina-HE) e a fração de colágeno (Picrosirius). O RNAm das proteínas da bomba de cálcio (Ca^{2+}) do retículo sarcoplasmático Ca^{2+} ATPase (SERCA2a), fosfolambam (PLB) e rianodina (RyR) do miocárdio foram avaliados por PCR em tempo real (qPCR). O marcador cardíaco dosado foi a creatina quinase fração músculo-cérebro (CK-MB), por meio da análise sérica.

Resultados- O PCF, peso do VE, a relação VE/PCF e a área dos cardiomiócitos, assim como, a expressão gênica SERCA2a, PLB e RyR, não teve diferença estatística, já para avaliação do colágeno, houve aumento do grupo TR ($p < 0,05$), quando contrastado com os demais grupos (CT, GH e TRGH). Na análise bioquímica da CK-MB houve diferença estatística dos grupos treinados em relação aos grupos não treinados.

Conclusão- O TR interfere na remodelação cardíaca aumentando o colágeno intersticial, que pode ser decorrente da necrose miocárdica caracterizada pelo aumento da CK-MB, porém, quando associado ao GH o colágeno não se alterou.

Palavras-chave: Expressão gênica, miocárdio, cálcio, hormônio do crescimento, treinamento.

BACKGROUND

Interventions such as resistance training (RT) and ergogenic resources have frequently been used to maintain good health and body esthetics. Amongst the ergogenic resources utilized, the growth hormone (GH) is highlighted. Although forbidden for competitive athletes by the World Anti-Doping agencies, this hormone is a widely spread drug [1-3].

GH affects the heart and causes cardiac hypertrophy without increasing fibrosis. This response is accompanied by increased contractility, changes in cardiac action potentials and peripheral vasodilatation genesis [4-5]. Some researches show the cardioprotective GH effect after a myocardial infarction, softening the pathological cardiac remodeling [4]. There are other studies reporting the damages of GH in individuals with chronic hypersecretion oh this hormone (acromegaly) leading to development of concentric cardiac hypertrophy with interstitial fibrosis and lymphomononuclear infiltrates and if the hormone overload is not controlled, it may evolve to heart failure (HF) [5-7]. Although other risk factors follow acromegaly, it is believed that the GH excess and its mediator insulin-like growth factor-1 (IGF-1), may be the major contributors to cardiovascular disease [7,8].

Regarding resistance training (RT), researches are controversial in relation to the cardiac effects. These exercises promote myocardial remodeling with left ventricular hypertrophy, increased capillary density, alteration in connective tissue and benefits affect heart function [9,10]. Some studies have demonstrated the influence of myocardial dysfunction followed by the practice of endurance exercise. However RT may also interfere with the markers of myocardial injury by causing overload of the cardiovascular system [11,12]. In a meta-analysis study, it was observed that resistance training does not show favorable effects on myocardial remodeling in HF as aerobic exercises does, the increase in blood pressure loads, systolic and diastolic, that takes place in resisted training, prevents the beneficial effects that the exercise could bring and may even exacerbate ventricular remodeling [13].

Therefore, depending on the GH dose, as well as the intensity and duration of RT, it may result in damages to the organism and in ventricular remodeling [5,14].

One of the mechanisms involved in the contractile function of heart remodeling is the gene expression associated with intracellular Ca^{2+} homeostasis [15]. However,

its role in cardiac remodeling is still not clear as well as GH administration, resistance exercise or association of both. The sarcoplasmic reticulum Ca^{2+} ATPase (SERCA2a), phospholamban (PLB) and ryanodine (RyR) are some of the proteins involved in the activation and regulation of several metabolic processes such as the balance between absorption, release of sarcoplasmic Ca^{+2} and the sarcolemmal output and by the heart contraction strength [16-17]. The RyR connects to Ca^{+2} at the beginning of the action potential by releasing it from the sarcoplasmic reticulum (SR), promoting and regulating muscle contraction [18]. The SERCA2a catalyzes and activates the transport of calcium into the SR through the cytoplasm vesicles and performs its role in muscles relaxation. This process is regulated by PLB, that remains dephosphorylated inhibiting the action of SERCA2a. This phosphorylation allows the reuptake of Ca^{2+} by SERCA2a [19].

Since GH has an important function in myocardial remodeling, its exogenous association to resistance training may interfere with cardiac remodeling. The hypothesis of this study is that the RT associated with GH modulates the morphology of the protein expression of the traffic of Ca^{2+} and marker of myocardial injury.

There are no researches evaluating the relationship between GH, RT, myocardial injury marker and gene transcription of molecular structures in myocardial calcium traffic. In order to evaluate these molecular mechanisms it is crucial to understand the physiological mechanisms involved in their association and provide clinical indicators to use them safely. Therefore, this study aimed to evaluate the effects of GH administration, RT and its association on cardiac remodeling in relation to morphology and levels of myocardial mRNA expression of proteins involved in Ca^{2+} homeostasis and cardiac marker.

MATERIAL AND METHODS

Animals and Procedures

28 male Wistar rats were used, with a mean weight of 235 ± 15,2 grams, 9 weeks old, from the Biotery of University of Oeste Paulista (UNOESTE), SP, Brazil. The animals were placed in 7 boxes with four animals each, with individual marking, free access to water and food (SupraLab®) and standard environmental conditions

were maintained, with light control (light cycles/dark 12 hours, light from 7am), temperature (21 ± 5 ° C) and relative air humidity (55 ± 5%).

This study was approved by UNOESTE Ethics Committee on Animal Experimentation (CEUA) under 1688 and 1689 protocols and was performed according to the Guide for the Care and Use of Laboratory Animals published by the National Research Council [20].

Study Design

Rats were conducted to the Biotery of Animal Experimentation, University of Oeste Paulista (UNOESTE) , SP, Brazil and after 7 days of acclimatization they were divided into four groups: Control (CT, n=7), Growth Hormone administration (GH, n=7) Resistance Training (RT, n=7) and Resistance Training associated with administration of Growth Hormone (RTGH, n=7).

Growth Hormone Administration

The animals subjected to the use of GH were administered at a 0.2 IU/kg dose of recombinant human GH (rhGH Saizen ® - Merck) subcutaneous route, every two days for 30 consecutive days [21]. Remaining animals received physiological solutions in similar volume.

Resistance Training (RT)

Physical training was conducted using a protocol of vertical jumps in the water, three times a week for 30 consecutive days. One week before starting the experiment, the rats were adapted to exercise in the water, increasing the number of sets every day of adaptation with 50% overload of their total body weight. Training took place inside a PVC tube with 25 cm in diameter and 38 cm depth with heated water (30° C) inside it, as described by de Mello Malheiro [22]. After this adaptation period the animals initiated the training protocol and each session consisted of four sets of 10 jumps with 1 minute interval for rest. The rats were weighed before each session in order to recalculate the load added (50% overload of their total body weight). The overload was applied through fixed weights with a vest with velcro

positioned on the anterior chest. At the end of each training the animals were dried to return to their boxes.

Analysis of Anatomical Parameters

At the end of 4 weeks, 72 hours after the last training session, the animals were weighed, anesthetized with ethyl ether and killed by exsanguination. The hearts of the animals were removed, weighed and subsequently the left ventricle (LV) was dissected and weighed again. The LV apex was frozen in liquid nitrogen and the superior portion in 10% formol solution for gene expression and morphological assessment, respectively. The wet weight of the LV, adjusted for the final body weight (FBW), was used as an index of ventricular hypertrophy. The anatomical variables used to characterize cardiac remodeling were FBW, LV weight and the FBW/LV ratio.

Morphological Study

Cardiac tissue samples were fixed in 10% formol solution for a period of 48 hours. After fixation, the tissue was embedded into paraffin blocks. Four-micron coronal histological sections were obtained. The histological sections were stained on slides with Hematoxylin-eosin (HE) solutions for measurement of the cross-sectional areas of myocytes, using microscope LEICA DM750 coupled to a video camera that sends digital images to a computer equipped with image Image-Pro plus analysis software (Media Cybernetics, Silver Spring, Maryland, USA) [23,24].

Histomorphometry - The images were obtained by binocular optical microscope. All images were captured by video camera at 40x magnification. A selection of images to capture and scanning was done visually. The morphometry of these images was performed using appropriate software for this purpose. Four LV sections were obtained from each animal. In each section captures of different fields were performed, chosen according to to the location where you could visualize more cells in a cross section area. Fifty cells were measured by ventricle analyzed. The selected myocytes were cross-sectioned and had a round shape, visible in the center of the cell nucleus and localized in the subendocardial layer of the muscular wall of the LV. This precaution was taken in order to standardize the maximum number of myocytes of different groups. The average cross-sectional areas obtained for each group were used as an indicator of cell size [25].

Slides with coronal histological sections of 6 microns and stained by Picro Sirius red technique specific for collagen visualization were made to assess the LV myocardial interstitium. The images of cardiac tissue were captured by Leica DM LS computer attached to a video camera that sends digital images to a computer with image analysis program Image-Pro Plus (Media Cyberetics, Silver Spring, Maryland, USA). Twenty fields per ventricle were analyzed using 40X objective. The fields chosen were distant from the perivascular region [26].

Relative gene expression of regulators of intracellular Ca2 +

Total RNA was extracted from the heart tissue (left ventricle) using Trizol (Invitrogen) and then treated with DNAse according to the manufacturer's instructions. The RNA integrity was assessed by electrophoresis. The High Capacity cDNA Reverse Transcription (Applied Biosystems, CA, USA) kit was used for the synthesis of complementary DNA (cDNA) from 1000 ng total RNA. We used RT-PCR to quantitatively measure mRNA relative levels of SERCA2a (Rn00568762_m1), RyR (Rn01470303_m1) and PLB (Rn01434045_m1). For this, we used TaqMan Universal PCR Master Mix (Applied Biosystems, CA, USA) according to manufacturer's instructions and the detection system Applied Biosystems StepOne Plus. All samples were assessed two times. The cycling conditions were as follows: enzyme activation at 50°C for 2 minutes; denaturation at 95°C for 10 minutes; cDNA amplification products by 40 cycles of denaturation at 95°C for 15 seconds; and annealing / extension at 60°C for 1 minute. Gene expression was quantified in relation to the values of control group and after normalization by an internal control β-actin (ACTB, Rn00667869_m1), being determined by the 2-ΔΔCt method, as previously described [27,28].

CK-MB Dosage

Blood samples were collected to perform serum biochemistry of creatine kinase muscle-brain fraction (CK-MB) in tubes (Vacutainer®) without anticoagulant. Next the blood was centrifuged at 3000 rpm (g = 1257). Serum was placed in plastic microtubes and maintained at -20°C. Serum biochemistry was performed using the automated kinetic UV method (Cobas C111, Roche ®) [29].

Data Analysis

In order to compare the investigated parameters between the experimental groups and validation of the assumptions of data normality and variance homogeneity the Shapiro-Wilk and Levene tests were performed respectively. For data with normal distribution, one way variance analysis (one-way ANOVA) with contrasts using Tukey test for data with normal distribution or Kruskal Wallis method for non-normal data. All analyzes were performed according to the statistical methods described by Maroco [30], using the SPSS v.13.0 software for Windows. The level of statistical significance for all analyzes was 5%. Data are expressed as mean ± standard deviation, median, minimum and maximum value.

RESULTS

The parameters indicating cardiac, anatomical and morphological remodeling are presented in Table 1. The values were expressed as mean ± standard deviation, median, minimum and maximum value. The final body weight, LV weight, LV/FBW ratio and cardiomyocytes area showed no statistical difference. However, for collagen evaluation (Figure 1) there was a statistical difference in the RT group, when compared to the other groups (CT, GH and RTGH). The expression of mRNA of regulatory proteins traffic Ca^{2+}, RyR, SERCA2a and PLB, showed no statistical difference. The values were expressed as mean ± standard deviation, and are presented in Figures 2, 3, and 4 respectively.

Regarding the dosage of CK-MB, there was statistically significant difference between the trained groups compared with non-trained groups (Figure 5).

DISCUSSION

This study was conducted in order to verify the influence of RT, GH and its association on morphology, gene expression of protein of Ca^{2+} traffic and marker cardiac damage, activity of CK-MB on the heart.. The main findings of this study were increase in collagen density in the left ventricle of the group that practiced isolated RT, decrease in collagen volume fraction when RT was associated with GH and myocardial injury caused by RT regardless of GH.

The fraction of collagen found in the RT group was higher when compared to other groups. However we do not know if this remodeling was a beneficial physiological effect of RT once the myocardial injury marker was increased. One of

the few studies that evaluated the influence of RT on myocardial remodeling reinforces these findings and adds that interstitial collagen formation was beneficial and may vary depending on the type of physical stimulus received: either aerobic or resistance [9]. A possible explanation for this finding is that cardiac pressure overload imposed by the mechanical stress of RT, may be related to the collagen degradation in the myocardium and synthesis takes place in response to the regulation of mRNA levels of collagen [31,32].

This component of increased cardiac remodeling may result from the cardiomyocyte injury [33] demonstrated in our experiment, by the elevation of CK-MB in the trained groups. This result corroborates with literature data on high-intensity exercise in half marathon athletes [34] marathon [35] and ultra marathon [36].

CK-MB is a marker of myocardial injury and it is associated with maintenance of plasmatic membrane integrity. This elevation of CK-MB, observed in our experiment, may be related to high exercise intensity, which leads to injury and cell death [37]. In this process occurs inflammatory reaction and expression of inflammatory mediators as the tumor necrosis factor alpha (TNF-alpha) and interleukin-6, which in response to this overtraining, may lead to a predominance of protein degradation over the protein synthesis impairing cardiac restructuring [38].

In our study GH softened collagen deposition, this fact resembles other studies that focus on the cardioprotective effect in pathological cardiac remodeling easing the formation of collagen type I and III [39,40]. However we did not evaluate what kind of collagen (Iα1, Iα2 and III) was modulated, since these may vary with the provided stimulus, physiological or pathological as well as by supplementation of substances and the intensity of exercise. Thus, further research is required for determining what type of collagen and which molecular pathway was activated in this response.

There was no cardiac hypertrophy in the evaluation of LV/FBW ratio and cardiomyocyte diameter neither in the use of GH or in isolated training and in combination. However, these results are controversial [40-45]. Moreira et al., [40] found no alterations in the LV/FBW of rats submitted to chronic pressure overload in aortic stenosis model after a short period of treatment with GH (1 mg/kg for 14 days). These authors showed as cardioprotective effect, just the attenuation of myocardial fibrosis, which indicates a specific effect of this hormone. Sugizaki et al., [41] did not evidence differences in LV/FBW in rats submitted to swimming with weight overload

(5% of body weight). These animals underwent five sessions of weekly swimming for 12 consecutive weeks. However, Barauna et al., [42] observed an increase in the diameter of cardiomyocytes in rats undergoing an endurance training protocol consisted in trunk extension with overload oh the equipment for 4 sets of 12 repetitions with 65% to 75% of one repetition maximum for 4 or 12 weeks. This type of training induced concentric cardiac hypertrophy without ventricular dysfunction and decreased chambers.

Although ventricular hypertrophy with increased cardiomyocyte diameter has been observed in others studies involving RT and GH [40-41], we highlight that a longer period of training, load, protocol and GH doses used may influence this fact [42,45].

The cellular and molecular mechanisms related to GH action, RT and its association in the heart have not been fully elucidated [42,43,45]. In this study there were no changes in genes related to the traffic of cardiac Ca^{2+}. The cardiomyocytes express receptors for both GH and IGF-1 and these receptors are influenced by hemodynamic changes and it is believed that both hormones have stimulatory effects on myocardial contractility [45]. Furthermore, these hormones and GH-releasing peptides such as ghrelin have beneficial cardiac effects [45-47]. The study of Ma et al., [15] showed that activation of the ghrelin receptor GHS-R1a produced an inotropic effect on ischemic cardiomyocytes from injury by ischemia/reperfusion. This is probably to protect or recover the proteins of traffic of Ca^{2+} as SERCA2a and PLB. It was expected in this study that this effect in cardiac remodeling could be mediated by elevated expression of the genes evaluated due to GH administration, since it stimulates protein synthesis of the cell and mRNA formation, however this did not occur in this study. Some hypotheses to explain this fact would be the lower sensitivity of cardiovascular tissue to direct GH action, as noted by Volterrani et al., [45], and also the negative feedback of GH and IGFs by its exogenous administration which may have interfered in the regulation of synthesis and secretion of GH [48].

RT promotes increase in Ca^{2+} concentration inside the cell by increasing the level of cardiomyocytes contractility, this fact leads to an overexpression of the SERCA2a target and consequently of its regulator, the PLB [49-50]. However, in our study, the genes of calcium trafficking did not undergo RT influences alone or associated with GH. This suggests that the period of four weeks was not enough to provide cardiac remodeling with alteration in expression of these genes in healthy

animals, however in another experiment that evaluated animals with cardiac dysfunction using a training protocol set in 8 weeks, there have been statistical differences in the cardiac calcium genes expression [51].

Other mechanisms may be involved in the modulation of cardiac contractility in response to RT, for example the neurohumoral factors, the sympathetic nervous system through adrenergic receptors (α1a, β1 and β2) and the endocrine system through angiotensin II (Ang II) [52,53], as well as the circulatory mechanisms neuromodulators by the action of nitric oxide (NO) [54]. Noteworthy is α- and β-MHC (myosin heavy chain) expression, since MHC is the major contractile protein of the heart, and is crucial for the efficiency of cardiac performance [55].

Evaluate the expression of these mechanisms is essential for a better understanding of the positive inotropic effect in cardiac remodeling. Therefore, further studies that analyze the same methodology but with a different approach regarding the mechanisms involved in morphologic, biochemical and cardiac contractile response are required.

This study did not assess the mechanisms that led to cardiac damage, so further investigations are required.

CONCLUSION

The RT promoted remodeling of the cardiac interstitial collagen, and tissue necrosis characterized by increased CK-MB, however, when RT was combined with GH collagen remained unchanged. The other results of this study showed that the RT associated with administration of GH did not promote changes in cardiac structure or an increase in mRNA of key genes of the cardiac Ca^{2+}, RyR, SERCA2a and PLB.

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Table 1. Anatomical parameters, cardiomyocytes cross-sectional area and interstitial collagen fraction expressed in mean ± standard deviation, median, minimum and maximum value.

Variables	Groups			
	CT	**GH**	**RT**	**RTGH**
FBW (g)	297,27 ± 22,14 305,10 [258,80 – 317,70]	313,81 ± 14,02 309 [295,70 – 334,20]	304,30 ± 29,08 307,10 [255,70 – 343,30]	292,05 ± 15,96 285,30 [273,40 – 314,20]
LV (g)	0,74 ± 0,31 0,61 [0,47 – 1,23]	0,87 ± 0,25 0,74 [0,58 – 1,21]	0,75 ± 0,26 0,67 [0,43 – 1,22]	0,77 ± 0,23 0,68 [0,55 – 1,14]
LV/FBW (mg/g)	2,48 ± 0,95 2,04 [1,74 – 4,03]	2,76 ± 0,79 2,35 [1,92 – 3,73]	2,55 ± 1,04 2,12 [1,26 – 4,13]	2,64 ± 0,81 2,38 [1,91 – 3,93]
SA (px^2)	8834 ± 1457 9458 [6979 - 10460]	9359 ± 1242 9118 [7537 - 10800]	10613 ± 2018 9550 [9082 - 13712]	8847 ± 910,8 8820 [7842 - 10234]
IC (%)	0.099 ± 0.043 0.106 [0.026 – 0.169]	0.087 ± 0.028 0.077 [0.056 – 0.135]	0.223 ± 0.102 ***** 0.221 [0.064 – 0.409]	0.112 ± 0.029 0.099 [0.082 – 0.147]

CT= without exercise and without growth hormone (control); GH= sedentary group and growth hormone; RT= resistance training; RTGH= resistance training and growth hormone; FBW= final body weight; LV= left ventricular weight; SA= sectional area of cardiomyocytes; IC= interstitial collagen fraction; * $p < 0.05$ vs. CT Group, GH, TRGH.

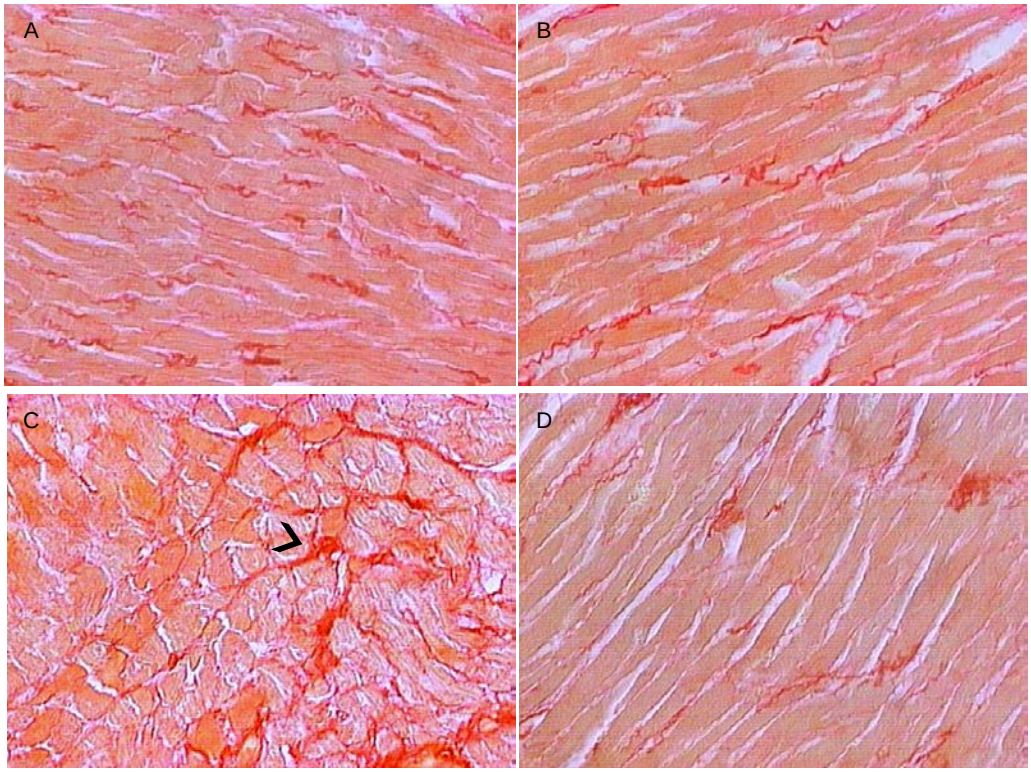

Figure 1. Picrosirius red myocardial collagen. Optical microscope on 40X objective. Collagen is stained in red. A - control group (CT); B - group of growth hormone (GH); C - group resistance training (RT); D - group resistance training and growth hormone.

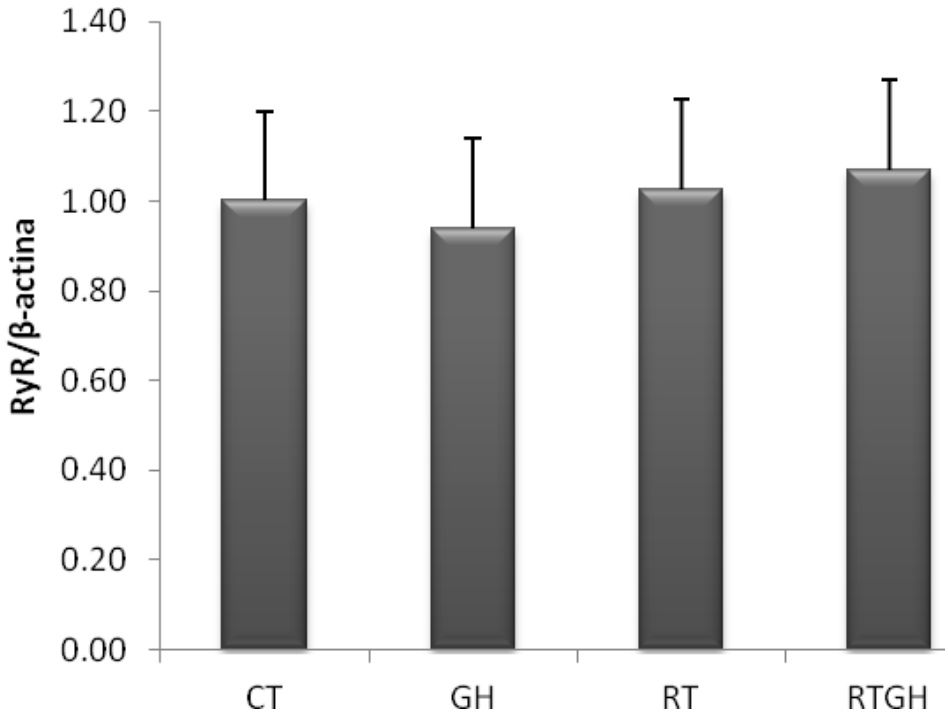

Figure 2. Relative levels of mRNA determined by qPCR of Ryanodine (RyR), expressed as mean ± standard deviation. CT = control group; Group GH = growth hormone; RT = resistance training group; RTGH = group resistance training and growth hormone.

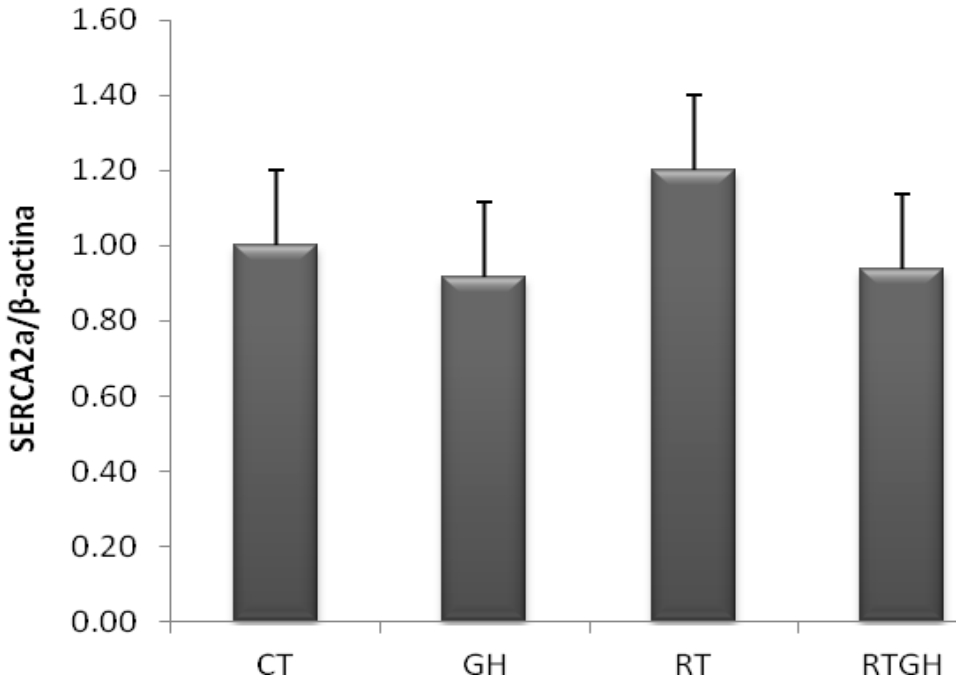

Figure 3. Relative levels of mRNA determined by RT-qPCR of the Sarcoplasmic Reticulum Ca^{2+} ATPase (SERCA2a), expressed as mean ± standard deviation. CT= control group; Group GH= growth hormone; RT= resistance training group; RTGH= group resistance training and growth hormone.

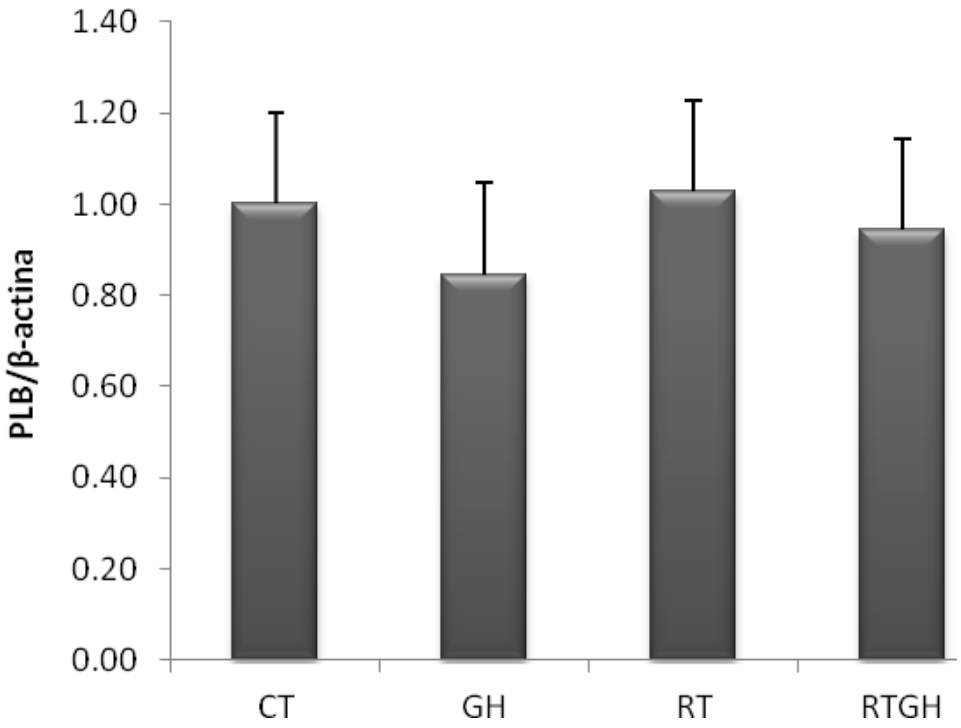

Figure 4. Relative levels of mRNA determined by RT-qPCR of phospholamban (PLB), expressed as mean ± standard deviation. CT= control group; Group GH= growth hormone; TR= resistance training group; TRGH= group resistance training and growth hormone.

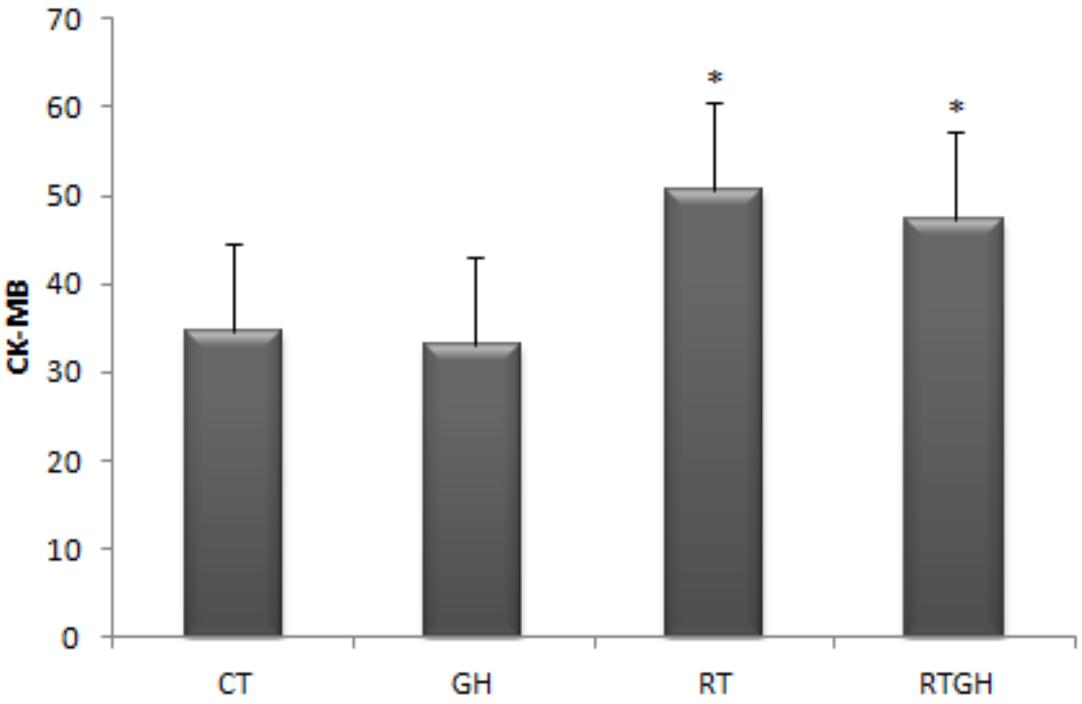

Figure 5. Determination of Creatine phosphokinase-muscle brain (CK-MB) by biochemical analysis expressed as mean ± standard deviation. CT= control group; Group GH= growth hormone; RT= resistance training group; RTGH= group resistance training and growth hormone. * $p < 0,05$ RT, RTGH vs. CT, GH.

ANEXO 2

GROWTH HORMONE & IGF RESEARCH

AUTHOR INFORMATION PACK

DESCRIPTION

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[1] J. van der Geer, J.A.J. Hanraads, R.A. Lupton, The art of writing a scientific article, J. Sci. Commun. 163 (2010) 51–59.

Reference to a book:

[2] W. Strunk Jr., E.B. White, The Elements of Style, fourth ed., Longman, New York, 2000.

Reference to a chapter in an edited book:

[3] G.R. Mettam, L.B. Adams, How to prepare an electronic version of your article, in: B.S. Jones, R.Z. Smith (Eds.), Introduction to the Electronic Age, E-Publishing Inc., New York, 2009, pp. 281–304.

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