UNIVERSIDADE FEDERAL DE VIÇOSA

JULIANO MAGALHÃES GUEDES

EFEITOS DO TREINAMENTO RESISTIDO E DA SUPLEMENTAÇÃO COM β-HIDROXI β-METILBUTIRATO (HMB) SOBRE A FORÇA MUSCULAR, COMPOSIÇÃO CORPORAL E METABOLISMO LIPÍDICO EM RATOS WISTAR

VIÇOSA - MINAS GERAIS

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Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Ciência da Nutrição, para obtenção do título de *Doctor Scientiae*.

Orientador: Antônio José Natali

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Assentimento:

Juliano Magalhães Guedes
Autor

Antônio José Natali
Orientador

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RESUMO

GUEDES, Juliano Magalhães, D.Sc., Universidade Federal de Viçosa, agosto de 2020. **Efeitos do treinamento resistido e da suplementação com β-hidroxi β-metilbutirato (HMB) sobre a força muscular, composição corporal e metabolismo lipídico em ratos Wistar.** Orientador: Antônio José Natali. Coorientadores: Maria do Carmo Gouveia Peluzio, Miguel Araújo Carneiro Júnior e Leandro Licursi de Oliveira.

Objetivo: O objetivo deste estudo foi verificar os efeitos do treinamento resistido (TR) e da suplementação com sal de cálcio do β-hidroxi β-metilbutirato (CaHMB) sobre a força muscular, composição corporal e metabolismo lipídico em ratos Wistar. **Métodos:** Ratos Wistar adultos (idade: 3 meses) foram aleatoriamente distribuídos em 4 grupos de 12 animais cada: sedentário controle (SC); sedentário com suplemento (SS); treinamento resistido controle (RTC) e treinamento resistido com suplemento (RTS). Os animais dos grupos SS e RTS receberam, por gavagem, 1 mL de CaHMB (320 mg/kg de peso corporal). Os animais dos grupos RTC e RTS realizaram um programa de TR (4 séries de 10 repetições com intervalo de 90 s entre as séries; 1 vez/dia, 5 dias/semana; carga de 80% de 1 repetição máxima – 1 RM), por 8 semanas. Todos os animais foram pesados e tiveram a circunferência abdominal mensurada e o índice de Lee calculado semanalmente e o consumo alimentar e a força muscular monitorados durante o período experimental. Ao final das intervenções foram determinadas: a composição corporal, o perfil lipídico, a expressão gênica de interleucinas (IL-6, IL-10 e IL-15), da proteína contendo o domínio 5 da fibronectina do tipo 3 (FNDC-5) no músculo esquelético e de IL-6 e proteína desacopladora mitocondrial do tipo 1 (UCP-1) no tecido adiposo branco (TAB) e a concentração de irisina no TAB. **Resultados:** O TR isolado aumentou a força muscular e reduziu o consumo alimentar, o ganho de peso, o índice de Lee, a circunferência abdominal, as percentagens de gordura e água, as concentrações plasmáticas de lipoproteínas de muito baixa densidade (VLDL), colesterol não lipoproteínas de alta densidade (Não-HDL), triglicerídeos, colesterol total e aumentou a expressão gênica de FNDC-5 no músculo sóleo e a concentração de irisina no TAB. A combinação do TR com o CaHMB potencializou a redução da circunferência abdominal (5,3 %), índice de Lee (2,4 %), porcentagem de gordura (24,4 %), concentrações plasmáticas de VLDL (16,8 %) e triglicerídeos (17 %) e aumentou a expressão gênica de IL-6 (47,4 %) e FNDC-5 (78.9 %) no sóleo e a concentração de irisina (26,9 %) no TAB. Isoladamente, tanto o TR quanto a suplementação com CaHMB não afetaram o percentual de proteína e a expressão gênica de IL-6 e UCP-1 no TAB e de IL-10 e IL-15 no gastrocnêmio. Não houve esteatose hepática com os diferentes tratamentos após a intervenção. **Conclusões:** A suplementação com CaHMB potencializou os efeitos benéficos do TR na redução de gordura corporal, em associação com

aumento da expressão gênica de IL-6 e de FNDC-5 muscular e da concentração de irisina no TAB, apesar de não ter afetado o conteúdo de proteínas e a força muscular.

Palavras-chave: Exercício. Suplementação. Tecido Adiposo. Irisina.

ABSTRACT

GUEDES, Juliano Magalhães, D.Sc., Universidade Federal de Viçosa, August, 2020. **Effects of resistance training and β-hydroxy β-methylbutyrate (HMB) supplementation on muscle strength, body composition and lipid metabolism in Wistar rats. Adviser**: Antônio José Natali. Co-advisers: Maria do Carmo Gouveia Peluzio, Miguel Araújo Carneiro Júnior and Leandro Licursi de Oliveira.

Objective: The aim of this study was to verify the effects of resistance training (RT) and calcium salt of β-hydroxy β-methylbutyrate (CaHMB) supplementation on muscle strength, body composition and lipid metabolism in Wistar rats. **Methods:** Three-month old male Wistar rats were randomly divided into 4 groups of 12 animals each: sedentary control (SC); sedentary with supplement (SS); resistance training control (RTC) and resistance training with supplementation (RTS). The animals from SS and RTS groups received, by gavage, 1 mL of CaHMB (320 mg / kg of body weight). The animals from RTC and RTS groups performed an RT program (4 sets of 10 repetitions with 90 s interval; 1 session / day, 5 days / week; load of 80% 1 maximum repetition - 1 RM), for 8 weeks. All animals were weighed and had abdominal circumference measured and Lee index calculated weekly and the food consumption and muscle strength monitored throughout the experimental period. By the end of interventions, the body composition, plasma lipid profile, the gene expression of interleukins (IL-6, IL-10 and IL-15) and fibronectin type III domain containing 5 (FNDC-5) in skeletal muscle and IL-6 and uncoupling protein-1 (UCP-1) in white adipose tissue (WAT) and the concentration of irisin in WAT were determined. **Results:** Resistance training alone increased total and relative muscle strength; reduced food intake, weight gain, Lee index, abdominal circumference, percentages of fat and water, plasma concentrations of very low-density lipoprotein (VLDL), non-high-density lipoprotein (Non-HDL) cholesterol, triglycerides, total cholesterol; and increased FNDC-5 gene expression in soleus muscle and the concentration of irisin protein in WAT. Compared to RT alone, the combination of CaHMB supplementation with RT further reduced abdominal circumference (5.3 %), Lee index (2.4 %), fat percentage (24.4 %), plasma VLDL-cholesterol (16.8%) and triglycerides (17%) and increased the gene expression of FNDC-5 (78.9 %) and IL-6 (47.4%) in gastrocnemius muscle and irisin concentration (26.9 %) in WAT. Neither RT nor CaHMB supplementation alone affected the protein percentage and the gene expression of IL-6 and UCP-1 in WAT and IL-10 and IL-15 in gastrocnemius muscle. There was no hepatic steatosis with the different treatments after the intervention. **Conclusions:** CaHMB supplementation increased the beneficial effects of RT on body fat reduction and was associated with increased

muscular genic expression of IL-6 and FNDC-5 and irisin concentration in WAT, despite no change in protein content and muscle strength.
Keywords: Exercise. Supplementation. Adipose Tissue. Irisin.

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LISTA DE ABREVIATURAS E SIGLAS

AKT - Threonine protein kinases

AMPK - AMP-activated protein kinase

ANOVA – Analysis of variance

BCAT - Branched chain amino acid aminotransferase

BCKD - Branched chain α-keto acid dehydrogenase

CaHMB – Calcium β-hidroxi β-metilbutirate

CPT-1 - Carnitine palmitoyl transferase 1

ELISA - Enzyme-linked immunosorbent assay

ERR-α - Estrogen-related α receptors

FA - Fatty acid

FNDC-5 - Fibronectin type III domain-containing protein 5

HDL – High-density lipoprotein

HIIT - High-intensity interval training

HMG–CoA - β-hydroxy β-methylglutaryl-CoA

HSL - Hormone-sensitive lipase

IL-6 - Interleukin 6

IL-10 - Interleukin 10

IL-15 - Interleukin 15

IGF-1 - Insulin-like growth factor

KIC - α-ketoisocaproate

LDL – Low-density lipoprotein

MCP – Monocyte chemotactic protein

mTOR - Mammalian target of rapamycin

NRF - Nuclear respiratory factor

1RM - one repetition maximum

PGC1-α - Peroxisome proliferator-activated receptor gamma coactivator 1-α

PPARs - Peroxisome proliferator-activated receptors

PYY - Peptide tyrosine tyrosine

$p70^{S6K}$ - Ribosome protein kinase 1 β

RT – Resistance training

RTC - Resistance training control

RTS - Resistance training with supplementation

RT-PCR - Real-time polymerase chain reaction

SC - Sedentary control

SS - Sedentary with supplement

SIRTs - Sirtuins

UCP-1 - Uncoupling protein 1

VLDL – Very low-density lipoprotein

WAT - White adipose tissue

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1. INTRODUÇÃO GERAL

O excesso de gordura corporal está normalmente associado ao maior risco de doenças cardiovasculares, o que compromete a qualidade de vida de pessoas de diferentes idades (1). Estima-se que, em breve, haverá mais crianças e adolescentes obesos que malnutridos no mundo (2). Desta forma, torna-se um desafio para os governantes, no que se refere à saúde pública, pensar em estratégias que previnam obesidade e promovam qualidade de vida.

Neste sentido, uma composição corporal adequada, ou seja, massa muscular e massa gorda em proporções adequadas, assim como a força muscular são relevantes tanto para atletas, voltados ao rendimento esportivo, como para não atletas que buscam a boa forma, pois estes fatores estão relacionados com uma boa saúde (3). Assim, exercício físico e suplementos nutricionais que podem afetar a composição corporal têm atraído o interesse de diferentes populações. Para ilustrar, estudos reportam que o treinamento resistido (TR) é benéfico para a prevenção do acúmulo de gordura corporal e para a melhora da saúde (4,5). Adicionalmente, o β-hidroxi β-metilbutirato (HMB), um metabólito resultante do aminoácido essencial leucina, vem sendo utilizado como suplemento por praticantes de atividade física de diferentes idades, que buscam otimizar o desempenho esportivo e/ou melhorar indicadores de saúde (6,7), como ganho de massa magra e perda de massa gorda. Desta forma, a combinação do TR com a suplementação proteica tem sido alvo de investigações científicas há alguns anos e é sugerida para aumentar a massa magra e a força muscular (8), apesar dos mecanismos ainda não estarem completamente esclarecidos.

Em relação ao HMB, estudos mostram que este aumenta a massa e a força muscular e reduz a gordura corporal (9–14) apesar de resultados divergentes (15,16). O aumento da massa muscular em resposta à suplementação com HMB parece ser devido ao aumento da síntese proteica pela estimulação da via de sinalização do alvo da rapamicina em mamíferos (mTOR) e a inibição da proteólise pela via do sistema catabólico ubiquitina-proteassoma (17,18), o que é influenciado por citocinas (19,20). Tem sido reportado que o HMB pode também estimular a termogênese no tecido adiposo por meio do aumento da oxidação lipídica e biogênese mitocondrial via expressão de coativador 1-α do receptor gama ativado por proliferador de peroxissoma (PGC-1α), proteína desacopladora do tipo 3 (UCP-3) e irisina (21–23).

Ainda nesse contexto, o metabolismo do HMB demonstra que uma fração do metabólito é convertida em colesterol no fígado sob ação da enzima β-Hidroxi β-Metilglutaril-CoA (HMG-CoA) redutase (24-25). Assim, um grande aumento na biossíntese

de colesterol pelos hepatócitos, em função do excesso de consumo, poderia resultar em acúmulo de gordura no fígado. Neste sentido, no presente estudo, foi realizada a análise de esteatose hepática nos animais experimentais (veja apêndice), onde observou-se que a dose de HMB usada não alterou a concentração de gordura nos hepatócitos dos animais.

Com relação aos efeitos do HMB no perfil lipídico, estudos sugerem que o aumento da biossíntese de colesterol via HMG-CoA, da oxidação lipídica e biogênese mitocondrial via PGC-1α, UCP-3 e irisina (21–23) poderia alterar as concentrações de colesterol no sangue (24). Entretanto, os resultados são controversos (24–27). Por exemplo, há relatos de nenhuma alteração (27), aumento (26) e redução (25) do colesterol de baixa densidade (LDL-c) ou colesterol total. Destaca-se aqui a falta de padronização dos protocolos dos estudos e dos métodos estatísticos para análise dos dados (28).

O HMB é comercializado em cápsulas ou em pó e nas formas de ácido livre (AL) sal de cálcio (Ca). Em estudo comparativo, observou-se em humanos que o HMB-AL apresenta maiores concentrações plasmáticas e maior disponibilidade em relação ao HMB-Ca (29). O consumo de HMB por humanos, normalmente, é feito com a dosagem de 38 mg/kg de peso corporal; ou 3 g/dia, ingeridas em 3 doses equivalentes ao longo do dia (30,31). A dosagem de 3 g/dia corresponde a, aproximadamente, 60 g de leucina (32). Em estudos com ratos, a dosagem de 320 mg/kg de peso corporal tem sido usada (33,34).

Quanto ao TR, sabe-se que este potencializa o desempenho esportivo por causar hipertrofia muscular e aumentar a força, resistência, coordenação intra e inter-muscular e a oxidação de substratos energéticos (5,35). O TR induz o aumento da síntese proteica ao estimular vias de sinalização de anabolismo e a diminuição da proteólise ao inibir vias de sinalização de catabolismo (36,37). O TR pode também reduzir a gordura corporal, pois aumenta a taxa metabólica basal (38,39) e a termogênese no tecido adiposo branco (TAB) (40,41). Durante o TR, as contrações musculares promovem a síntese e secreção de citocinas tais como interleucinas (IL), proteína contendo o domínio 5 da fibronectina do tipo 3 (FNDC-5) e irisina que exercem efeitos locais e em outros órgãos como o fígado e tecido adiposo para suprir demandas de reparação muscular, hipertrofia e metabolismo energético (42–44).

Especificamente, a IL-6 age como um “sensor de energia na célula” por estimular positivamente a captação de glicose pelo músculo via aumento da sensibilidade da insulina (45,46), ativa a diferenciação de células satélites no músculo esquelético (46,47), diminui a expressão proteica de citocinas pró-inflamatórias (fator de necrose Tumoral alfa - TNF-α) e aumenta a expressão proteica de citocinas anti-inflamatórias (IL-10 e IL1-ra) (48,49). No TAB, a IL-6 estimula a termogênese via ativação da expressão gênica do RNA mensageiro

(mRNA) da UCP-1 aumentando o gasto energético e, consequentemente, o potencial oxidativo de gorduras (50).

A IL-10, quando expressa no músculo, causa mudanças no fenótipo de macrófagos de fenótipo M1 para M2 promovendo crescimento celular e regeneração tecidual (51). Ademais, a IL-10 também estimula a resposta imunológica por aumentar a concentração de mastócitos e inibir a produção de interferon-gama (IFN-γ) pelas células natural killer (52).

A IL-15 é uma citocina com ação anti-inflamatória por inibir a expressão da proteína c-reativa (PCR) e TNF-α (53), está positivamente associada a hipertrofia muscular (54,55), inibição da degradação proteica e apoptose nuclear (56), melhora do metabolismo da glicose (aumento da sensibilidade a insulina) e oxidação dos lipídeos (57,58). Em cultura de célula muscular humana a inoculação de IL-15 induziu a formação da cadeia pesada de miosina em miotubos (59,60).

Diferentemente da ação anabólica da IL-6, IL-10 e IL-15 no músculo esquelético, a irisina tem grande potencial termogênico no TAB (61,62). Essa citocina vem sendo muito estudada por desempenhar papel promissor no tratamento de patologias como dislipidemia, diabetes mellitus tipo 2, obesidade e/ou doença gordurosa do fígado (63–66). Em nível molecular, a via de sinalização para formação da irisina demonstra que o exercício físico promove o aumento da expressão do PGC1-α no músculo que, por sua vez, estimula a produção da FNDC-5, logo, o FNDC-5 é clivado dando origem a irisina. Depois de secretada no sangue pelo músculo, a irisina é conduzida ao TAB estimulando a ação da UCP-1 no interior da mitocôndria aumentando o *browning do* TAB e a produção de calor (63). Nesse contexto, apesar do FNDC-5 / irisina ser uma citocina secretada, principalmente, pelo músculo durante o exercício físico, sua ação no metabolismo energético está longe de ser totalmente compreendido (63).

Portanto, a combinação do TR com a suplementação parece promissora para o ganho de força e para a composição corporal. De fato, estudos demonstram que o TR associado à suplementação com HMB aumenta a massa e a força muscular e reduz a gordura corporal (12,14,15). Todavia, existem resultados divergentes ao não encontrarem tais efeitos (67,68) e que a eficiência desta combinação depende da população estudada (69,70). Resultados controversos também são encontrados em estudos que testaram os efeitos desta combinação sobre citocinas relacionadas à hipertrofia muscular (71,72) e à atividade metabólica no tecido adiposo (23,73). Assim, estas divergencias estimulam novas investigações, especialmente sobre os mecanismos envolvidos.

O modelo de TR para ratos adotado no presente estudo (74) está bem consolidado na literatura (75–78) e apresenta algumas vantagens: 1) simula o aparelho de barra guiada utilizado por humanos; 2) exige uma forte ativação concêntrica da musculatura esquelética de membros inferiores durante a fase de extensão dos joelhos; 3) recruta principalmente fibras musculares de contração rápida (tipo IIA); e 4) utiliza, predominantemente, o metabolismo energético anaeróbico. Porém, uma desvantagem desse modelo é que a fase excêntrica do movimento de flexão dos joelhos ocorre muito rapidamente. Nesse contexto, para protocolos de TR que visam hipertrofia muscular, é preciso que a escolha desse modelo seja analisada com cautela.

REFERÊNCIAS

1. World Health Organization (WHO). Global status report on non communicable diseases. Geneva: World Health Organization; 2010.
2. Bentham J, Di Cesare M, Bilano V, Bixby H, Zhou B, Stevens GA, et al. Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128·9 million children, adolescents, and adults. The Lancet. 2017;390(10113):2627–42.
3. Wolfe RR. The underappreciated role of muscle in health and disease. The American Journal of Clinical Nutrition. 2006;84(3):475–82.
4. Kraemer WJ, Ratamess NA. Fundamentals of resistance training: progression and exercise prescription. Medicine and science in sports and exercise. 2004;36(4):674–88.
5. American College of Sports Medicine (ACSM). Progression Models in Resistance Training for Healthy Adults. Medicine & Science in Sports & Exercise. 2009;41(3):687–708.
6. Juhn MS. Popular Sports Supplements and Ergogenic Aids. Sports Medicine. 2003;33(12):921–39.
7. Hoffman J. Dietary supplementation in sport and exercise : evidence, safety and ergogenic benefits. 1ª Edição. Routledge; 2019.
8. Kerksick CM, Wilborn CD, Roberts MD, Smith-Ryan A, Kleiner SM, Jäger R, et al. ISSN exercise & sports nutrition review update: research & recommendations. Journal of the International Society of Sports Nutrition. 2018;15(1):1–38.
9. Romero FG, Guimarães-Ferreira L, Yonamine CY, Salgueiro RB, Nunes MT. Effects of beta-hydroxy-beta-methylbutyrate (HMB) on the expression of ubiquitin ligases, protein synthesis pathways and contractile function in extensor digitorum longus (EDL) of fed and fasting rats. The Journal of Physiological Sciences. 2018;68(2):165–74.
10. Berton L, Bano G, Carraro S, Veronese N, Pizzato S, Bolzetta F, et al. Effect of oral beta-hydroxy-beta-methylbutyrate (HMB) supplementation on physical performance in healthy old women over 65 years: An open label randomized controlled trial. PLOS ONE. 2015;10(11):1–14.
11. Gerlinger-Romero F, Guimarães-Ferreira L, Giannocco G, Nunes MT. Chronic supplementation of beta-hydroxy-beta methylbutyrate (HMβ) increases the activity of the GH/IGF-I axis and induces hyperinsulinemia in rats. Growth Hormone & IGF Research. 2011;21(2):57–62.
12. Stout JR, Fukuda DH, Kendall KL, Smith-Ryan AE, Moon JR, Hoffman JR. β-Hydroxy-β-methylbutyrate (HMB) supplementation and resistance exercise significantly reduce abdominal adiposity in healthy elderly men. Experimental gerontology. 2015;64(1):33–4.
13. Durkalec-Michalski K, Jeszka J. The efficacy of a β-hydroxy-β-methylbutyrate supplementation on physical capacity, body composition and biochemical markers in elite rowers: a randomised, double-blind, placebo-controlled crossover study. Journal of the International Society of Sports Nutrition. 2015;12(1):1–31.
14. Asadi A, Arazi H, Suzuki K. Effects of β-hydroxy-β-methylbutyrate-free acid supplementation on strength, power and hormonal adaptations following resistance training. Nutrients. 2017;9(12):1–1316.
15. McIntosh ND, Love TD, Haszard J, Osborne H, Black KE. β-hydroxy β-methylbutyrate (HMB) supplementation effects on body mass and performance in elite

male rugby union players. Journal of Strength and Conditioning Research. 2018;32(1):19–26.
16. Slater G, Jenkins D, Logan P, Lee H, Vukovich M, Rathmacher JA, et al. Beta-hydroxy-beta-methylbutyrate (HMB) supplementation does not affect changes in strength or body composition during resistance training in trained men. International journal of sport nutrition and exercise metabolism. 2001;11(3):384–96.
17. Eley HL, Russell ST, Baxter JH, Mukerji P, Tisdale MJ. Signaling pathways initiated by beta-hydroxy-beta-methylbutyrate to attenuate the depression of protein synthesis in skeletal muscle in response to cachectic stimuli. AJP: Endocrinology and Metabolism. 2007;293(4):E923–31.
18. Smith HJ, Mukerji P, Tisdale MJ. Attenuation of proteasome-induced proteolysis in skeletal muscle by B-hydroxy-B-methylbutyrate in cancer-induced muscle loss. Cancer research. 2005;65(1):277–83.
19. Eley HL, Russell ST, Tisdale MJ. Mechanism of attenuation of muscle protein degradation induced by tumor necrosis factor- and angiotensin II by -hydroxy- -methylbutyrate. AJP: Endocrinology and Metabolism. 2008;295(6):E1417–26.
20. Eley HL, Russell ST, Tisdale MJ. Attenuation of depression of muscle protein synthesis induced by lipopolysaccharide, tumor necrosis factor, and angiotensin II by β-hydroxy-β-methylbutyrate. American Journal of Physiology Endocrinology and Metabolism. 2008;295(6):E-1409-16.
21. Stancliffe RA. Role of Beta-Hydroxy-Beta-Methylbutyrate (HMB) in Leucine Stimulation of Mitochondrial Biogenesis and Fatty Acid Oxidation. Master's Thesis, University of Tennessee, Knoxville, 2012.
22. Baggett B, Bruckbauer A, Zemel M. Synergistic Effects of Leucine and its Metabolites with Polyphenols on Irisin in Myotubes and Diet-induced Obese Mice. FASEB. 2013;27(1):11–637.
23. Shirvani H, Rahmati-Ahmadabad S, Broom DR, Mirnejad R. Eccentric resistance training and β-hydroxy-β-methylbutyrate free acid affects muscle PGC-1α expression and serum irisin, nesfatin-1 and resistin in rats. The Journal of Experimental Biology. 2019;222(10):jeb198424.
24. Arazi H, Taati B, Suzuki K. A Review of the Effects of Leucine Metabolite (β-Hydroxy-β-methylbutyrate) Supplementation and Resistance Training on Inflammatory Markers: A New Approach to Oxidative Stress and Cardiovascular Risk Factors. Antioxidants (Basel, Switzerland). 2018;7(10).
25. Nissen S, Sharp RL, Panton L, Vukovich M, Trappe S, Fuller JC. β-hydroxy-β-methylbutyrate (HMB) supplementation in humans is safe and may decrease cardiovascular risk factors. The Journal of nutrition. 2000;130:1937–45.
26. Holecek M, Muthny T, Kovarik M, Sispera L. Effect of beta-hydroxy-beta-methylbutyrate (HMB) on protein metabolism in whole body and in selected tissues. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association. 2009;47(1):255–9.
27. Hsieh L-C, Chow C-J, Chang W-C, Liu T-H, Chang C-K. Effect of beta-hydroxy-beta-methylbutyrate on protein metabolism in bed-ridden elderly receiving tube feeding. Asia Pacific journal of clinical nutrition. 2010;19(2):200–8.
28. Arazi H, Rohani H, Ghiasi A, Davaran M. The Effect of HMB Supplementation on Cardiovascular Risk Factors after Four Weeks of Resistance Training in Amateur Athletes. International Cardivascular Research Journal. 2015;9(2):89–93.
29. Fuller JC, Sharp RL, Angus HF, Baier SM, Rathmacher JA. Free acid gel form of β-hydroxy-β-methylbutyrate (HMB) improves HMB clearance from plasma in human subjects compared with the calcium HMB salt. British Journal of Nutrition.

2011;105:367–72.
30. Wilson JMJJM, Fitschen PJPPJ, Campbell B, Wilson GJ, Zanchi N, Taylor L, et al. International Society of Sports Nutrition Position Stand: beta-hydroxy-beta-methylbutyrate (HMB). Journal of the International Society of Sports Nutrition. 2013;10(1):1–6.
31. Rahimi MH, Mohammadi H, Eshaghi H, Askari G, Miraghajani M. The Effects of Beta-Hydroxy-Beta-Methylbutyrate Supplementation on Recovery Following Exercise-Induced Muscle Damage: A Systematic Review and Meta-Analysis. Journal of the American College of Nutrition. 2018;37(7):640–9.
32. Holeček M. Beta-hydroxy-beta-methylbutyrate supplementation and skeletal muscle in healthy and muscle-wasting conditions. Journal of cachexia, sarcopenia and muscle. 2017;8(1):1–13.
33. Pinheiro CH da J, Gerlinger-Romero F, Guimarães-Ferreira L, de Souza-Jr AL, Vitzel KF, Nachbar RT, et al. Metabolic and functional effects of beta-hydroxy-beta-methylbutyrate (HMB) supplementation in skeletal muscle. European journal of applied physiology. 2012;112(7):2531–7.
34. Christofoletti DC. Influência da suplementação com β-hidroxi-β-metilbutirato (HMB) em músculo esquelético de ratos submetidos à desnervação. 2014.
35. Fleck S, Kraemer W. Fundamentos do Treinamento de Força Muscular. 4ª edição. Artmed; 2017.
36. Schoenfeld BJ. The Mechanisms of Muscle Hypertrophy and Their Application to Resistance Training. Journal of Strength and Conditioning Research. 2010;24(10):2857–72.
37. Mejías-Peña Y, Estébanez B, Rodriguez-Miguelez P, Fernandez-Gonzalo R, Almar M, de Paz JA, et al. Impact of resistance training on the autophagy-inflammation-apoptosis crosstalk in elderly subjects. Aging. 2017;9(2):408–18.
38. Byrne HK, Wilmore JH. The effects of a 20-week exercise training program on resting metabolic rate in previously sedentary, moderately obese women. International Journal of Sport Nutrition. 2001;11(1):15–31.
39. Stavres J, Zeigler M, Bayles MP. Six Weeks of Moderate Functional Resistance Training Increases Basal Metabolic Rate in Sedentary Adult Women. International Journal of Exercise Science. 2018;11(2):32–41.
40. Thirupathi A, da Silva Pieri BL, Queiroz JAMP, Rodrigues MS, de Bem Silveira G, de Souza DR, et al. Strength training and aerobic exercise alter mitochondrial parameters in brown adipose tissue and equally reduce body adiposity in aged rats. Journal of Physiology and Biochemistry. 2019;75(1):101–8.
41. Reisi J, Ghaedi K, Rajabi H, Marandi SM. Can Resistance Exercise Alter Irisin Levels and Expression Profiles of FNDC5 and UCP1 in Rats? Asian journal of sports medicine. 2016;7(4):e35205.
42. Zhao J, Su Z, Qu C, Dong Y. Effects of 12 Weeks Resistance Training on Serum Irisin in Older Male Adults. Frontiers in Physiology. 2017;8(171):1–4.
43. Calle MC, Fernandez ML. Effects of resistance training on the inflammatory response. Nutrition research and practice. 2010;4(4):259–69.
44. Reisi J, Rajabi H, Ghaedi K, Seyed-Mohammad M, Mohammad-Reza D. Effect of Acute Resistance Training on Plasma Irisin Protein Level and Expression of Muscle FNDC5 and Adipose Tissue UCP1 Genes in Male Rats. Journal of Isfahan Medical School. 2013;31(256):1657–66.
45. Pedersen BK, Fischer CP. Beneficial health effects of exercise – the role of IL-6 as a myokine. Trends in Pharmacological Sciences. 2007;28(4):152–6.
46. Costa EM da, Werutsky CA. IL-6: Sinalização na Hipertrofia Muscular e efeitos

Metabólicos Sistêmicos. International Journal of Nutrology. 2015;8(4):95–100.
47. Trenerry MK, Della Gatta PA, Larsen AE, Garnham AP, Cameron-Smith D. Impact of resistance exercise training on interleukin-6 and JAK/STAT in young men. Muscle & Nerve. 2011;43(3):385–92.
48. Steensberg A, Fischer CP, Keller C, Møller K, Pedersen BK. IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. American Journal of Physiology-Endocrinology and Metabolism. 2003;285(2):E433–7.
49. Bruunsgaard H. Physical activity and modulation of systemic low-level inflammation. Journal of Leukocyte Biology. 2005;78(4):819–35.
50. Knudsen JG, Murholm M, Carey AL, Biensø RS, Basse AL, Allen TL, et al. Role of IL-6 in exercise training- and cold-induced UCP1 expression in subcutaneous white adipose tissue. PloS one. 2014;9(1):e84910.
51. Deng B, Wehling-Henricks M, Villalta SA, Wang Y, Tidball JG. IL-10 Triggers Changes in Macrophage Phenotype That Promote Muscle Growth and Regeneration. The Journal of Immunology. 2012;189(7):3669–80.
52. Zhang J-M, An J. Cytokines, inflammation, and pain. International anesthesiology clinics. 2007;45(2):27–37.
53. Bazgir B, Salesi M, Koushki M, Amirghofran Z. Effects of Eccentric and Concentric Emphasized Resistance Exercise on IL-15 Serum Levels and Its Relation to Inflammatory Markers in Athletes and Non-Athletes. Asian Journal of Sports Medicine. 2015;6(3):1–7.
54. Pistilli EEE, Bogdanovich S, Garton F, Yang N, Gulbin JP, Conner JD, et al. Loss of IL-15 receptor α alters the endurance, fatigability, and metabolic characteristics of mouse fast skeletal muscles. Journal of Clinical Investigation. 2011;121:3120–32.
55. Oh SL, Kim H, Kim YA, Seong J kyung, Song W. Effects of acute and chronic resistance exercise training on IL-15 expression in rat skeletal muscle. International Journal of Applied Sports Sciences. 2013;25(2):85–90.
56. Figueras M, Busquets S, Carbó N, Barreiro E, Almendro V, Argilés JM, et al. Interleukin-15 is able to suppress the increased DNA fragmentation associated with muscle wasting in tumour-bearing rats. FEBS Letters. 2004;569(1–3):201–6.
57. Quinn LS, Anderson BG, Strait-Bodey L, Stroud AM, Argiles JM. Oversecretion of interleukin-15 from skeletal muscle reduces adiposity. AJP: Endocrinology and Metabolism. 2008;296(1):E191–202.
58. Ye J. Beneficial metabolic activities of inflammatory cytokine interleukin 15 in obesity and type 2 diabetes. Frontiers of Medicine. 2015;9(2):139–45.
59. Nielsen AR, Mounier R, Plomgaard P, Mortensen OH, Penkowa M, Speerschneider T, et al. Expression of interleukin-15 in human skeletal muscle - effect of exercise and muscle fibre type composition. The Journal of Physiology. 2007;584(1):305–12.
60. Furmanczyk PS, Quinn LS. Interleukin-15 increases myosin accretion in human skeletal myogenic cultures. Cell biology international. 2003;27(10):845–51.
61. Villarroya F. Irisin, Turning Up the Heat. Cell Metabolism. 2012;15(3):277–8.
62. Norheim F, Langleite TM, Hjorth M, Holen T, Kielland A, Stadheim HK, et al. The effects of acute and chronic exercise on PGC-1α, irisin and browning of subcutaneous adipose tissue in humans. FEBS Journal. 2014;281(3):739–49.
63. Arias-Loste M, Ranchal I, Romero-Gómez M, Crespo J. Irisin, a Link among Fatty Liver Disease, Physical Inactivity and Insulin Resistance. International Journal of Molecular Sciences. 2014;15(12):23163–78.
64. Xiong X-Q, Chen D, Sun H-J, Ding L, Wang J-J, Chen Q, et al. FNDC5 overexpression and irisin ameliorate glucose/lipid metabolic derangements and enhance lipolysis in obesity. Biochimica et biophysica acta. 2015;1852(9):1867–75.

65. Chen N, Li Q, Liu J, Jia S. Irisin, an exercise-induced myokine as a metabolic regulator: an updated narrative review. Diabetes/Metabolism Research and Reviews. 2016;32(1):51–9.
66. Boström P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, et al. A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature. 2012;481(7382):463–8.
67. Teixeira FJ, Matias CN, Monteiro CP, Valamatos MJ, Reis JF, Batista A, et al. No effect of HMB or α-HICA supplementation on training-induced changes in body composition. European Journal of Sport Science. 2019;19(6):802–10.
68. Tritto AC, Bueno S, Rodrigues RMP, Gualano B, Roschel H, Artioli GG. Negligible effects of β-Hydroxy-β-methylbutyrate free acid and calcium salt on strength and hypertrophic responses to resistance training: A randomized, placebo-controlled study. International Journal of Sport Nutrition and Exercise Metabolism. 2019;29(5):505–11.
69. Albert FJ, Morente-Sánchez J, Ortega FB, Castillo MJ, Gutiérrez Á. Usefulness of B-Hydroxy-B-Methylbutyrate (Hmb) Supplementation in Different Sports: an Update and Practical Implications. Nutricion hospitalaria. 2015;32(01):20–33.
70. Kuriyan R, Lokesh DP, Selvam S, Jayakumar J, Philip MG, Shreeram S, et al. The relationship of endogenous plasma concentrations of β-Hydroxy β-Methyl Butyrate (HMB) to age and total appendicular lean mass in humans. Experimental Gerontology. 2016;81:13–8.
71. Kraemer WJ, Hatfield DL, Comstock BA, Fragala MS, Davitt PM, Cortis C, et al. Influence of HMB Supplementation and Resistance Training on Cytokine Responses to Resistance Exercise. Journal of the American College of Nutrition. 2014;33(4):247–55.
72. Rahimi R, Nejad HS. Effects of β -Hydroxy- β -Methylbutyrate Supplementation on IL-4 , IL-10 and TGF- β 1 during Resistance Exercise in Athletes. Preprints. 2017;23(5):1–10.
73. Kim H-J, So B, Choi M, Kang D, Song W. Resistance exercise training increases the expression of irisin concomitant with improvement of muscle function in aging mice and humans. Experimental gerontology. 2015;70:11–7.
74. Melo SFS. Seleção de microRNA e Proteína Alvo Envolvidos com a Função Cardíaca de Ratos Infartados em Resposta ao Treinamento Aeróbico. Universidade de São Paulo, São Paulo-SP; 2014.
75. Aguiar AF. Adaptações morfofuncionais e respostas moleculares do músculo esquelético de ratos submetidos ao treinamento resistido. Universidade Esdadual de São Paulo (UNESP), Botucatu-SP; 2011.
76. Melo SFS, Amadeu MA, Magalhães F de C, Fernandes T, Carmo EC do, Barretti DLM, et al. Exercício de força ativa a via AKT/mTor pelo receptor de angiotensina II tipo I no músculo cardíaco de ratos. Revista Brasileira de Educação Física e Esporte. 2011;25(3):377–85.
77. Carmo EC do, Bueno Junior CR, Fernandes T, Barretti D, Soares SF, Silva Junior ND da, et al. O papel do esteroide anabolizante sobre a hipertrofia e força muscular em treinamentos de resistência aeróbia e de força. Revista Brasileira de Medicina do Esporte. 2011;17(3):212–7.
78. Barauna VG, Junior MLB, Costa Rosa LFB, Casarini DE, Krieger JE, Oliveira EM. Cardiovascular adaptations in rats submitted to a resistance -training model. Clinical and Experimental Pharmacology and Physiology. 2005;32(4):249–54.

2. OBJETIVOS

2.1 Objetivo Geral

Verificar os efeitos do treinamento resistido e da suplementação com sal de cálcio do β-hidroxi β-metilbutirato sobre a força muscular, composição corporal e metabolismo lipídico em ratos Wistar.

2.2 Objetivos específicos

Verificar se um programa de treinamento resistido e a suplementação com sal de cálcio do β-hidroxi β-metilbutirato afetam, em ratos Wistar:

Força máxima e relativa;

Circunferência abdominal e índice de Lee;

Percentual de proteína, gordura e água;

Expressão gênica de IL-6, IL-10, IL-15 e FNDC-5 no músculo esquelético;

Expressão gênica de IL-6 e UCP-1 no TAB;

Concentração de irisina no TAB; e

Perfil lipídico.

HIPÓTESES

H0: A suplementação com CaHMB não afeta os efeitos do TR na força muscular, composição corporal e metabolismo lipídico em ratos Wistar.

H1: A suplementação com CaHMB afeta os efeitos do TR na força muscular, composição corporal e metabolismo lipídico em ratos Wistar.

3. ARTIGOS CIENTÍFICOS

3.1 Artigo 1: Revisão

Artigo submetido à Revista Chilena de Nutrición (Qualis: B2).

Data de submissão: 18 / 02 / 2020

EFFECTS OF B-HYDROXY-B-METHYLBUTYRATE AND RESISTANCE TRAINING ON BODY FAT AND SIGNALING PATHWAYS OF LIPID METABOLISM: AN UPDATE OF THE LITERATURE

EFECTOS DE B-HYDROXY-B-METHYLBUTYRATE (HMB) Y ENTRENAMIENTO DE RESISTENCIA EN GRASA CORPORAL Y CAMINOS DE SEÑALIZACIÓN DEL METABOLISMO LÍPIDO: UNA ACTUALIZACIÓN DE LA LITERATURA

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ABSTRACT

The excess body fat is a risk for cardiovascular diseases such as obesity and diabetes which compromises human health and quality of life worldwide. In this sense, nutritional supplements and exercise are related to changes of body composition and have been currently used by different populations. β-hydroxy-β-methylbutyrate (HMB) has demonstrated positive effects on body fat reduction and resistance training (RT) is thought to increase lean body mass and reduce body fat. However, the effects of combining HMB supplementation with RT related to adipose tissue metabolic activity are controversial. This review updated the signaling pathways (AMPK, PGC1- α, irisin and SIRTs) of lipid metabolism underlying the effects of the combination of HMB supplementation with RT on the body composition.
Keywords: resistance training; HMB; body composition; lipid metabolism.

RESUMEN

El exceso de grasa corporal es un problema grave para aumentar el riesgo de enfermedades cardiovasculares como la obesidad, comprometiendo la salud y la calidad de vida de la población. En este sentido, el entrenamiento de resistencia (ER) es un tipo de ejercicio físico que mejora la composición corporal al aumentar la masa corporal magra y reducir la masa corporal grasa. La ER en combinación con la nutrición (e.g. la suplementación con proteínas) es una intervención clave para mejorar el metabolismo de las grasas corporales y reducir la obesidad. Con respecto a la suplementación de proteínas, el β-hidroxi-β-metilbutirato (HMB) es un metabolito del aminoácido de cadena ramificada leucina que ha demostrado efectos positivos en la reducción de grasa corporal. Sin embargo, los efectos de combinar suplementos de HMB con ER relacionados con la actividad metabólica del tejido adiposo son controvertidos y justifican investigaciones adicionales. Este estudio analizó los efectos de los suplementos de HMB asociados con la ER en la concentración de grasa corporal y las vías de señalización del metabolismo de los lípidos.
Palabras clave: entrenamiento de resistencia; HMB; composición corporal; metabolismo de los lípidos.

INTRODUCTION

The excess of body fat, especially visceral fat, is related to the increased risk for several cardiovascular and metabolic diseases such as stroke, atherosclerosis, obesity and diabetes, which compromises the health and quality of life of individuals of different ages worldwide (1). It is estimated that the world will have more obese than malnourished children and adolescents by the year 2022 (2). Thus, the reduction of body fat and the enhancement of energy metabolism are common goals for both government agencies and the general population that seek strategies to prevent obesity and improve other physiological factors related to high quality of life.

In this context, nutritional supplements related to changes of body composition have been currently used by different populations. For instance, the β-hydroxy-β-methylbutyrate (HMB) has been used by sedentary individuals, elderly and even athletes (3-5). Moreover, epidemiological studies have shown that resistance training (RT) is a suitable type of physical exercise for the prevention of body fat accumulation and for health improvement (6,7). Therefore, the combination of a balanced diet based on proteins and amino acids with RT has been shown to optimize the reduction of body fat (8).

In this sense, the molecular signaling pathways of lipid metabolism such as AMP-activated protein kinase (AMPK), peroxisome proliferator-activated receptor gamma co-activator 1-α (PGC1-α), irisin and sirtuins (SIRTs) which are either activated or inhibited by the combination of HMB supplementation with RT has been investigated, though not fully understood. Thus, in this review we thought to update the signaling pathways (AMPK, PGC1-α, irisin and SIRTs) of lipid metabolism underlying the effects of the combination of HMB supplementation with RT on the body composition.

β -HIDROXI β –METILBUTIRATO (HMB)

The search for nutritional strategies to increase muscle mass gain and reduce body fat has been the focus of study in the last decades (8). The increase of muscle strength and improvement of body composition are attributes related to good health for different populations (9,10), such as children, adults and elderly of different physical fitness level, health or metabolic diseases.

In this context, the β-hydroxy β-methylbutyrate (HMB) is a metabolite resulting from the essential amino acid leucine that has gained notoriety due to its beneficial effects in

improving physiological and morphological parameters related to strength gain, muscle hypertrophy and body fat reduction (3-5), although there are some results in contrast (11,12).

HMB metabolism

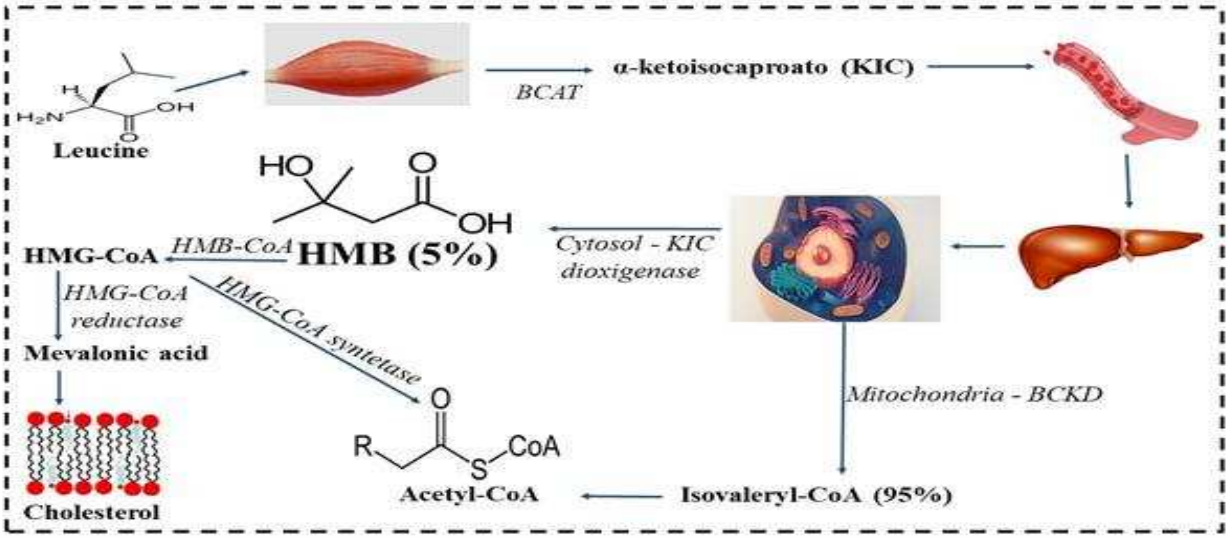
Initially, the leucine is transaminated in α-ketoisocaproate (KIC) mainly in skeletal muscles by the action of the branched chain amino acid aminotransferase enzyme (BCAT). The KIC increases rapidly in the systemic circulation and becomes readily available to the liver. In the liver, the KIC has 2 pathways: 5% is converted into HMB by the cytosol of hepatocytes, through the action of the enzyme KIC dioxigenase and 95% is converted into isovaleryl-CoA by the mitochondria of hepatocytes, through the action of the enzyme branchedchain α-keto acid dehydrogenase (BCKD) (13).

Subsequently, the HMB-CoA enzyme converts the HMB formed in the liver into β-Hydroxy β-Methylglutaryl-CoA (HMG-CoA). The HMG-CoA has two different pathways: 1st - cholesterol synthesizes, through the action of the HMG-CoA enzyme reductase that converts HMG-CoA into mevalonic acid that is directed to cholesterol biosynthesis; and 2nd - acetyl-CoA synthesis, through the action of the enzyme HMG-CoA synthetase.

Moreover, a significant amount (i.e. 14% to 29%) of the HMB formation is excreted in the urine. This percentage of metabolite loss change with specific dosages. The higher the dosage, the greater the excretion (14).

The isovaleryl-CoA pathways of HMB formation follows a series of reactions that results in acetyl-CoA synthesis, where it will be directed to the supply of energy (4,14). Figure 1 illustrates the HMB metabolism.

Figure 1. β-hydroxy-β-methylbutyrate metabolism.

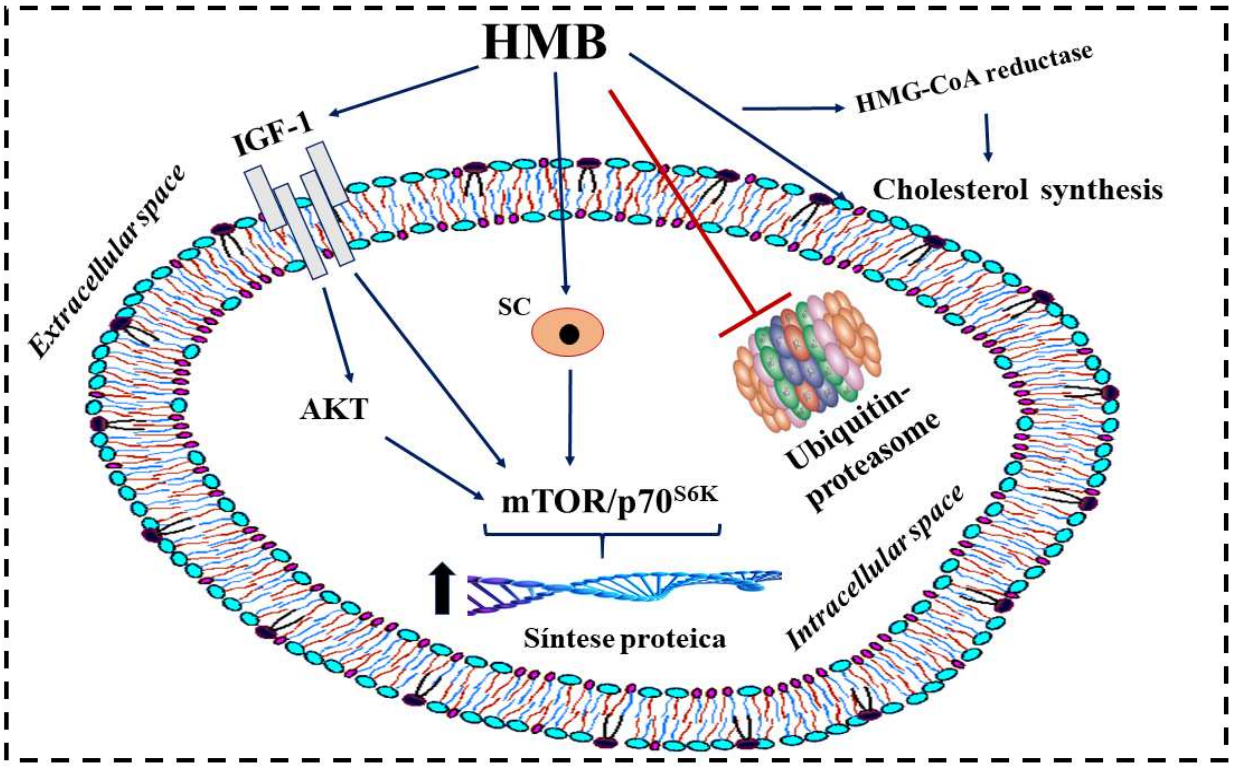


Legend: KIC, α-ketoisocaproate; HMB, β-hidroxi β-metilbutirato; BCAT, branched chain amino acid aminotransferase enzyme; HMG-CoA, β-Hidroxi β-Metilglutaril-CoA; BCKD, branchedchain α-keto acid dehydrogenase enzyme.

Mechanisms of action

The HMB acts through different mechanisms: 1st - inhibition of the action of the ubiquitin-proteolytic proteasome pathway (15); 2nd - increases in the expression of the HMG-CoA reductase enzyme accelerating the cholesterol synthesis of skeletal muscle tissue membrane (13,16); 3rd – increases in the insulin-like growth factor (IGF-1) gene transcription (17,18) which stimulates muscle growth via serine/threonine-protein kinases (AKT) phosphorylation; 4rd- increases in the proliferation of fast-twitch fiber satellite cells (19); and 5th – increases in the phosphorylation and activation of the AKT / mammalian target of rapamycin (m-TOR) / ribosome protein kinase 1 β ($p70^{S6K}$) pathway (17,20). Figure 2 illustrates the mechanisms of action of the HMB.

Figure 2. β-hydroxy-β-methylbutyrate mechanisms of action.



Legend: HMB, β-hidroxi β-metilbutirato; HMG, CoA-β-Hidroxi β-Metilglutaril-CoA; IGF-1, insulin-like growth factor; SC, satellite cells; AKT/m-TOR/p70^{S6K}, serine/threonine-protein kinases / mammalian target of rapamycin / ribosome protein kinase 1 β.

Endogenous HMB and supplementation

The liver is the main organ that produces HMB. Skeletal muscle and other tissues also produce HMB in low amounts (13). An adult individual of 70-kg synthesizes an average of 0.3-0.4 g per day of HMB (21). In this sense, to obtain the ergogenic effects of HMB in the human body, it would be necessary to consume it in concentrated amounts in the form of supplements.

The most used dosage in human studies is 3 g / day, taken immediately after exercise or in 3 doses of 1 g / day. This dosage corresponds to approximately 60 grams of leucine (3). In rats, the most found dosage in studies is 320 mg / kg of body weight, dissolved in water (5,22). In humans, this dose would be equivalent to 38 mg / kg weight / day (5).

β-hydroxy-β-methylbutyrate is commercialized on the market by capsules and powder and it is found in the form of free acid (FA) or in the form of calcium salt (Ca). In humans, supplementation with HMB-FA showed higher plasma concentrations and greater availability compared to HMB-Ca, without significant differences in the urine metabolite excretion, indicating greater retention of HMB-FA in the body (23).

RESISTANCE TRAINING

Resistance training enhances sports performance by increasing the strength, hypertrophy, resistance and oxidation of energetic substrates in skeletal muscle (7), as well as promoting health benefits by reducing body fat, plasma triglycerides, blood pressure and risk of type 2 diabetes (24,25), which contributes positively to the increase in quality of life (26). In addition, chronic RT improves the immune system, through modulation of pro-inflammatory and anti-inflammatory cytokines (27,28), thus contributing to the prevention of diseases caused by various types of microorganisms.

HMB AND RESISTANCE TRAINING

Athletes from different sports use HMB as supplement to gain lean body mass, muscle strength, and reduce fat mass (3-5,17,19) In fact, several studies have shown that HMB supplementation associated with RT for a period of time more than 3 weeks improves the body composition of adults with low to moderate physical fitness level (29), elderly (16,30,31) and trained individuals (32,33).

The study of Stout et al. (31) with elderly evaluated the effects of HMB supplementation on body fat mass for 12 weeks. After the intervention, the HMB-RT group reduced abdominal fat compared to that of the RT group only. These results suggested that HMB supplementation optimized the metabolic capacity of using fatty acids by muscle fibers. In addition, the study of Shirvani et al. (34) with elderly rats, demonstrated that supplementation with HMB-FA associated with RT for 8 weeks increased muscle strength, gene expression of the PGC1-α in skeletal muscle and plasma irisin concentration. The PGC1-α and irisin are important molecules related to the control of lipid metabolism.

The proliferator-activated receptor gamma co-activator 1α is synthetized after RT (35) activating the peroxisome proliferator-activated receptors (PPARs), nuclear respiratory factors (NRFs) 1 and 2 and estrogen-related α receptors (ERR-α)(36). These factors increase β-oxidation and mitochondrial biogenesis through increased activity of fatty acid transport proteins into the mitochondria and activation the nuclear transcription of oxidative enzymes (36). In addition, one of the important proteins that increases in adipose tissue after RT (35) activating the PGC1-α is the AMPK. The AMPK increases the enzyme carnitine palmitoyl transferase 1 (CPT-1). This enzyme is responsible for transporting fatty acids from the sarcoplasm to the mitochondria to be oxidized (37) (Figure 3).

The signaling pathways of irisin demonstrate that physical exercise promotes in the skeletal muscle an increase of PGC-1-α, which, in turn, stimulates the production of the fibronectin type III domain-containing protein 5 (FNDC-5), therefore, FNDC-5 is cleaved forming irisin. Once produced, irisin is transported through the blood to the adipose tissue stimulating thermogenesis via activation of the mitochondrial membrane uncoupling protein (UCP-1). This action increases the browning of white adipose tissue, converting it to brown adipose tissue and, consequently, increasing the white thermogenesis and oxidation of fatty acids (38,39) (Figure 3).

Considering the importance of AMPK, PGC1- α and irisin in the control of lipid metabolism (34,36,38,39) and HMB supplementation associated with RT in body fat loss (30,31,33), as shown in the figure 3, it is relevant that other studies investigate the combination of this type of intervention in the activation of metabolic pathways of adipose tissue.

Another signaling pathway of adipose tissue is the sirtuin (SIRT). Sirtuins are classes of proteins that are related to mitochondrial biogenesis and activation of enzymes involved in glucose metabolism and lipid oxidation such as PGC1- α (40) (Figure 3).

According to Vargas-Ortiz (41), SIRT-1 is activate with high-intensity interval training (HIIT), but not, with RT. Another study by the same group (42), discussed that RT did not induce a sufficient metabolic stress to activate oxidative phosphorylation, and consequently, the SIRT pathway.

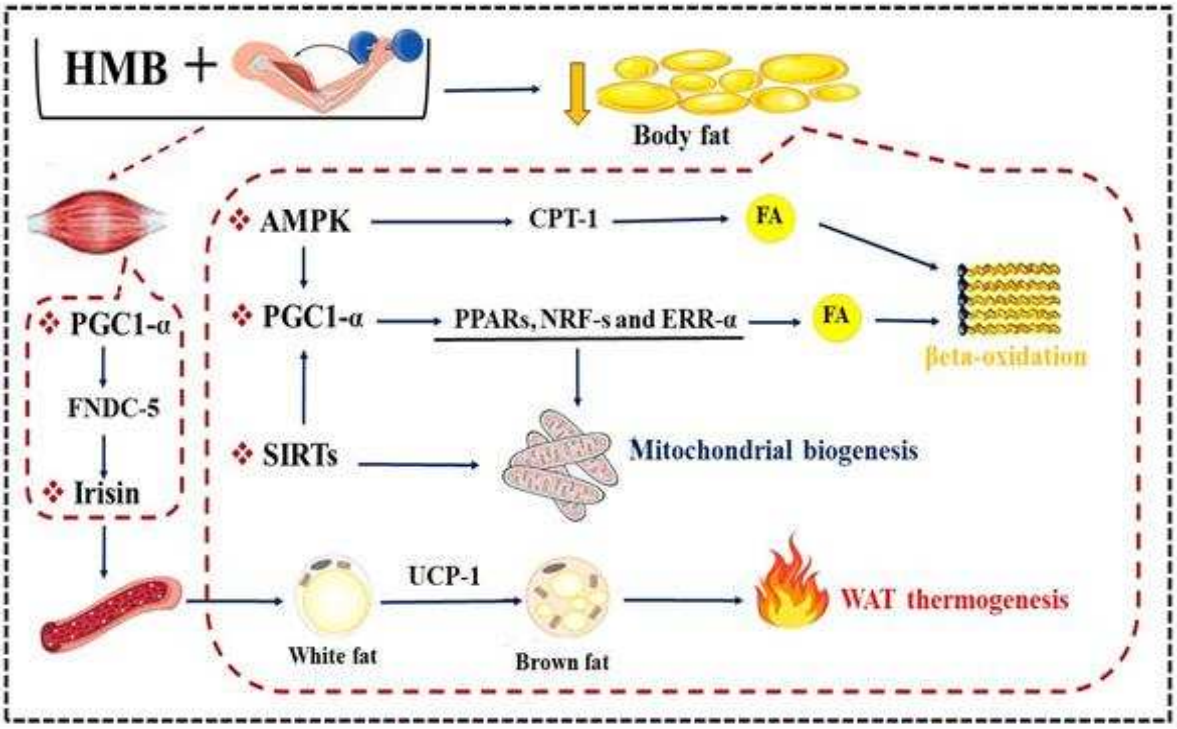
In relation to HMB, the study of Baggett et al. (43) demonstrated that HMB combined with resveratrol increased SIRT activity. Furthermore, regardless of RT, other studies demonstrated that HMB supplementation increases the transport of free fatty acids into the cell as a result of activation of PGC1-α (43,44), hormone-sensitive lipase (HSL) (45) and mitochondrial biogenesis (44).

Therefore, the cellular signaling pathways of AMPK, PGC1- α, irisin and SIRTs are determinants in the control of lipid metabolism and, consequently, weight loss, via improvement of thermogenesis and / or mitochondrial biogenesis (34,36,38-40). However, it is not known yet the magnitude of activation of these pathways by combining HMB supplementation with RT. It is possible that HMB supplementation optimizes the effects of RT on body fat reduction through activation of AMPK, PGC1- α, irisin and SIRTs, as shown in figure 3.

It is important to highlight that the reduction of body fat is important for several reasons: protection against cardiovascular and metabolic diseases caused by excess adipose

tissue (1); improvement of body composition and sports performance in several modalities (46,47); and reduction of psycho-social problems (48). In this context, HMB supplementation associated with RT emerges as an interesting intervention for the reduction of body fat, which, in turn, is an important factor of improving health and sports performance. Figure 3 summarizes the effect of HMB supplementation associated with RT on body fat and the possible signaling pathways of lipid metabolism.

Figure 3. Effects of β-hydroxy-β-methylbutyrate supplementation associated with resistance training on body fat and the possible signaling pathways of lipid metabolism.



Legend: HMB, β-hidroxi β-metilbutirato; PGC1-α, proliferator-activated receptor gamma co-activator 1α; AMPK, adenosine monophosphate activated protein kinase; SIRTs, sirtuins; FNDC-5, fibronectin type III domain-containing protein 5; UCP-1, type 1 mitochondrial uncoupling protein; PPARs, peroxisome proliferator-activated receptors, NRFs, nuclear respiratory factors 1 and 2; ERR-α, estrogen-related α receptors; CPT-1, carnitine palmitoil transferase 1; FA, fatty acids; WAT, white adipose tissue.

In relation to trained and competitive athletes, a meta-analysis study found no effect of HMB supplementation on strength and body composition in trained and competitive athletes (49). It is possible that HMB does not alter the metabolic activity of adipose tissue in these

individuals due to their high level of physiological adaptations in skeletal muscle and lipid metabolism.

The results of studies on the effectiveness of HMB associated with RT in the metabolic activity of adipose tissue should be analyzed carefully since there are variations among studies' methodologies, dosage supplement (HMB-AL or HMB-Ca), duration of intervention and populations. Thus, it is essential that other studies are carried out to address this question.

CONCLUSION

In this review we updated the signaling pathways (AMPK, PGC1- α, irisin and SIRTs) of lipid metabolism underlying the effects of the combination of HMB supplementation with RT on the body composition. Although HMB supplementation associated with RT is thought to reduce body fat and enhance lean body mass, the framework of lipid metabolism signaling pathways is not completely known which warrants further investigations, especially on AMPK, PGC1- α, irisin and SIRTs.

REFERENCES

1. World Health Organization. Global status report on non communicable diseases. Geneva: WHO, 2011.

2. Risk Factor Collaboration. Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128·9 million children, adolescents, and adults. Lancet 2016; 390(10113): 2627-2642.

3. Holeček M. Beta-hydroxy-beta-methylbutyrate supplementation and skeletal muscle in healthy and muscle-wasting conditions. Journal of cachexia, sarcopenia and muscle 2017; 8(4): 529-541.

4. Wilson GJ, Wilson JM, Manninen AH. Effects of beta-hydroxy-beta-methylbutyrate (HMB) on exercise performance and body composition across varying levels of age, sex, and training experience: A review. Nutrition & Metabolism 2008; 3: 5-1.

5. Wilson JM, Fitschen PJ, Campbell B, Wilson GJ, Zanchi N, Taylor L, et al. International Society of Sports Nutrition Position Stand: beta-hydroxy-beta-methylbutyrate (HMB). Journal of the International Society of Sports Nutrition 2013; 10: 1-6.

6. Kraemer WJ, Ratamess NA. Fundamentals of resistance training: progression and exercise prescription. Medicine and science in sports and exercise 2004; 364: 674–88.

7. American College of Sports Medicine (ACSM). Progression Models in Resistance Training for Healthy Adults. Medicine & Science in Sports & Exercise 2009; 41(3): 687–708.

8. Kerksick CM, Wilborn CD, Roberts MD, Smith-Ryan A, Kleiner SM, Jäger R, et al. ISSN exercise & sports nutrition review update: research & recommendations. Journal of the International Society of Sports Nutrition 2018; 15(1): 1-38.

9. Celis-Morales CA, Welsh P, Lyall DM, Steell L, Petermann F, Anderson J, et al. Associations of grip strength with cardiovascular, respiratory, and cancer outcomes and all cause mortality: prospective cohort study of half a million UK Biobank participants. BMJ 2018; 361: k1651.

10. Wolfe RR. The underappreciated role of muscle in health and disease. The American Journal of Clinical Nutrition 2006; 84(3): 475–82.

11. Sanchez-Martinez J, Santos-Lozano A, Garcia-Hermoso A, Sadarangani KP, Cristi-Montero C. Effects of beta-hydroxy-beta-methylbutyrate supplementation on strength and body composition in trained and competitive athletes: A meta-analysis of randomized controlled trials. Journal of Science and Medicine in Sport 2018; 21(7): 727–35.

12. Ransone J, Neighbors K, Lefavi R, Chromiak J. The effect of beta-hydroxy beta-methylbutyrate on muscular strength and body composition in collegiate football players. Journal of strength and conditioning research 2003; 17(1): 34–9.

13. Nissen SL, Abumrad NN. Nutritional role of the leucine metabolite β-hydroxy β-methylbutyrate (HMB). The Journal of Nutritional Biochemistry 1997; 8(6): 300–11.

14. Zanchi NE, Gerlinger-Romero F, Guimarães-Ferreira L, de Siqueira Filho MA, Felitti V, Lira FS, et al. HMB supplementation: clinical and athletic performance-related effects and mechanisms of action. Amino Acids 2011; 40(4): 1015–25.

15. Romero FG, Guimarães-Ferreira L, Yonamine CY, Salgueiro RB, Nunes MT. Effects of beta-hydroxy-beta-methylbutyrate (HMB) on the expression of ubiquitin ligases, protein synthesis pathways and contractile function in extensor digitorum longus (EDL) of fed and fasting rats. The Journal of Physiological Sciences 2018; 68(2): 165–74.

16. Berton L, Bano G, Carraro S, Veronese N, Pizzato S, Bolzetta F, et al. Effect of Oral Beta-Hydroxy-Beta-Methylbutyrate (HMB) Supplementation on Physical Performance in Healthy Old Women Over 65 Years: An Open Label Randomized Controlled Trial. PloS one 2015; 10(11): e0141757.

17. Albert FJ, Morente-Sánchez J, Ortega FB, Castillo MJ, Gutiérrez Á. Usefulness of B-Hydroxy-B-Methylbutyrate (HMB) Supplementation in Different Sports: an Update and Practical Implications. Nutricion hospitalaria 2015; 32(1): 20–33.

18. Gerlinger-Romero F, Guimarães-Ferreira L, Giannocco G, Nunes MT. Chronic supplementation of beta-hydroxy-beta methylbutyrate (HMβ) increases the activity of the GH/IGF-I axis and induces hyperinsulinemia in rats. Growth Hormone & IGF Research 2011;21(2): 57–62.

19. Asadi A, Arazi H, Suzuki K. Effects of β-hydroxy-β-methylbutyrate-free acid supplementation on strength, power and hormonal adaptations following resistance training. Nutrients 2017; 9(12): e1316.

20. Molfino A, Gioia G, Rossi Fanelli F, Muscaritoli M. Beta-hydroxy-beta-methylbutyrate supplementation in health and disease: A systematic review of randomized trials. Amino Acids 2013; 45(6): 1273–92.

21. Van Koevering M, Nissen S. Oxidation of leucine and alpha-ketoisocaproate to beta-hydroxy-beta-methylbutyrate in vivo. The American journal of physiology 1992; 262(1): E27-31.

22. Caperuto EC, Tomatieli RV, Colquhoun A, Seelaender MC, Costa Rosa LF. Beta-hydoxy-beta-methylbutyrate supplemen- tation affects Walker 256 tumor-bearing rats in a

time-dependent manner. Clin Nutr 2007; 26: 117–122.

23. Fuller JC, Sharp RL, Angus HF, Baier SM, Rathmacher JA. Free acid gel form of β-hydroxy-β-methylbutyrate (HMB) improves HMB clearance from plasma in human subjects compared with the calcium HMB salt. British Journal of Nutrition 2011;105(3): 367–72.

24. Kraemer WJ, Ratamess NA, French DN. Resistance training for health and performance. Current Sports Medicine Reports 2002; 1(3): 165–71.

25. Westcott WL. Resistance Training is Medicine. Current Sports Medicine Reports 2012; 11(4): 209–16.

26. Hart PD, Buck DJ. The effect of resistance training on health-related quality of life in older adults: Systematic review and meta-analysis. Health Promotion Perspectives 2019; 9(1): 1–12.

27. Della Gatta PA, Garnham AP, Peake JM, Cameron-Smith D. Effect of exercise training on skeletal muscle cytokine expression in the elderly. Brain, Behavior, and Immunity 2014; 39:80–6.

28. Kraemer WJ, Hatfield DL, Comstock BA, Fragala MS, Davitt PM, Cortis C, et al. Influence of HMB Supplementation and Resistance Training on Cytokine Responses to Resistance Exercise. Journal of the American College of Nutrition 2014; 33(4): 247–55.

29. Nissen S, Sharp R, Ray M, Rathmacher JA, Rice D, Fuller JC, et al. Effect of leucine metabolite beta-hydroxy-beta-methylbutyrate on muscle metabolism during resistance-exercise training. Journal of applied physiology 1996; 81(5): 2095–104.

30. Stout JR, Smith-Ryan AE, Fukuda DH, Kendall KL, Moon JR, Hoffman JR, et al. Effect of calcium β-hydroxy-β-methylbutyrate (CaHMB) with and without resistance training in men and women 65+yrs: a randomized, double-blind pilot trial. Experimental gerontology 2013; 48(11): 1303–10.

31. Stout JR, Fukuda DH, Kendall KL, Smith-Ryan AE, Moon JR, Hoffman JR. β-Hydroxy-β-methylbutyrate (HMB) supplementation and resistance exercise significantly reduce abdominal adiposity in healthy elderly men. Experimental gerontology 2015; 64: 33–4.

32. Wilson JM, Lowery RP, Joy JM, Andersen JC, Wilson SMC, Stout JR, et al. The effects of 12 weeks of beta-hydroxy-beta-methylbutyrate free acid supplementation on muscle mass, strength, and power in resistance-trained individuals: a randomized, double-blind, placebo-controlled study. European journal of applied physiology 2014; 114(6): 1217–27.

33. Panton LB, Rathmacher JA, Baier S, Nissen S. Nutritional supplementation of the leucine metabolite beta-hydroxy-beta-methylbutyrate (hmb) during resistance training. Nutrition 2000; 16(9): 734–9.

34. Shirvani H, Rahmati-Ahmadabad S, Broom DR, Mirnejad R. Eccentric resistance training and β-hydroxy-β-methylbutyrate free acid affects muscle PGC-1α expression and serum irisin, nesfatin-1 and resistin in rats. The Journal of Experimental Biology 2019; 222(10): jeb198424.

35. Thirupathi A, da Silva Pieri BL, Queiroz JAMP, Rodrigues MS, de Bem Silveira G, de Souza DR, et al. Strength training and aerobic exercise alter mitochondrial parameters in brown adipose tissue and equally reduce body adiposity in aged rats. Journal of Physiology and Biochemistry 2019; 75(1): 101–8.

36. Scarpulla RC. Nucleus-encoded regulators of mitochondrial function: integration of respiratory chain expression, nutrient sensing and metabolic stress. Biochimica et biophysica acta 2012; 1819(9–10): 1088–97.

37. O'Neill HM, Holloway GP, Steinberg GR. AMPK regulation of fatty acid metabolism and mitochondrial biogenesis: implications for obesity. Molecular and cellular endocrinology 2013; 366(2): 135–51.

38. Arias-Loste M, Ranchal I, Romero-Gómez M, Crespo J. Irisin, a Link among Fatty Liver Disease, Physical Inactivity and Insulin Resistance. International Journal of Molecular Sciences 2014; 15(12): 23163–78.

39. Boström P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, et al. A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature 2012; 481(7382): 463–8.

40. Chalkiadaki A, Guarente L. Sirtuins mediate mammalian metabolic responses to nutrient availability. Nature reviews Endocrinology. 2012 8(5): 287–96.

41. Vargas-Ortiz K, Pérez-Vázquez V, Macías-Cervantes MH. Exercise and sirtuins: A way to mitochondrial health in skeletal muscle. Internat. Journal of Molecular Sciences 2019; 20(11): e2717.

42. Vargas-Ortiz K, Pérez-Vázquez V, Figueroa A, Díaz FJ, Montaño-Ascencio PG, Macías-Cervantes MH. Aerobic training but no resistance training increases SIRT3 in skeletal muscle of sedentary obese male adolescents. European Journal of Sport Science 2018; 1892): 226–34.

43. Baggett B, Bruckbauer A, Zemel M. Synergistic Effects of Leucine and its Metabolites with Polyphenols on Irisin in Myotubes and Diet-induced Obese Mice. FASEB 2013; 27: 1.

44. Stancliffe RA. Role of Beta-Hydroxy-Beta-Methylbutyrate (HMB) in Leucine Stimulation of Mitochondrial Biogenesis and Fatty Acid Oxidation. Masters Theses 2012.

45. Pinheiro CHJ, Guimarães-Ferreira L, Gerlinger-Romero F, Curi R. An Overview on Beta-hydroxy-beta-methylbutyrate (HMB) Supplementation in Skeletal Muscle Function and

Sports Performance. In: Nutrition and Enhanced Sports Performance. Elsevier 2013; 455–463.

46. Fields JB, Merrigan JJ, White JB, Jones MT. Body composition variables by sport and sport-position in elite collegiate athletes. Journal of Strength and Conditioning Research 2018; 32(11): 3153–9.

47. Reale R, Burke LM, Cox GR, Slater G. Body composition of elite Olympic combat sport athletes. European Journal of Sport Science 2019; 28: 1–10.

48. Fuentes S, Brondeel R, Franco M, Sureda X, Traissac P, Cleary LK, et al. Psycho-social factors related to obesity and their associations with socioeconomic characteristics: the RECORD study. Eating and Weight Disorders 2019; 7: 1-11.

49. Sanchez-Martinez J, Santos-Lozano A, Garcia-Hermoso A, Sadarangani KP, Cristi-Montero C. Effects of beta-hydroxy-beta-methylbutyrate supplementation on strength and body composition in trained and competitive athletes: A meta-analysis of randomized controlled trials. J Sci Med Sport 2018; 21(7): 727-735.

3.2 Artigo 2: Original

Artigo aceito - Biology of Sport (Qualis: B2)

Data do aceite: 21/ 07 / 2020

β-HYDROXY β -METHYLBUTYRATE SUPPLEMENTATION BENEFITS THE EFFECTS OF RESISTANCE TRAINING ON BODY FAT REDUCTION VIA INCREASED IRISIN EXPRESSION IN WHITE ADIPOSE TISSUE

Head Title

RESISTANCE TRAINING AND HMB SUPPLEMENTATION

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AUTHORS' CONTRIBUTIONS

All authors contributed to study design, interpretation of outcomes and preparation of the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

ABSTRACT

The effects of resistance training (RT) associated with calcium β-hydroxy-β-methylbutyrate (CaHMB) supplementation on the body composition and gene expression of cytokines related to skeletal muscle hypertrophy and adipose tissue metabolism were studied in rats. Male Wistar rats were divided into four groups of 12 animals: sedentary control (SC); sedentary supplemented (SS); resistance training control (RTC) and resistance training supplemented (RTS). Rats from RTC and RTS groups were submitted to a RT program and those from SS and RTS groups received 1 mL of CaHMB (320 mg kg^{-1} day^{-1}) by gavage, for 8 weeks. We evaluated: body composition; plasma lipid profile; the gene expression of interleukin (IL)-6, IL-10, IL-15 and fibronectin type III domain-containing protein 5 (FNDC-5) in skeletal muscle, and IL-6, mitochondrial uncoupling protein 1 (UCP-1) in white adipose tissue (WAT); and the concentration of irisin in WAT. Compared to RTC alone, the combination of CaHMB with RT (RTS) further reduced abdominal circumference (5.3 %), Lee index (2.4 %), fat percentage (24.4 %), plasma VLDL-cholesterol (16.8%) and triglycerides (17%) and increased the gene expression of FNDC-5 (78.9 %) and IL-6 (47.4%) in skeletal muscle and irisin concentration (26.9 %) in WAT. Neither RT nor CaHMB affected the protein percentage and the gene expression of IL-6 and UCP-1 in WAT and IL-10, IL-15 in skeletal muscle. In conclusion, CaHMB supplementation increased the beneficial effects of RT on body fat reduction and was associated with muscular genic expression of IL-6 and FNDC-5 and irisin concentration in WAT, despite no change in protein mass and maximal strength.

Key words: resistance training, HMB, body composition, irisin, cytokine

INTRODUCTION

Optimal body composition and muscle strength are relevant for athletes and non-athletes as these attributes are associated with ideal health [1]. Resistance training (RT) in combination with protein supplementation has been shown to promote muscular hypertrophy and increase muscular strength [2].

Resistance training is known to increase muscle mass and strength and reduce body fat [3]. Muscle hypertrophy induced by RT triggers an increase in muscle protein synthesis via upregulation of anabolic signaling pathways and an attenuation in proteolysis via downregulation of catabolic signaling pathways [4]. Body fat is also reduced by RT because it increases basal metabolic rate [3,5] and thermogenesis in white adipose tissue (WAT) [5]. Skeletal muscle contraction during RT triggers the production and release of cytokines such as interleukin (IL) and irisin which exert effects both locally and in a crosstalk manner with other organs, such as, the liver and adipose tissue, to cope with muscle repair, hypertrophy and fuel metabolism [6]. For instance, the pro-inflammatory cytokine IL-6 mediates lipid oxidation [7], induces the production of anti-inflammatory interleukins during exercise (e.g. IL-10 and IL-1ra) [8] and stimulates muscle growth [9]. Interleukin-15 exerts local anabolic/anti-catabolic effects [10] and increases glucose uptake, thus inhibiting lipid deposition [10,11]. Through action of peroxisome proliferator-activated receptor gamma coactivator 1α (PGC-1α), skeletal muscle expresses fibronectin type III domain-containing protein 5 (FNDC-5), which encodes a membrane protein that is cleaved and secreted as irisin. FNDC-5 is known to drive brown-fat-like cells development in WAT, which enhances lipid metabolism/oxidation via mitochondrial uncoupling protein 1 (UCP-1) activity [12]. Additionally, higher irisin concentrations are associated with favorable lipid profile and reduction of metabolic diseases in the general population [13].

Previous studies demonstrated that RT and the metabolite of the branched-chain amino acid leucine, β-hydroxy-β-methylbutyrate (HMB), increased muscle mass and strength and reduced body fat [14-18], despite recent findings [19,20]. HMB has been shown to increase muscle mass through enhancement of protein synthesis via upregulation of mTOR signaling pathways and attenuation of proteolysis via downregulation of ubiquitin-proteasome system catabolic pathway [21,22], which is influenced by cytokines [23,24]. HMB may also stimulate thermogenesis in adipose tissue by increasing lipid oxidation and mitochondrial biogenesis through the expression of PGC-1α, UCP-3 and Irisin [25,26].

The combination of RT with HMB supplementation has shown to augment muscle mass and strength and reduce body fat [27]**.** However, there are divergent results showing no such effects [28] and the efficiency of this combination appears to be population-specific [27]. Moreover, such differing results also are reported in studies on effects of RT associated with HMB supplementation on cytokines related to skeletal muscle hypertrophy [29] and adipose tissue metabolic activity [30-32], which warrants further investigations. Therefore, this study assessed the effects of RT associated with CaHMB supplementation on the body composition and gene expression of cytokines related to skeletal muscle hypertrophy and adipose tissue metabolic activity in an animal model.

MATERIALS AND METHODS

Animals and experimental design

Three-month-old male Wistar rats were randomly divided into groups: sedentary control (SC); sedentary supplementation (SS); resistance training control (RTC); and resistance training supplemented (RTS). The animals were housed in individual cages, in a temperature-controlled room (22°C ± 2°C) with a 12/12 light/dark cycle and received water and standard commercial chow (Presence, Paulínia, SP - Brazil) *ad libitum.*

Maximum load test and resistance training program

All animals were adapted for 2 weeks and on the last day of adaptation, the maximum weight lifted [one repetition maximum (1RM)] was measured with the squat-training apparatus. The 1RM was defined as the minimum load that rats were unable to jump following electrical stimulation. The maximum load measurement was repeated on weeks 2, 4 and 6 in animals from resistance training groups in order to update the training load. After the 8th week of RT, all animals had their maximum load measured and it was used as our index of maximal strength.

Animals from resistance training groups were submitted to a RT program as described previously [33,34] which was adapted from Tamaki et al. [35]. In each training session the animals performed 4 sets of 10 repetitions (Load: 80% of 1RM) with a 90-s interval between sets. The animals performed 5 sessions/week, for 8 weeks. Animals from sedentary groups performed the same training sets without additional load.

Calcium β-hydroxy-β-methylbutyrate supplementation

Animals from SS and RTS groups were supplemented with CaHMB (Metabolic Technologies Inc., Ames, IA, USA – purity: 99.5 %). A daily dose of 320 mg/kg of body weight of CaHMB was administrated by gavage (volume: 1 mL saline) for 8 weeks, immediately prior to the RT session (08:00 a.m.) on week days and at the same time on weekends. Animals from SC and RTC groups received the same volume of saline at the same times. This dose of CaHMB was shown to increase oxidative metabolism and muscle strength [36] and is considered safe for rats [37]. CaHMB was administered prior to the RT in order to avoid any interference of exercise stress in the HMB intake.

Body weight and food intake

All animals were weighed weekly and food intake was monitored and calculated daily by diminishing the residual (dirty waste + clean waste) from the amount of food offered.

Determination of body composition

The Lee index was calculated by dividing the body weight cubic root by the naso-anal length [38]. The abdominal circumference was measured immediately anterior the hind paws [39]. These indirect measures were performed before, after the 4th and the 8th weeks of intervention, under anesthesia.

After euthanasia, the skin and viscera were separated from muscles and bones (empty carcass) and head and tail were disposed. The water percentage was evaluated using the gravimetric method by evaporation of water in an oven (Fanem, Guarulhos - SP, Brazil) at 105°C for 24 h. The water percentage was determined by the difference between the pre- and post-drying weight. The fat percentage was determined by the gravimetric process using Soxhlet equipment, with the use of petroleum ether as solvent for the 8-hour extraction, and the fat percentage was calculated. The percentage of protein was calculated by the indirect method of nitrogen determination [Protein (g) = nitrogen (g) × 6.25] and the Kjeldahl method [40]. The remaining percentage was defined as mineral residue (i.e. mineral and carbohydrate).

Sample collection

After an 8-hour fast, trained animals performed the last RT session and two hours later they were euthanized by decapitation. Immediately, the blood samples were collected from the torso in tubes with heparin (BD Vacutainer®, São Paulo, Brazil) and then centrifuged at 3400 rpm, for 15 minutes at 5°C to obtain plasma for analysis. The soleus and gastrocnemius muscles were dissected and stored at -80°C. The gastrocnemius muscle was used for the gene expression analyses of IL-6, IL-10 and IL-15, while the soleus muscle was used for the analysis of FNDC-5. A sample of the epididymal WAT were removed and stored at -80°C for the gene expression analysis of IL-6 and UCP-1 and irisin levels.

Determination of gene expression

Samples of skeletal muscle and WAT (30–50 mg) were homogenized to isolate total RNA using TRizol reagent (Invitrogen, São Paulo, SP, Brazil) following manufacturer's instruction. RNA purity (180/130 nm ratio) and concentration (ng/mL) were determined spectrophotometrically by NanoDrop 2000 (Thermo Scientific, Rockford, IL, USA), and RNA integrity was checked electrophoretically by 1% agarose gel stained with Nancy-520 (Sigma-Aldrich, Sao Paulo, SP, Brazil). Messenger RNA (mRNA) levels of IL-6, IL-10, IL-15, FNDC-5 and UCP-1 genes were assessed by quantitative real-time polymerase chain reaction (qRT-PCR). For this purpose, cDNA was synthetized from 2 µg of total RNA using oligo dT (0.5 µg), RiboLockTM RNAse inhibitor (20 U), 1mM of dNTP Mix, RevertAidTM Reverse Transcriptase (200U), totaling a solution with a final volume of 20 µl (Fermentas, Glen Burnie, MD, EUA). After cDNA synthesis, qRT-PCR for target genes and endogenous reference gene Ppia were run separately, and amplifications were performed with an ABI Prism 7500 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) by using Power SYBR Green PCR (Thermo Fisher Scientific, EUA). The amplification conditions included a denaturation step of 2 min at 95°C, followed by 40 cycles of 0.1 sec of denaturation at 95°C, 5 sec of annealing at 60°C, and 15 s of elongation at 72°C, followed by a single fluorescent measurement and finally 25 sec of final elongation. Amplification was followed by a melting curve analysis between 65 and 95°C and finally a cooling step for 1 min at 40°C. Results were expressed using the comparative cycle threshold (Ct) method as described by the manufacturer. The ΔCt values were calculated in every sample for each gene of interest as Ctgene of interest minus Cthousekeeping, using Ppia as housekeeping. The

calculation of the relative changes in the expression level of one specific gene (ΔΔCt) was performed by subtraction of the average ΔCt from the SC group to the ΔCt from each sample, and fold-change determined as 2(-ΔΔCt) [41]. The following primers were used: IL-6 (accession number: NM_012589.2) - forward TCCTACCCCAACTTCCAATGCTC and reverse TTGGATGGTCTTGGTCCTTAGCC; IL-10 (accession number: NM_012854.2) - forward TTGAACCACCCGGCATCTAC and reverse CCAAGGAGTTGCTCCCGTTA; IL-15 (accession number: XM_017601189.1) - forward GCTGTGTCAGTGTAGGTCTCC and reverse AGGAGAAAGCAGT-TCATTGCAG; FNDC-5 (accession number: NM_001270981.1) - forward AGAAGGCACAAGTCCGTGAG and reverse TGATGGAGTCGGAACCCTGA; UCP-1 (accession number: NM_012682.2) - forward GGCGATCCGGGCTTAAAGAG and reverse AGCCACCAGGGCTATTTGTG; and Ppia (accession number: NM_017101.1) - forward TGGCAAGCATGTGGTCTTTGGGAAG and reverse GGTGA TCTTCTTGCTGGTCTTGCCATTC.

Determination of irisin protein concentration

The quantification of irisin in epididymal WAT was performed using Rat Irisin enzyme-linked immunosorbent assay (ELISA) Kit - detection range: 0.78–50 ng/mL (Express Biotech International, USA), based on sandwich ELISA technology. Anti-irisin antibody was pre-coated onto 96-well plates. The biotin conjugated anti-irisin antibody was used as detection antibodies.

Determination of plasma lipid profile (mg/dL)

The plasma total cholesterol, triglycerides and high-density lipoprotein cholesterol (HDL-c) were determined by the enzymatic colorimetric method (Cobas®, c 111 analyzer, Roche, USA) using commercial kits (Bioclin, Belo Horizonte, MG, Brazil), according to the manufacturer's instructions. The low-density lipoprotein cholesterol (LDL-c) and very low-density lipoprotein (VLDL-c) were calculated by the Friedewald formula: LDL-c = total cholesterol – (HDL-c + triglycerides / 5) and VLDL-c = triglycerides / 5 [42]. The non-HDL-c was calculated by the difference between total cholesterol and HDL-c: non-HDL-c = (total cholesterol – HDL-c) [43].

Statistical analyses

The Shapiro-Wilk test was use to assess the data distribution and the Levene's test was used to assess the equality of variances. Repeated-measures ANOVA was used to identify differences in rat body weight gain, food intake, 1RM, Lee index and abdominal circumference during the experimental period. Two-way ANOVA followed by the Tukey post-hoc test was used to compare data for gene expression and protein concentration. Effect size (ES) was reported to emphasize the size of the difference among groups. The effect sizes (ES) were interpreted as partial eta squared (η2): small (ES ≥ 0.01 < 0.06); medium (ES ≥ 0.06 < 0.14); large (ES ≥ 0.14) [44]. Data are presented as means ± SD, and significance was accepted if $p \leq 0.05$. All statistical analyses were performed using SPSS 17.0 software (SPSS Inc., Chicago, Ill., USA).

Ethics

The experimental procedures were approved by the Ethics Committee for Animal Use of the Universidade Federal de Viçosa (process number 28/2016) and were conducted in accordance with the national guidelines for the care and use of animals.

RESULTS

Maximal strength

The mean weekly absolute maximal load lifted (1RM) (Fig. 1A) was significantly higher in RTC than in SC group in week 4 (1250.00 ± 52.22 vs 883.33 ± 57.74 g, $F_{3,44} = 125.76$, $p = 0.001$; ES = 0.896) and week 8 (1783.33 ± 93.74 vs 966.67 ± 88.76 g, $F_{3,44} = 284.12$, $p = 0.001$; ES = 0.951). Likewise, the relative 1RM (Fig. 1B) was higher in RTS versus SS group in the 4th week (3.14 ± 0.39 vs 2.16 ± 0.21 g, $F_{3,44} = 33.42$, $p = 0.001$; ES = 0.695) and in the 8th week (4.46 ± 0.76 vs 2.31 ± 0.39 g, $F_{3,44} = 56.87$, $p = 0.001$; ES = 0.795).

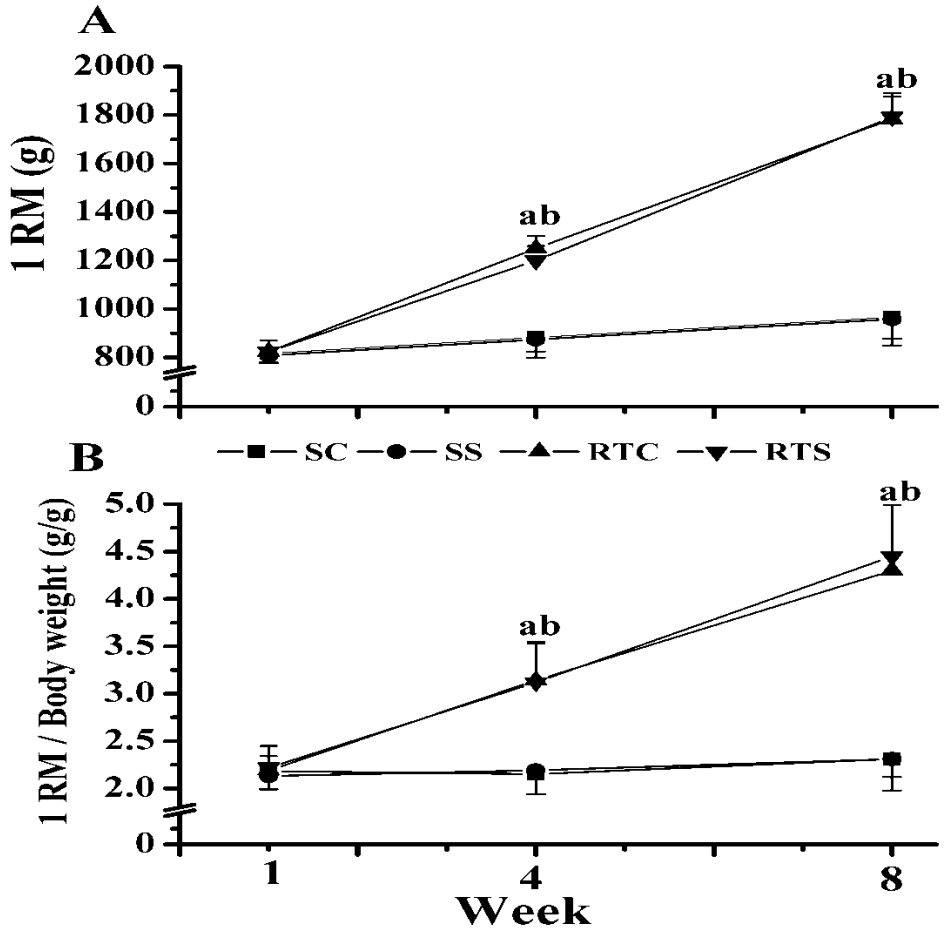

Figure 1. Maximum load lifted in one repetition. SC, sedentary control; SS, sedentary supplementation; RTC, resistance training control; RTS, resistance training supplementation. 1RM, one repetition maximum. [a]$p < 0.05$ vs. SC; [b]$p < 0.05$ vs SS.

Food intake and weight gain

The food intake (Fig. 2A) was lower in RTC than in SC group (80.25 ± 4.90 vs. 92.42 ± 8.70 g vs, F3,44 = 11.35, p = 0.001; ES = 0.436); and lower in RTS than in SS group (75.50 ± 6.91 vs. 88.25 ± 7.42 g, F3,44 = 31.11, p = 0.001; ES = 0.414) in week 8. The weight gain (Fig. 2B) was lower in RTC versus SC group (21.83 ± 6.47 vs. 35.92 ± 9.09 g, F3,44 = 5.53, p = 0.02; ES = 0.112); and lower in RTS versus SS group (17.25 ± 6.06 vs. 30.58 ± 9.74 g, F3,44 = 4.96, p = 0.03; ES = 0.101) in week 4. Additionally, weight gain was lower in RTC versus SC group (36.17 ± 8.47 vs. 56.88 ± 8.25 g, F3,44 = 6.36, p = 0.02; ES = 0.126) in week 8.

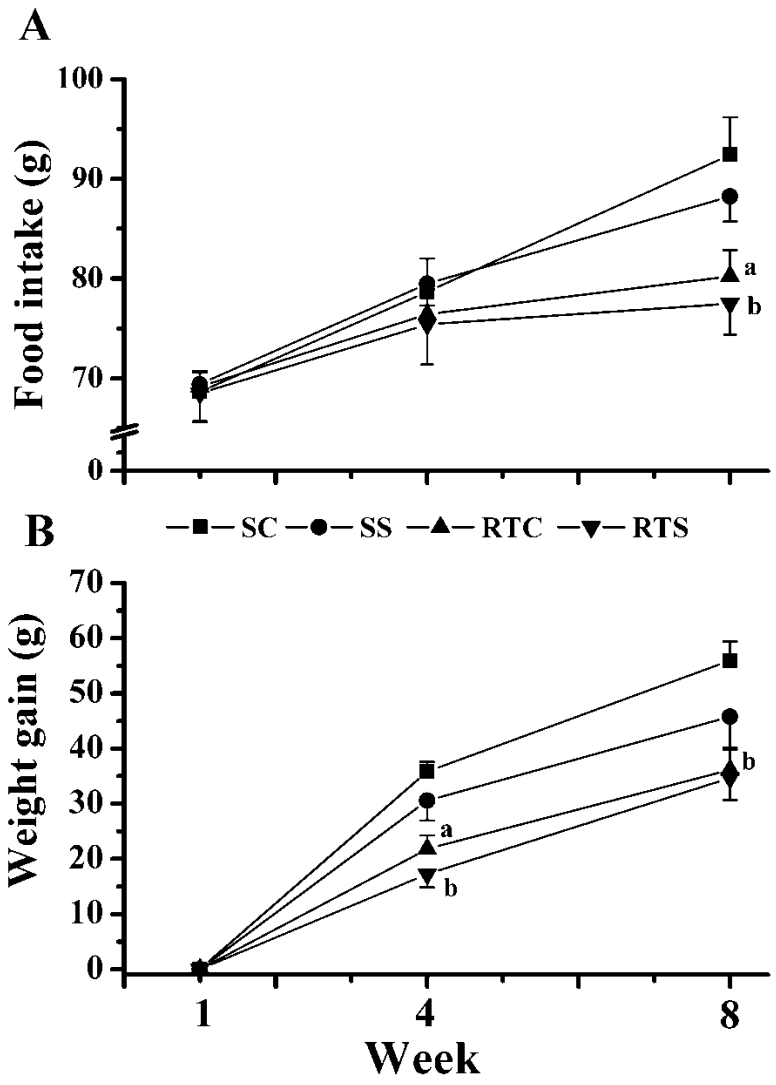

Figure 2. Food intake and weight gain. SC, sedentary control; SS, sedentary supplementation; RTC, resistance training control; RTS, resistance training supplementation**.** 2-way ANOVA followed by Tukey post hoc test: $^{a}p < 0.05$ vs. SC; $^{b}p < 0.05$ vs SS.

Body composition

The abdominal circumference was lower in RTC group than SC group (16.72 ± 0.98 vs. 18.27 ± 0.52 cm, F3,44 = 34.14, p = 0.001; ES = 0.437) in week 8 (Fig. 3A). The RTS group (15.83 ± 0.54 cm) presented lower abdominal circumference versus SS group (18.05 ± 0.44 cm) (F3,44 = 70.358, p = 0.001; ES = 0.615) and RTC group (16.71 ± 0.97 cm) (F3,44 = 11.30, p = 0.002; ES = 0.204) in week 8. The Lee index (Fig. 3B) was higher in SS group (311.62 ± 8.67) than in SC group (300.58 ± 7.42) (F3,44 = 10.62, p = 0.002; ES = 0.194) and in RTS group (301.06 ± 5.86) (F3,44 = 9.77, p = 0.003; ES = 0.182) in week 1. The RTS group (296.62 ± 7.11) presented lower Lee index compared to SS group (306.24 ± 9.88) (F3,44 =

10.14, p = 0.003; ES = 0.187) and to RTC group (305.83 ± 6.47 (F3,44 = 9.29, p = 0.004; ES = 0.174) in week 4. Similarly, the Lee index in RTS group (298.23 ± 5.24) was lower compared to SS group (309.83 ± 6.54) (F3,44 = 18.21, p = 0.001; ES = 0.293) and to RTC group (305.59 ± 7.85) (F3,44 = 7.34, p = 0.01; ES = 0.143) in week 8.

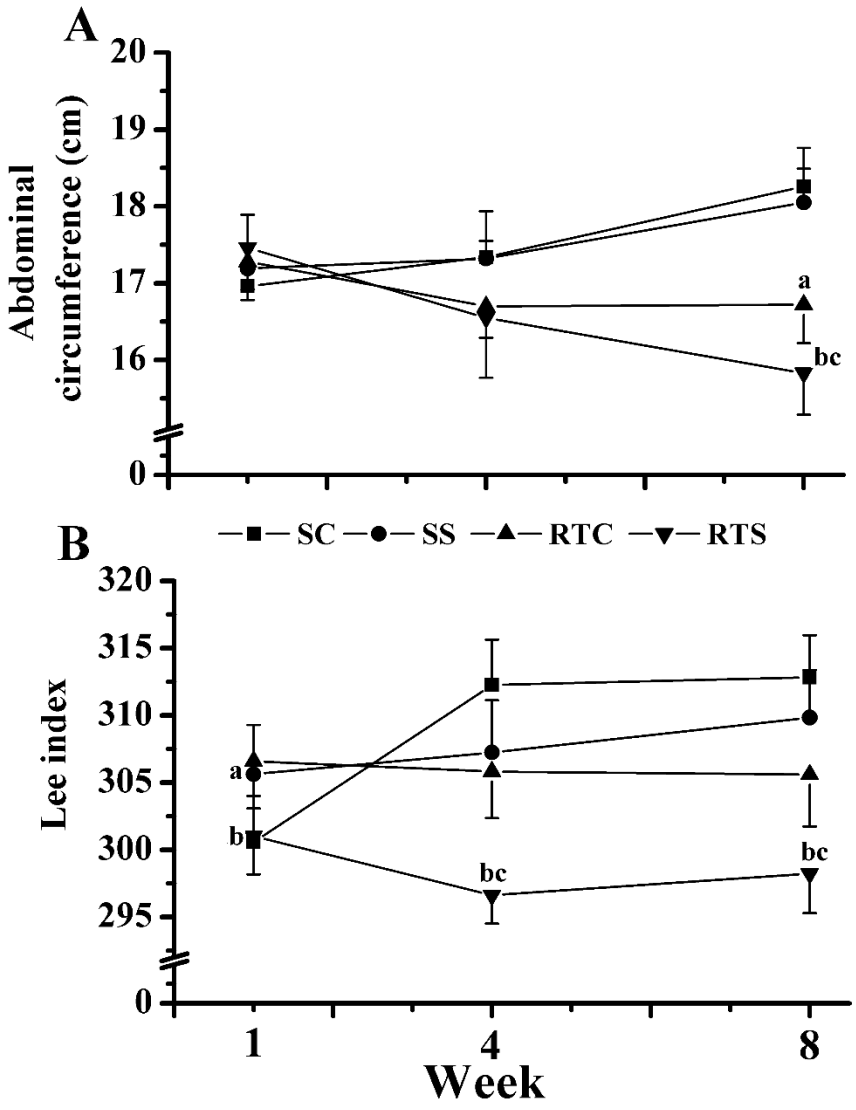

Figure 3. Abdominal circumference and Lee index. SC, sedentary control; SS, sedentary supplementation; RTC, resistance training control; RTS, resistance training supplementation. [a]$p < 0.05$ vs. SC; [b]$p < 0.05$ vs. SS; [c]$p < 0.05$ vs. RTC.

The fat percentage was lower in training groups (RTC and RTS) compared to sedentary groups (F1,44 = 40.71, p = 0.001; ES = 0.53) (Table 1). Supplemented groups (SS and RTS) had lower fat percentage than control groups (F1,44 = 6.19, p = 0.01; ES = 0.143). The RTS

group exhibited lower fat percentage versus RTC group (F1,44 = 7.83, p= 0.03; ES = 0.175). The percentage of water was higher in training groups (RTC and RTS) than sedentary groups (F1,44 = 73.73, p = 0.001; ES = 0.666). The percentage of residues in RTS group was higher than in SS group (F1,44 = 25.54, p = 0.001; ES = 0.408).

Table 1. Body composition.

	SC	SS	RTC	RTS
Fat (%)	16.44 ± 2.27	15.69 ± 3.41	12.68 ± 1.77[a]	9.58 ± 1.97[bc]
Protein (%)	17.87 ± 0.98	18.85 ± 1.95	18.60 ± 0.99	18.28 ± 1.86
Water (%)	61.38 ± 2.13	60.42 ± 2.01	63.16 ± 3.50[a]	64.38 ± 2.47[b]
Other (%)	4.31 ± 0.34	5.04 ± 0.42	5.56 ± 0.51	7.76 ± 0.36[b]

SC, sedentary control. SS, sedentary supplemented. RTC, resistance training control. RTS, resistance training supplemented. Other, residues of mineral and carbohydrate. [a]$p < 0.05$ vs. SC; [b]$p < 0.05$ vs SS. [c]$p < 0.05$ vs RTC.

Gene expression

The gene expression of IL-6 in gastrocnemius muscle (Fig. 4A) was higher in RTS group than in RTC group (1.16 ± 0.23 vs 0.61 ± 0.25, F1,44 = 14.749, p = 0.001; ES = 0.251). The expression of IL-10 (Fig. 4B) did not differ between groups (F1,44 = 0.110, p = 0.92; ES = 0.025), neither did the gene expression of IL-15 (Fig. 4C) (F1,44 = 0.67, p = 0.98; ES =0.002). The gene expression of FNDC-5 in the soleus muscle (Fig. 4D), was higher in training groups (RTC and RTS) than in sedentary groups (F1,44 = 35.29, p = 0.001; ES = 0.445). FNDC-5 gene expression was higher in supplemented groups (SS and RTS) than in control groups (F1,44 = 27.44, p = 0.001; ES = 0.38). The RTS group presented higher FNDC-5 gene expression than RTC group (3.32 ± 1.08 % vs 1.83 ± 0.36, F1,44 = 35.28, p = 0.001; ES =0.445).

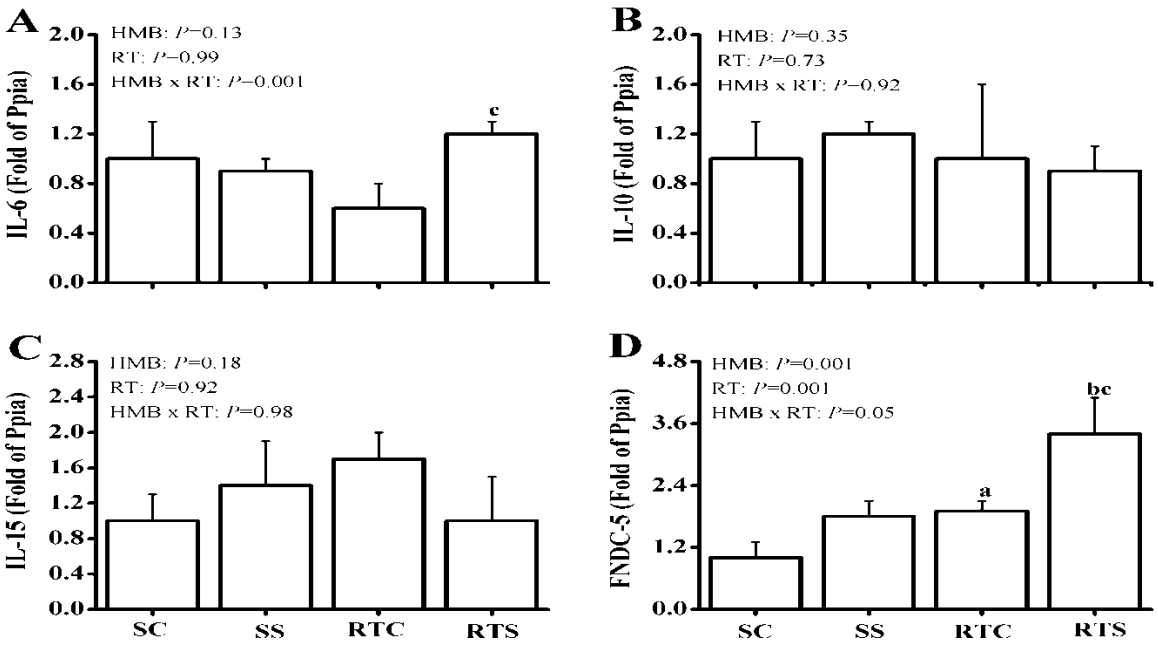

Figure 4. Gene expression of interleukin and fibronectin type III domain-containing protein 5 in skeletal muscle. SC, sedentary control; SS, sedentary supplementation; RTC, resistance training control; RTS, resistance training supplementation. IL, interleukin (gastrocnemius muscle); FNDC-5, fibronectin type III domain-containing protein 5 (soleus muscle). HMB, β-hydroxy β-methylbutyrate; RT, resistance training. $^{a}p < 0.05$ vs. SC; $^{b}p < 0.05$ vs. SS; $^{c}p < 0.05$ vs. RTC.

There was no difference between groups either in IL-6 (Fig. 5A) ($F_{1,44} = 0.03$, $p = 0.75$; ES = 0.002) or in UCP-1 gene expression (Fig. 5B) ($F_{1,44} = 0.06$, $p = 0.73$; ES = 0.002) in epididymal WAT.

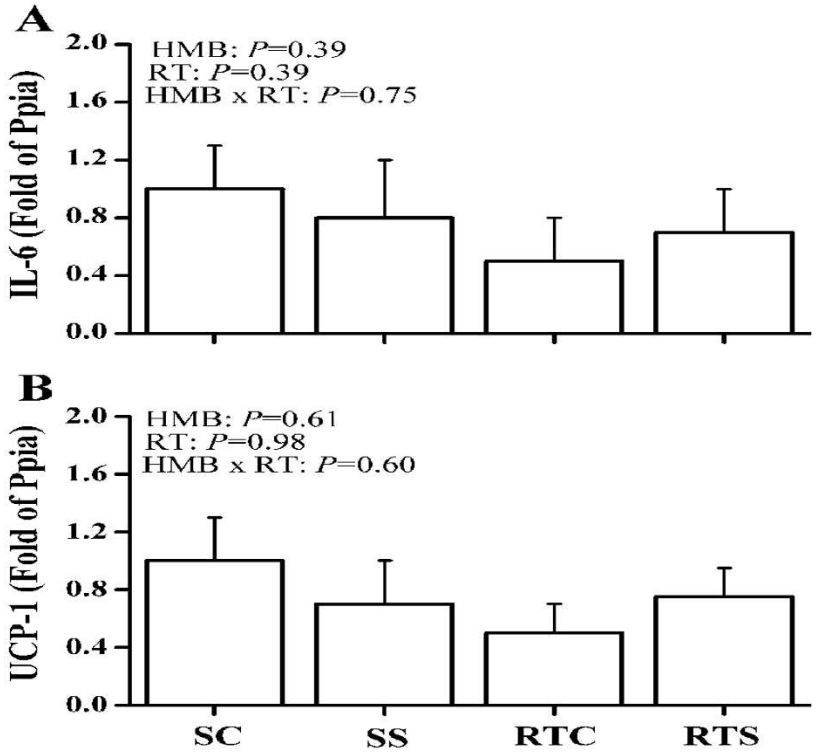

Figure 5. Gene expression of interleukin and mitochondrial uncoupling protein 1 in epididymal adipose tissue. SC, sedentary control; SS, sedentary supplementation; RTC, resistance training control; RTS, resistance training supplementation. IL, interleukin; UCP-1, mitochondrial uncoupling protein 1.

Irisin protein concentration

The concentration of irisin in epididymal WAT was higher in training groups (RTC and RTS), compared to sedentary groups ($F_{1,44} = 7.98$, $p = 0.007$; ES = 0.153) (Fig. 6). The supplemented groups (SS and RTS) had higher irisin concentration than control groups ($F_{1,44} = 7.10$, $p = 0.001$; ES = 0.139). In addition, the RTS group exhibited higher irisin concentration versus the RTC group (124.93 ± 31.33 ng/mL vs. 98.11 ± 19.36 ng/mL, $F_{1, 44} = 9.22$, $p = 0.004$; ES = 0.173).

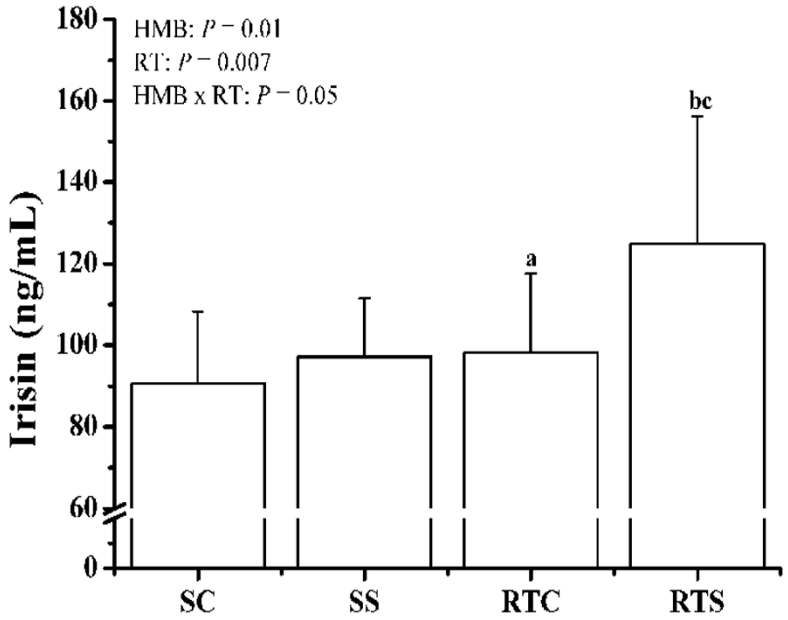

Figure 6. Concentration of irisin in epididymal adipose tissue. SC, sedentary control; SS, sedentary supplementation; RTC, resistance training control; RTS, resistance training supplementation. [a]$p < 0.05$ vs. SC; [b]$p < 0.05$ vs. SS; [c]$p < 0.05$ vs. RTC.

Plasma lipid profile

Plasma concentration of VLDL-c was lower in training groups (RTC and RTS), compared to sedentary groups (F1,44 = 130.72, p = 0.001; ES = 0.748) (Table 2). The supplemented groups (SS and RTS) had lower plasma VLDL-c concentration than control groups (F1,44 = 7.99, p = 0.007; ES = 0.154). Plasma non-HDL-c concentration was lower in training groups (RTC and RTS) than in sedentary groups (F1,44 = 32.98, p = 0.001; ES = 0.428). Plasma concentration of triglycerides was lower in training groups (RTC and RTS), compared to sedentary groups (F1,44 = 130.72, p = 0.001; ES = 0.748). The supplemented groups (SS and RTS) exhibited lower plasma triglycerides concentration than control groups (F1,44 = 7.99, p = 0.007; ES = 0.154). Plasma total cholesterol was lower in training groups (RTC and RTS) than in sedentary groups (F1,44 = 81.44, p = 0.001; ES = 0.649).

Table 2. Plasma lipid profile.

	SC	SS	RTC	RTS
LDL-c (mg/dL)	38.88 ± 8.41	38.50 ± 7.09	36.00 ± 4.20	37.18 ± 6.36
HDL-c (mg/dL)	45.25 ± 3.98	44.91 ± 5.28	43.83 ± 4.21	44.00 ± 3.90
VLDL-c (mg/dL)	25.00 ± 3.68	24.00 ± 1.66	17.80 ± 2.68[a]	14.80 ± 1.65[bc]
Non-HDL-c (mg/dL)	63.90 ± 7.55	62.50 ± 5.74	53.80 ± 4.43[a]	52.00 ± 6.67[b]
Triglycerides (mg/dL)	125.16 ± 18.37	120.00 ± 8.31	89.16 ± 11.89[a]	74.08 ± 8.22[bc]
Total cholesterol (mg/dL)	109.16 ± 5.52	107.41 ± 3.80	97.66 ± 3.33[a]	96.00 ± 4.60[b]

LDL-c, low-density lipoprotein cholesterol. HDL-c, high-density lipoprotein cholesterol. VLDL-c very low-density lipoprotein cholesterol SC, sedentary control. SS, sedentary supplemented. RTC, resistance training control. RTS, resistance training supplemented. [a]$p < 0.05$ vs SC; [b]$p < 0.05$ vs SS; [c]$p < 0.05$ vs RTC.

DISCUSSION

The present study demonstrated that RT improved body composition by reducing the Lee index, abdominal circumference and body fat, with no change in mass protein. Moreover, despite no isolated effect of CaHMB supplementation on body composition, combining CaHMB with RT amplified the effects of RT on body composition. It is important to consider here trained animals experienced similar food intake and weight gain over the experimental period. Thus, our results suggest that CaHMB supplementation magnifies the effect of RT on body fat reduction. It is noteworthy that RT animals presented lower food intake and hence weight gain than sedentary ones. It is conceivable that RT has diminished the animals' appetite by affecting hormones such as acylated ghrelin, leptin and peptide tyrosine tyrosine (PYY) [45].

We further assessed the synergistic effect of RT and CaHMB on cytokines related to fat metabolism in skeletal muscle and adipose tissue. It is important to point out that cytokine expression has been reported to be different between gastrocnemius and soleus muscles [46]. Furthermore, the secretion of FNDC-5 is around 40 % higher in slow-oxidative fiber-type muscle (i.e. soleus) than in fast-glycolytic fiber-type muscle (i.e. gastrocnemius) [47]. Even though the combination of RT with CaHMB supplementation reduced body fat, gene expression of IL-10 and IL-15 in gastrocnemius muscle, and of IL-6 and UCP-1 in WAT was not altered by treatments. However, the combined treatments increased the gene expression of IL-6 in gastrocnemius, FNDC-5 in soleus muscles, and irisin concentration in WAT. RT has

been reported to increase FNDC-5, irisin and UCP-1 [5,48], and HMB has been suggested to increase lipid oxidation in adipose tissue via increase in the gene and protein expression of hormone-sensitive lipase [49], mitochondrial biogenesis [25] and activation of the PGC-1α [31,32]. Additionally, HMB associated with polyphenols was suggested to activate sirtuin signaling and FNDC-5/irisin pathway, resulting in significant increases in fatty acid oxidation in adipocytes and myotubes [26].

In this context, the results of this study indicate that CaHMB supplementation amplifies the effect of RT on body fat reduction by activating FNDC-5/irisin pathway related thermogenesis in WAT. Although eccentric RT associated with HMB free acid increased the serum irisin levels in rats [32], our study is the first to demonstrate reduction in body fat in response to RT associated with CaHMB linked to activation of the FNDC-5/irisin pathway.

Our data showed no increase in UCP-1 gene expression in WAT. Although Bostrom et al. [50] reported that irisin reduced body fat via increasing the expression of UCP-1 in adipose tissue, Norheim et al. [51] demonstrated that UCP-1 mRNA did not correlate with gene expression of FNDC-5 in skeletal muscle, adipose tissue and serum irisin levels in response to endurance and resistance exercise training. In this sense, it is conceivable that irisin may act on WAT by increasing energy expenditure, fatty acids oxidation and thermogenesis through other mechanisms beyond UCP-1 activity. Indeed, we observed increased FNDC-5 gene expression in soleus muscle and irisin expression in WAT along with the reduction in body fat. Other studies have reported effects of irisin on body fat reduction through other mechanisms, such as improved glucose tolerance and lipid oxidation via phophorylation of AMP-activated protein kinase (AMPK) and acetyl-CoA-carboxylase [52,53]. Therefore, our results suggest an alternative effect of CaHMB supplementation on WAT metabolism.

The RT model used here augmented the maximal strength in trained animals, independent of CaHMB. Such increase in strength observed here is probably due to neuromuscular adaptations to the exercise [54,55], inasmuch as no change in protein was observed in these animals as a result of RT. Aligned with such absence of effect on protein mass, our results also showed no effect of treatments on the gene expression of IL-10 and IL-15 in gastrocnemius muscle, although the treatments increased the gene expression of IL-6.

Finally, alongside the reduction in body fat, the applied RT reduced the plasma concentrations of VLDL-c, non-HDL-c, triglycerides and total cholesterol. Like in body fat, the combination of CaHMB with RT amplified the effects of RT on plasma lipid profile, since VLDL-c and triglycerides concentrations were ~ 17 % greater in RTS than in RTC group. RT is reported to reduce serum triglycerides and total cholesterol [56,57], mainly due to

stimulation of lipid metabolism via reduction of free fatty acid synthesis and increase in lipid oxidation [5] in the liver. The CaHMB supplementation also was efficient in reducing serum triglycerides in resistance trained individuals [58].

This study has a limitation. We evaluated the gene expression of cytokines and irisin concentration only in specific tissues with high activities of these cytokines. We believe that evaluation of IL-6, IL-10, IL-15, FNDC-5 and irisin in different muscles and adipose tissues could generate more on this issue. Despite that, the findings presented here are of clinical relevance and have practical implications since it reinforces the usefulness of combining CaHMB supplementation and RT to promote attributes associated with good health.

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CONCLUSIONS

The supplementation with CaHMB improved the benefits of RT on reducing body fat and was associated with increased muscular genic expression of IL-6 and FNDC-5, and irisin concentration in WAT, despite no change in body protein and strength.

REFERENCES

1. Celis-Morales CA, Welsh P, Lyall DM, Steell L, Petermann F, Anderson J, et al. Associations of grip strength with cardiovascular, respiratory, and cancer outcomes and all cause mortality: prospective cohort study of half a million UK Biobank participants. BMJ. 2018; 361:k1651.
2. Morton RW, Murphy KT, McKellar SR, Schoenfeld BJ, Henselmans M, Helms E, et al. A systematic review, meta-analysis and meta-regression of the effect of protein supplementation on resistance training-induced gains in muscle mass and strength in healthy adults. Br J Sports Med. 2018;52(6):376–84.
3. ACSM. Progression Models in Resistance Training for Healthy Adults. ACSM - American College of Sports Medicine. Med Sci Sports Exerc. 2009; 41(3): 687–708.
4. Egerman MA, Glass DJ. Signaling pathways controlling skeletal muscle mass. Crit Rev Biochem Mol Biol. 2014; 49(1):59–68.
5. Reisi J, Ghaedi K, Rajabi H, Marandi SM. Can resistance exercise alter irisin levels and expression profiles of FNDC5 and UCP1 in rats? Asian J Sports Med. 2016; 7(4):35205.
6. Pedersen BK. Muscles and their myokines. J Exp Biol. 2011; 214:337–46.
7. Van Hall G, Steensberg A, Sacchetti M, Fischer C, Keller C, Schjerling P, et al. Interleukin-6 stimulates lipolysis and fat oxidation in humans. J Clin Endocrinol Metab. 2003; 88(7):3005–10.
8. Steensberg A, Fischer CP, Keller C, Møller K, Pedersen BK. IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. Am J Physiol Endocrinol Metab. 2003; 285(2):433–7.
9. Serrano AL, Baeza-Raja B, Perdiguero E, Jardí M, Muñoz-Cánoves P. Interleukin-6 is an essential regulator of satellite cell-mediated skeletal muscle hypertrophy. Cell Metab. 2008; 7(1):33–44.
10. Carbó N, López-Soriano J, Costelli P, Alvarez B, Busquets S, Baccino FM, et al. Interleukin-15 mediates reciprocal regulation of adipose and muscle mass: a potential role in body weight control. Biochim Biophys Acta. 2001; 1526(1):17–24.
11. Quinn LS, Anderson BG, Strait-Bodey L, Stroud AM, Argiles JM. Oversecretion of interleukin-15 from skeletal muscle reduces adiposity. Am J Physiol Endocrinol Metab. 2008; 296(1):191–202.
12. Boström P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, et al. A PGC1-α-

dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature. 2012; 481(7382):463–8.

13. Oelmann S, Nauck M, Völzke H, Bahls M, Friedrich N. Circulating Irisin Concentrations Are Associated with a Favourable Lipid Profile in the General Population. Plos One. 2016; 11(4):e0154319. https://doi.org/10.1371/journal.pone.0154319.
14. Wilson JM, Fitschen PPJ, Campbell B, Wilson GJ, Zanchi N, Taylor L, et al. International society of sports nutrition position stand: beta-hydroxy-beta-methylbutyrate (HMB). J Int Soc Sports Nutr. 2013; 10(1):1-6.
15. Wilson JJM, Lowery RP, Joy JM, Andersen JC, Wilson SMC, Jeffrey R Stout JR et al. The effects of 12 weeks of beta-hydroxy-beta-methylbutyrate free acid supplementation on muscle mass, strength, and power in resistance trained individuals: a randomized, double-blind, placebo-controlled study. Eur. J Appl. Physiol. 2014; 114(6):1217-27.
16. Nissen S, Sharp R, Ray M, Rathmacher JA, Rice D, Fuller Jr JC, et al. Effect of leucine metabolite b-hydroxy b-methylbutyrate on muscle metabolism during resistance-exercise training. J Appl Physiol. 1996; 81(5):2095-2104.
17. Vukovich MD, Stubbs NB, Bohlken RM. Body composition in 70-year old adults responds to dietary b-hydroxy-b-methylbutyrate (HMB) similar to that of young adults. J Nutr. 2001; 131(7):2049-52.
18. Stout JR, Fukuda DH, Kendall KL, Smith-Ryan AE, Moon JR, Hoffman JR. Beta-Hydroxy-beta-methylbutyrate (HMB) supplementation and resistance exercise significantly reduce abdominal adiposity in healthy elderly men. Exp Gerontol. 2015; 64: 33-4.
19. Teixeira FJ, Matias CN, Monteiro CP, Valamatos MJ, Reis JF, Tavares F. Leucine metabolites do not enhance training-induced performance or muscle thickness. Med Sci Sports Exerc. 2018; 51(1):56-64.
20. Jakubowski JS, Wong EPT, Nunes EA, Noguchi KS, Vandeweerd JK, Murphy KT, et al. Equivalent hypertrophy and strength gains in HMB- or leucine-supplemented men. Med Sci Sports Exerc. 2018; 51(1):65-75.
21. Eley H L, Russell ST, Baxter JH, Mukerji P, Tisdale MJ. Signaling pathways initiated by b-hydroxy-b-methylbutyrate to attenuate the depression of protein synthesis in skeletal muscle in response to cachectic stimuli. Am J Physiol Endocrinol Metab. 2007; 293(4):E923-31.

22. Smith HJ, Mukerji P, Tisdale MJ. Attenuation of proteasome-induced proteolysis in skeletal muscle by b-hydroxy-b-methylbutyrate in cancer-induced muscle loss. Cancer Res. 2005; 65(1):277-283.

23. Eley H L, Russell ST, Tisdale MJ. Attenuation of depression of muscle protein synthesis induced by lipopolysaccharide, tumor necrosis factor and angiotensin II by b-hydroxy-b-methylbutyrate. Am J Physiol Endocrinol Metab. 2008; 295:E1409-16.

24. Eley HL, Russell ST, Tisdale MJ. Mechanism of attenuation of muscle protein degradation induced by tumor necrosis factor-alpha and angiotensin II by beta-hydroxy-beta-methylbutyrate. Am J Physiol Endocrinol Metab. 2008; 295(6): E1417-26.

25. Stancliffe RA. Role of Beta-Hydroxy-Beta-Methylbutyrate (HMB) in Leucine Stimulation of Mitochondrial Biogenesis and Fatty Acid Oxidation. Masters Theses. 2012.

26. Baggett B, Bruckbauer A, Zemel M. Synergistic Effects of Leucine and its Metabolites with Polyphenols on Irisin in Myotubes and Diet-induced Obese Mice. FASEB J. 2013;27(1).

27. Wilson JJM, Fitschen PPJ, Campbell B, Wilson GJ, Zanchi N, Taylor L, et al. International society of sports nutrition position stand: beta-hydroxy-beta-methylbutyrate (HMB). J Int Soc Sports Nutr. 2013; 10(1):1-6.

28. Thomson JS, Watson PE, Rowlands DS. Effects of nine weeks of beta-hydroxy-beta-methylbutyrate supplementation on strength and body composition in resistance trained men. J Strength Cond Res. 2009; 23(3):827–35.

29. Silva VR, Belozo FL, Micheletti TO, Conrado M, Stout JR, Pimentel GD, et al. B-Hydroxy-B-Methylbutyrate Free acid supplementation may improve recovery and muscle adaptations after resistance training: a systematic review. Nutr Res. 2017; 45:1–9.

30. Portal S, Zadik Z, Rabinowitz J, Pilz-Burstein R, Adler-Portal D, Meckel Y, et al. The effect of HMB supplementation on body composition, fitness, hormonal and inflammatory mediators in elite adolescent volleyball players: a prospective randomized, double-blind, placebo-controlled study. Eur J Appl Physiol. 2011; 111(9):2261–9.

31. He X, Duan Y, Yao K, Li F, Hou Y, Wu G, et al. β-Hydroxy-β-methylbutyrate, mitochondrial biogenesis, and skeletal muscle health. Amino Acids. 2016; 48(3):653–64.

32. Shirvani H, Rahmati-Ahmadabad S, Broom DR, Mirnejad R. Eccentric resistance training and β-hydroxy-β-methylbutyrate free acid affects muscle PGC-1α expression and serum irisin, nesfatin-1 and resistin in rats. J Exp Bio. 2019;222(10):198424.
33. Melo S, Barauna V, Júnior M, Bozi L, Drummond L, Natali A, et al. Resistance training regulates cardiac function through modulation of miRNA-214. Int J Mol Sci. 2015; 16(12):6855–67.
34. Rios L, Del RJ, Silva Da KA, Rodrigues AC, Soares PNP, Gomes TNP, et al. Enhanced femoral neck strength in response to weightlifting exercise training in maturing male rats. ISMJ. 2013;14(3):155–67. https://hdl.handle.net/10520/EJC142366
35. Tamaki T, Uchiyama S, Nakano S. A weight-lifting exercise model for inducing hypertrophy in the hindlimb muscles of rats. Med Sci Sports Exerc. 1992; 24(8):881–6.
36. Pinheiro CH da J, Gerlinger-Romero F, Guimarães-Ferreira L, de Souza-Jr AL, Vitzel KF, Nachbar RT, et al. Metabolic and functional effects of beta-hydroxy-beta-methylbutyrate (HMB) supplementation in skeletal muscle. Eur J Appl Physiol. 2012; 112(7):2531–7.
37. Baxter JH, Carlos JL, Thurmond J, Rehani RN, Bultman J, Frost D. Dietary toxicity of calcium β-hydroxy-β-methyl butyrate (CaHMB). Food Chem Toxicol. 2005; 43(12):1731–41.
38. Lee MO. Determination of the surface area of the white rat with application to the expression of metabolic results. Am J Physiol. 1929; 89(1):24–33.
39. Novelli ELB, Diniz YS, Galhardi CM, Ebaid GMX, Rodrigues HG, Mani F, et al. Anthropometrical parameters and markers of obesity in rats. Lab Anim. 2007; 41(1):111–9.
40. AOAC (1998) Official Method of Analysis. 15th Edition, Association of Official Analytical Chemists, Washington DC.
41. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2 -DDCt method. Methods. 2001; 25:402–408.
42. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18(6):499–502.
43. Frost PH, Havel RJ. Rationale for use of non-high-density lipoprotein cholesterol rather than low-density lipoprotein cholesterol as a tool for lipoprotein cholesterol screening and assessment of risk and therapy. Am J Cardiol. 1998; 81(4A):26B-31B.
44. Cohen J. Statistical power analysis for the behavioural sciences. 2nd ed. Hillsdale (NJ):

Lawrence Erlbaum Associates; 1988.

45. Larsen P, Marino F, Melehan K, Guelfi KJ, Duffield R, Skein M. High-intensity interval exercise induces greater acute changes in sleep, appetite-related hormones, and free-living energy intake than does moderate-intensity continuous exercise. Appl Physiol Nutr Metab. 2019; 44(5):557–66.
46. Tominaga T, Ma S, Saitou K, Suzuki K. Glucose Ingestion Inhibits Endurance Exercise-Induced IL-6 Producing Macrophage Infiltration in Mice Muscle. Nutrients. 2019; 11(7): 149.
47. Roca-Rivada A, Castelao C, Senin LL, Landrove MO, Baltar J, Belén Crujeiras A, et al. FNDC5/irisin is not only a myokine but also an adipokine. PLoS One. 2013; 8(4): e60563.
48. Reisi J, Rajabi H, Ghaedi K, Seyed-Mohammad M, Mohammad-Reza D. Effect of Acute Resistance Training on Plasma Irisin Protein Level and Expression of Muscle FNDC5 and Adipose Tissue UCP1 Genes in Male Rats. IUMS. 2013;31256:1657–66.
49. Pinheiro CHJ, Guimarães-Ferreira L, Gerlinger-Romero F, Curi R. An Overview on Beta-hydroxy-beta-methylbutyrate (HMB) Supplementation in Skeletal Muscle Function and Sports Performance. In: Nutrition and Enhanced Sports Performance. Elsevier; 2013; 1(47): 455–63. https://doi.org/10.1016/B978-0-12-396454-0.00047-3.
50. Boström P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, et al. A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature. 2012; 481(7382):463–8.
51. Norheim F, Langleite TM, Hjorth M, Holen T, Kielland A, Stadheim HK, et al. The effects of acute and chronic exercise on PGC-1α, irisin and browning of subcutaneous adipose tissue in humans. FEBS J. 2014; 281(3):739–49.
52. Xin C, Liu J, Zhang J, Zhu D, Wang H, Xiong L, et al. Irisin improves fatty acid oxidation and glucose utilization in type 2 diabetes by regulating the AMPK signaling pathway. Int J Obes. 2016; 40(3):443–51.
53. So WY, Leung PS. Irisin ameliorates hepatic glucose/lipid metabolism and enhances cell survival in insulin-resistant human HepG2 cells through adenosine monophosphate-activated protein kinase signaling. Int J Biochem Cell Biol. 2016; 78:237–47.
54. Duncan ND, Williams DA, Lynch GS. Adaptations in rat skeletal muscle following long-term resistance exercise training. Eur J Appl Physiol. 1998; 77(4):372–8.
55. Kraemer WJ, Fleck SJ, Evans WJ. Strength and power training: physiological

mechanisms of adaptation. Exerc Sport Sci Rev. 1996; 24:363–97.

56. Kelley GA, Kelley KS. Impact of progressive resistance training on lipids and lipoproteins in adults: A meta-analysis of randomized controlled trials. Prev Med. 2009; 48(1):9–19.

57. Elliott KJ, Sale C, Cable NT. Effects of resistance training and detraining on muscle strength and blood lipid profiles in postmenopausal women. Br J Sports Med. 2002; 36(5):340–4.

58. Arazi H, Taat B, Suzuki K. A Review of the effects of leucine metabolite (β-Hydroxy-β-methylbutyrate) supplementation and resistance training on inflammatory markers: A new approach to oxidative stress. Antioxidants. 2018; 7(10):148.

4. LIMITAÇÕES DO ESTUDO

Esse estudo com modelo animal forneceu evidências sobre a eficácia da combinação do TR com a suplementação com CaHMB na redução da gordura corporal, melhora do perfil lipídico e sobre o envolvimento de uma importante via de sinalização celular relacionada ao metabolismo lipídico. Assim, estes resultados possuem relevância clínica e implicações práticas, pois apontam benefícios de combinar TR com suplementação com CaHMB em prol de atributos relacionados à saúde como redução da gordura corporal e melhora do perfil lipídico.

Apesar da relevância, os achados deste estudo devem ser analisados com cautela, pois existem limitações: primeiro, foi avaliada expressão gênica de citocinas e concentração de irisina apenas em tecidos específicos com alta atividade destas moléculas. Possivelmente, a avaliação de todas as citocinas (IL-6, IL-10, IL-15, FNDC-5 e irisina) em diferentes tipos de fibras musculares (tipo I, tipo IIa e tipo IIb) e porções do tecido adiposo (subcutâneo, inguinal e retroperitonial) geraria mais informações sobre seus efeitos sistêmicos nos animais desse estudo; e segundo, as amostras para estas análises foram coletadas logo após uma sessão de TR. Os tempos para as citocinas analisadas alcançarem o pico de concentração no músculo e no TAB são diferentes. Dessa forma, outras informações importantes poderiam ser obtidas com coletas de amostras em tempos distintos após a sessão de TR.

5. CONCLUSÕES GERAIS

Com base nos resultados da revisão de literatura, as vias de sinalizações celulares da AMPK, do PGC1- α, da FNDC-5/irisina e das sirtuínas são determinantes no controle do metabolismo lipídico por estarem relacionadas ao aumento da termogênese do tecido adiposo e/ou biogênese mitocondrial e, consequentemente, do emagrecimento e de melhoras de parâmetros de saúde. Todavia, apesar das evidências que a suplementação com CaHMB associada ao TR pode reduzir gordura corporal e aumentar massa corporal magra, as vias de sinalização do metabolismo lipídico envolvidas ainda não estão bem esclarecidas, o que estimula novas investigações, especialmente sobre AMPK, PGC1- α, irisina e sirtuínas.

De acordo com os resultados do estudo original, o TR aumentou a força muscular, melhorou o perfil lipídico e a composição corporal ao reduzir a gordura corporal, com possível envolvimento da via FNDC-5/irisina no TAB, mas não alterou a massa magra. A suplementação com CaHMB, quando associada ao TR, potencializou os benefícios do TR sobre o perfil lipídico, a composição corporal e o metabolismo do TAB, em associação com aumento da expressão gênica de IL-6 e FNDC-5 muscular e da concentração de irisina no TAB, apesar de não ter afetado a massa magra e a força muscular. Desta forma, os resultados confirmaram parcialmente a hipótese do estudo.

6. APÊNDICES

Análise de esteatose hepática

Após eutanásia, foram coletados fragmentos do fígado, que foram fixados em formalina a 10% por 24 horas. Em seguida, os fragmentos foram desidratados em etanol, clarificados em xilol e embebidos em parafina. Os blocos foram seccionados transversalmente em cortes histológicos de 4 µm de espessura e posteriormente corados com hematoxilina-eosina (H & E) e montados em lâminas de histologia. Para evitar análises repetidas da mesma área histológica, as seções foram avaliadas em semi-séries, usando uma de cada 10 secções. As lâminas foram visualizadas e as imagens capturadas usando um microscópio de luz (Olympus AX-70, Tóquio, Japão) conectado a uma câmera digital (Olympus Q Color-3, Tóquio, Japão).

As análises semiquantitativas das esteatoses micro e macrovesiculares, além do grau de inflamação verificado pelas células de Kupffer, foram realizadas com a contagem dos quadrantes (grade com 266 interseções) em que houve presença desses tipos de esteatoses utilizando-se o software Image-Pro-Plus 4.5 (Media Cybernetics, Silver Spring, MD, USA). Foram usadas 12 imagens aleatórias de cada animal. Os resultados foram interpretados de acordo com os critérios estabelecidos por Brunt et al. (1). Os graus de esteatose vesicular foram categorizados como: 0(ausente) 1; (< 10%); 2(10-33%); 3 (33-66%); e (4 > 66%).

Os resultados são apresentados na Figura 1. Não houve diferença estatística ($p > 0.05$) entre os grupos para o grau de esteatose hepática. Aproximadamente 91.2% dos grupos apresentaram grau 0 (SC - 81.7%, SS - 91.6%, RTC - 98.3% e RTS - 93.3%), grau 1 ~ 5.4% (SC – 8.3%, SS – 5.0%, RTC – 1.7% e RTS – 6.7%), grau 2 ~ 2.1% (SC – 6.6%, SS – 1.8%, RTC e RTS - 0%), grau 3 ~ 1.2% (SC – 3.3%, SS – 1.7%, RTC e RTS - 0%) e grau 4 nenhum grupo. Pode-se observar também que a dose usada nesse experimento é segura, pois não causa esteatose hepática.

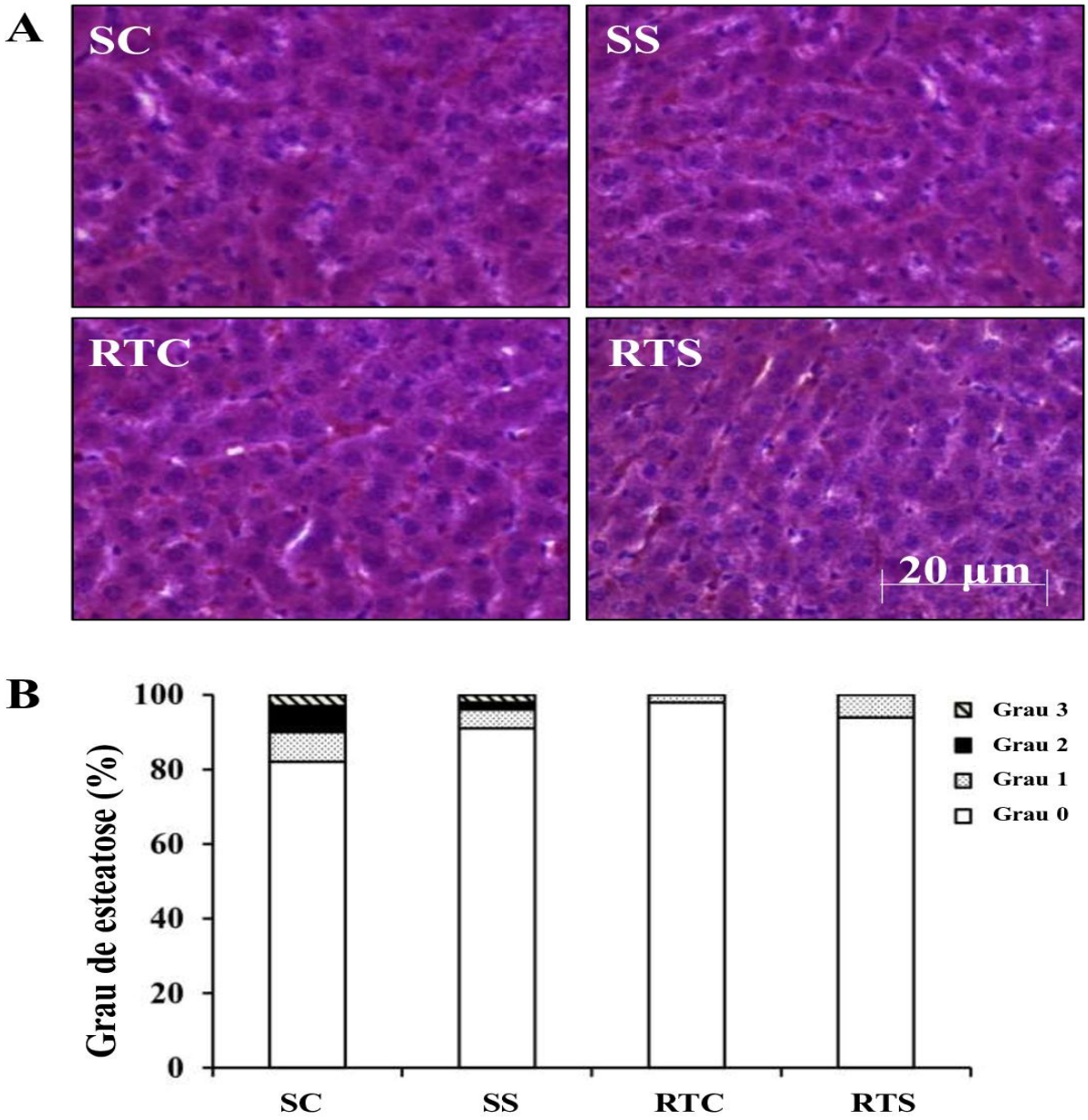

Figura 1. Esteatose hepática. (A) Fotomicrografia representativa de lâminas histológicas do fígado dos animais. (B) Grau de esteatose. SC, sedentário controle; SS, sedentário suplementado; RTC, treinamento resistido controle; RTS, treinamento resistido suplementação. Valores (média de 60 imagens por grupo). ANOVA de uma entrada, seguida do teste *post hoc* de Tukey.

REFERÊNCIA

BRUNT, E. M., *et al.* Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. **Am J Gastroenterol**, v. 94, n. 9, p. 2467-2474, 1999.

7. ANEXOS

Anexo 1 – Aprovação do comitê de ética

MINISTÉRIO DA EDUCAÇÃO
UNIVERSIDADE FEDERAL DE VIÇOSA
COMISSÃO DE ÉTICA NO USO DE ANIMAIS - CEUA
Campus Universitário - Viçosa, MG - 36570-000 - Telefone: (31) 3899-3783

Viçosa, 08 de junho de 2016

Ilmo. Prof.
Antônio José Natali
Coordenador do projeto
DES/UFV

Sr. Coordenador,

Após avaliação da Metodologia utilizada no Projeto de Pesquisa intitulado "Efeitos crônicos da suplementação com beta-hidroxi beta-metilbutirato (HMB) e do treinamento resistido nos marcadores inflamatórios e composição corporal de ratos wistar", aqui nomeado Processo 28/2016, a CEUA/UFV emite parecer favorável ao protocolo de utilização de animais proposto, tendo como base para análise a Legislação vigente (Lei Nº 11.794, de 08 de outubro de 2008), as Resoluções Normativas editadas pelo CONCEA/MCTI, bem como a DBCA (Diretriz Brasileira de Prática para o Cuidado e a Utilização de Animais para Fins Científicos e Didáticos) e as Diretrizes da Prática de Eutanásia preconizadas pelo CONCEA/MCTI.

Acresce a esse Parecer a exigência de Relatório Final de Atividades conforme itens a seguir:

RESUMO DOS RESULTADOS FINAIS OBTIDOS A PARTIR DOS EXPERIMENTOS ENVOLVENDO A ***UTILIZAÇÃO DE ANIMAIS*** *NO PROJETO DE PESQUISA*

1 Número do protocolo de submissão do projeto de pesquisa à CEUA/UFV:
2 Metodologia completa obrigatoriamente com:
- Local (is) Geral (is) e específico (s) oficial (is) onde ocorreu a experimentação;
- O nome científico do animal em questão;
- Número total de animais utilizados na pesquisa.
3 Resultados:
4 Nome do Coordenador do Projeto:
Assinatura:
5 Nome do Responsável Técnico:
Assinatura:
Inscrição em CRMV:

Profª. Átima Clemente Alves Zuanon
Presidente
Comissão de Ética no Uso de Animais – CEUA/UFV

Anexo 2 – Doação do suplemento

Material Transfer Agreement

This Agreement, made and entered into this 10 day of 02, 2017 (hereinafter referred to as the "Effective Date") by and between Metabolic Technologies, Inc., having its principal address at 2711 South Loop Drive, Suite 4400, Ames, Iowa 50010 (hereinafter referred to as "Provider") and ANTÔNIO JOSÉ NATALI, having its principal address at DEPARTMENT OF PHYSICAL EDUCATION, FEDERAL UNIVERSITY OF VICOSA, 36.570-VICOSA, MG, BRAZIL (hereinafter referred to as "Recipient") (collectively the "Parties"),

WITNESSETH:

WHEREAS, Provider has developed a material known as Calcium Beta-hydroxy-Beta-methylbutyrate (CaHMB) ("Material").

WHEREAS, Recipient desires Material for the purpose of internal research in the nature of testing and development efforts and Provider is willing to provide Material to Recipient for this purpose.

THEREFORE, for and in consideration of the mutual understandings by the Parties, it is hereby agreed:

1. The Material is the confidential and proprietary property of Provider and is to be used by Recipient solely for internal research in the nature of testing and development efforts at Recipient's facilities. Recipient shall not reverse engineer or analyze the Material by any means for its physical and chemical properties without the express written authorization of Provider. No further license or permission is granted by Provider, and all other rights in the Material are expressly withheld. Provider shall be free, in its sole discretion, to distribute the Material to others and to use it for its own purposes.

2. All disclosures of and related to the Material by Provider to Recipient shall be considered proprietary and confidential. Recipient shall not transfer the Material to anyone or use the Material for any purpose other than for the purposes stated in this Agreement. Recipient shall treat the Material and any disclosure related thereto with the same degree of care as Recipient accords to its own proprietary and confidential material but no less than reasonable care.

3. Provider retains ownership of the Material and Recipient agrees that nothing herein shall be deemed to grant to Recipient any rights to use the Material for any products or processes for profit-making or commercial purposes. Recipient acknowledges that the Material is or may be the subject of a patent application(s). No express or implied licenses or other rights are provided to the Recipient under any patents, patent applications, trade secrets or other proprietary rights of the Provider, including any altered forms of the Material made by the Provider. The Material will not be used in research that is subject to consulting or licensing obligations of Recipient to another individual, institution or business entity unless prior

written permission is obtained from Provider. At the request of Provider, Recipient will return all unused Material.

4. Recipient acknowledges and understands that any invention or discovery made by Recipient while using the Material which is or may be patentable ("Inventions"), and any substance made by Recipient which contains/incorporates the Material ("Modifications") shall be the sole property of Provider, and Recipient shall promptly disclose any such Invention or Modification to Provider and agrees to assist and cooperate with Provider in any efforts to protect the intellectual property rights therein. No express or implied licenses or other rights are provided to use the Material, Modifications or any related patents of the Provider for commercial purposes.

5. Any Material delivered pursuant to this Agreement is understood to be experimental in nature and may have hazardous properties. NOTHING IN THIS AGREEMENT SHALL BE DEEMED TO BE A REPRESENTATION OR WARRANTY BY PROVIDER OF THE ACCURACY, SAFETY, OR USEFULNESS FOR ANY PURPOSE OF THE MATERIAL AT ANY TIME MADE AVAILABLE BY PROVIDER. The Material is provided WITHOUT WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE OR ANY OTHER WARRANTY, EXPRESS OR IMPLIED. PROVIDER MAKES NO REPRESENTATION OR WARRANTY THAT THE USE OF THE MATERIAL WILL NOT INFRINGE ANY PATENT OR OTHER PROPRIETARY RIGHT. Neither Provider nor any affiliated company shall have any liability whatsoever to Recipient or any other person for or on account of any injury, loss, claim or damage of any kind or nature sustained by, or any damage assessed or asserted against, or any other liability incurred by or imposed upon Recipient or any other person, arising out of or in connection with or resulting from the use of any Material.

6. Recipient may not publish any information relating to the Material, Inventions, Modifications, or the work performed under this Agreement without the express written consent of Provider.

7. Recipient will use the Material in compliance with all laws, governmental regulations and guidelines applicable to the Material, including Public Health Service and National Institutes of Health regulations and guidelines such as, for example, those relating to research involving the use of animals or recombinant DNA. Except to the extent prohibited by law, the Recipient assumes all liability for damages which may arise from its use, storage or disposal of the Material.

8. This Agreement will terminate on the earliest of the following dates: (a) on completion of the Recipient's current research with the Material, or (b) on thirty (30) days written notice by either party to the other. This Agreement shall be terminable by Provider upon any material breach of the Agreement by Recipient. The provisions of this Agreement that contain rights and obligations that would naturally extend beyond the term of this

Agreement shall survive termination. Upon termination or expiration of this Agreement, the Recipient will discontinue its use of the Material and will, upon direction of the Provider, return or destroy any remaining Material.

9. This Agreement is not assignable, whether by operation of law or otherwise, without the prior written consent of Provider.

10. This Agreement shall be governed by and construed in accordance with the laws of the State of Iowa and any action brought to enforce any provision or obligation hereunder shall be brought in a court of competent jurisdiction in the State of Iowa.

11. This Agreement represents the total understanding between the Parties and supersedes any and all other understandings or agreements. Should any part of this Agreement be found unenforceable, the remaining provisions of the Agreement shall remain in full force and effect.

IN WITNESS WHEREOF, the Parties have caused this Agreement to be executed by their respective authorized representatives.

METABOLIC TECHNOLOGIES, INC.

By: _____

Printed Name: SHAWN BAKER

Title: C.O.O.

Date: 2/13/2017

By: _____

Printed Name: ANTONIO JOSE NATALI

Title: Ph.D

Date: 10 FEBRUARY 2017

Anexo 3 – Carta de aceite do artigo

BIOLSPORT-01450-2019-03

Biology of Sport

Authors:
JULIANO GUEDES, Maria do Carmo Peluzio, John Rathmacher, Tiago Leal, Miguel Carneiro Júnior, Diego de Carvalho, Leandro de Oliveira, Antônio Natali

Decision letter:
July 21, 2020
BIOLSPORT-01450-2019-03
β-HYDROXY β -METHYLBUTYRATE SUPPLEMENTATION BENEFITS THE EFFECTS OF RESISTANCE TRAINING ON BODY FAT REDUCTION VIA INCREASED IRISIN EXPRESSION IN WHITE ADIPOSE TISSUE

Dear Prof. JULIANO GUEDES,

I am pleased to inform you that your manuscript, entitled: β-HYDROXY β -METHYLBUTYRATE SUPPLEMENTATION BENEFITS THE EFFECTS OF RESISTANCE TRAINING ON BODY FAT REDUCTION VIA INCREASED IRISIN EXPRESSION IN WHITE ADIPOSE TISSUE, has been finally accepted for publication in our journal.

Thank you for submitting your work to us.

Piotr Zmijewski, PhD
Editor-in-Chief
Biology of Sport
Institute of Sport in Warsaw, Poland