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β-hydroxy-β-methylbutyrate supplementation benefits the effects of resistance training on body fat reduction via increased irisin expression in white adipose tissue

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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 an RT programme 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 or 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 the lack of change in protein mass and maximal strength.

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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 signalling pathways and attenuation of proteolysis via downregulation of catabolic signalling pathways [4]. Body fat is also reduced by RT because it increases the 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 the 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 development of brown-fat-like cells in WAT, which enhances lipid metabolism/oxidation via mitochondrial

uncoupling protein 1 (UCP-1) activity [12]. Additionally, higher irisin concentrations are associated with favourable 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 signalling pathways and attenuation of proteolysis via downregulation of the 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 been 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 programme

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 in 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 an RT programme 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 an 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 administered by gavage (volume: 1 mL of saline) for 8 weeks, immediately prior to the RT session (08:00 a.m.) on week days and at the same time at 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 to the hind paws [39]. These indirect measures were performed before and after the 4th and the 8th weeks of intervention, under anaesthesia.

After euthanasia, the skin and viscera were separated from muscles and bones (empty carcass) and the head and tail were disposed of. 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 a 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 was 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 the 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), RiboLock RNase inhibitor (20 U), 1 mM of dNTP Mix, RevertAid Reverse Transcriptase (200 U), making a solution with a final volume of 20 μL (Fermentas, Glen Burnie, MD, EUA). After cDNA synthesis, qRT-PCRs for target genes and the 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 s of denaturation at 95°C, 5 s of annealing at 60°C, and 15 s of elongation at 72°C, followed by a single fluorescent measurement and finally 25 s 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 the housekeeping gene. 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 $2^{(-\Delta\Delta Ct)}$ [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 epidydimal 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 precoated onto 96-well plates. The biotin conjugated anti-irisin antibody was used as the detection antibody.

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 analyser, 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 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 the RTC than in the 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 the RTS versus the 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).

Food intake and weight gain

The food intake (Fig. 2A) was lower in the RTC than in the SC group (80.25 ± 4.90 vs. 92.42 ± 8.70 g vs, $F_{3,44}$ = 11.35, p = 0.001; ES = 0.436); and lower in the RTS than in the SS group (75.50 ± 6.91 vs. 88.25 ± 7.42 g, $F_{3,44}$ = 31.11, p = 0.001; ES = 0.414) in week 8. The weight gain (Fig. 2B) was lower in the RTC versus the SC group (21.83 ± 6.47 vs. 35.92 ± 9.09 g, $F_{3,44}$ = 5.53, p = 0.02; ES = 0.112); and lower in the RTS versus the SS group (17.25 ± 6.06 vs. 30.58 ± 9.74 g, $F_{3,44}$ = 4.96, p = 0.03; ES = 0.101) in week 4. Additionally, weight gain was lower in the RTC versus the SC group (36.17 ± 8.47 vs. 56.88 ± 8.25 g, $F_{3,44}$ = 6.36, p = 0.02; ES = 0.126) in week 8.

Body composition

The abdominal circumference was lower in the RTC group than the SC group (16.72 ± 0.98 vs. 18.27 ± 0.52 cm, $F_{3,44}$ = 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 the SS group (18.05 ± 0.44 cm) ($F_{3,44}$ = 70.358, p = 0.001; ES = 0.615) and RTC group (16.71 ± 0.97 cm) ($F_{3,44}$ = 11.30, p = 0.002; ES = 0.204) in week 8. The Lee index (Fig. 3B) was higher in the SS group (311.62 ± 8.67) than in the SC group (300.58 ± 7.42) ($F_{3,44}$ = 10.62, p = 0.002; ES = 0.194) and in the RTS group (301.06 ± 5.86) ($F_{3,44}$ = 9.77, p = 0.003; ES = 0.182) in week 1. The RTS group (296.62 ± 7.11) presented a lower Lee index value compared to the SS group (306.24 ± 9.88) ($F_{3,44}$ = 10.14, p = 0.003; ES = 0.187) and to the RTC group (305.83 ± 6.47 ($F_{3,44}$ = 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 the SS group (309.83 ± 6.54) ($F_{3,44}$ = 18.21, p = 0.001; ES = 0.293) and to the RTC group (305.59 ± 7.85) ($F_{3,44}$ = 7.34, p = 0.01; ES = 0.143) in week 8.

The fat percentage was lower in training groups (RTC and RTS) compared to sedentary groups ($F_{1,44}$ = 40.71, p = 0.001; ES = 0.53) (Table 1). Supplemented groups (SS and RTS) had a lower fat percentage than the control groups ($F_{1,44}$ = 6.19, p = 0.01; ES = 0.143). The RTS group exhibited a lower fat percentage versus the RTC group ($F_{1,44}$ = 7.83, p = 0.03; ES = 0.175). The percentage of water was higher in training groups (RTC and RTS) than sedentary groups ($F_{1,44}$ = 73.73, p = 0.001; ES = 0.666). The percentage of residues in the RTS group was higher than in the SS group ($F_{1,44}$ = 25.54, p = 0.001; ES = 0.408).

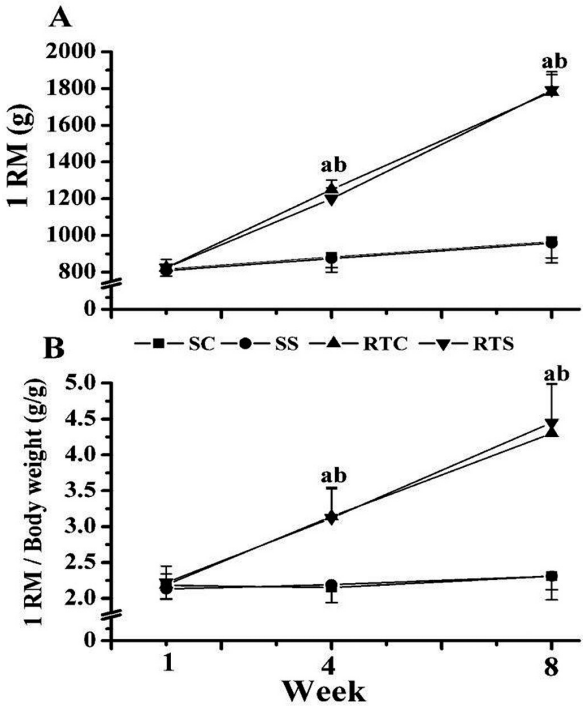

FIG. 1. Maximum load lifted in one repetition. SC, sedentary control; SS, sedentary supplemented; RTC, resistance training control; RTS, resistance training supplemented; 1RM, one repetition maximum; [a]p < 0.05 vs. SC; [b]p < 0.05 vs. SS.

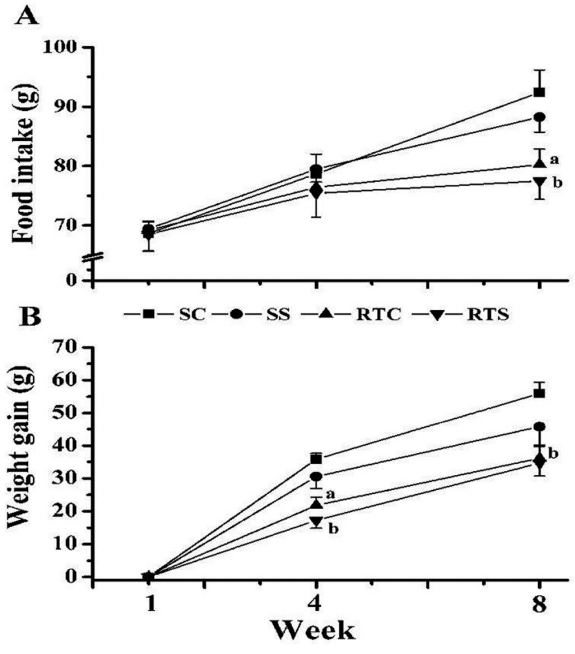

FIG. 2. Food intake and weight gain. SC, sedentary control; SS, sedentary supplemented; RTC, resistance training control; RTS, resistance training supplemented. 2-way ANOVA followed by Tukey post hoc test: [a]p < 0.05 vs. SC; [b]p < 0.05 vs. SS.

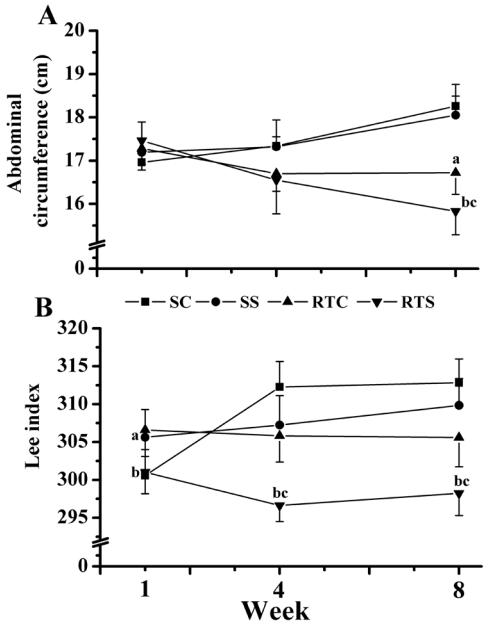

FIG. 3. Abdominal circumference and Lee index. SC, sedentary control; SS, sedentary supplemented; RTC, resistance training control; RTS, resistance training supplemented. [a]$p < 0.05$ vs. SC; [b]$p < 0.05$ vs. SS; [c]$p < 0.05$ vs. RTC.

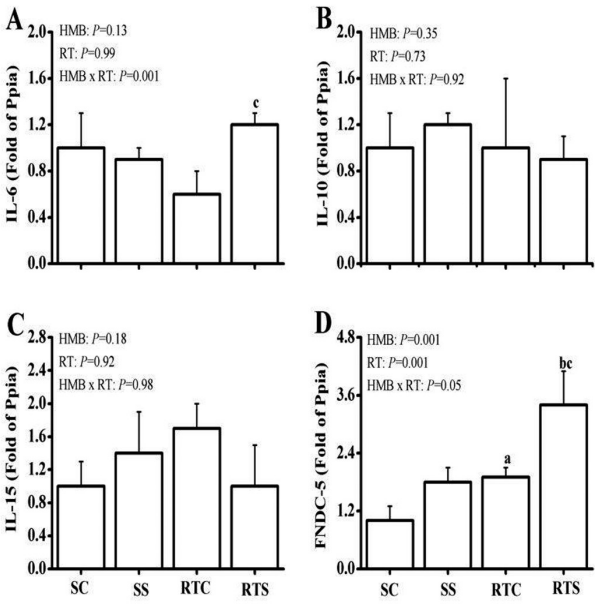

FIG. 4. Gene expression of interleukin and fibronectin type III domain-containing protein 5 in skeletal muscle. SC, sedentary control; SS, sedentary supplemented; RTC, resistance training control; RTS, resistance training supplemented. IL, interleukin (gastrocnemius muscle); FNDC-5, fibronectin type 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.

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 the RTS group than in the RTC group (1.16 ± 0.23 vs 0.61 ± 0.25, $F_{1,44} = 14.749$, $p = 0.001$; ES = 0.251). The expression of IL-10 (Fig. 4B) did not differ between groups ($F_{1,44} = 0.110$, $p = 0.92$; ES = 0.025); nor did the gene expression of IL-15 (Fig. 4C) ($F_{1,44} = 0.67$, $p = 0.98$; 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 ($F_{1,44} = 35.29$, $p = 0.001$; ES = 0.445). FNDC-5 gene expression was higher in supplemented groups (SS and RTS) than in control groups ($F_{1,44} = 27.44$, $p = 0.001$; ES = 0.38). The RTS group presented higher FNDC-5 gene expression than the RTC group (3.32 ± 1.08% vs 1.83 ± 0.36, $F_{1,44} = 35.28$, $p = 0.001$; ES = 0.445).

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.

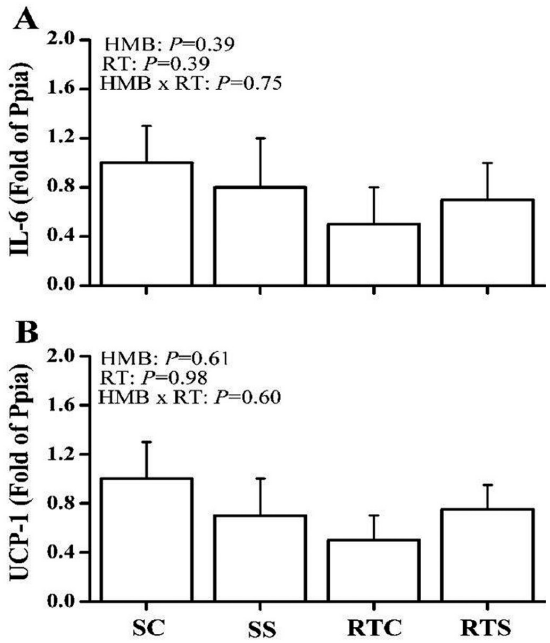

FIG. 5. Gene expression of interleukin and mitochondrial uncoupling protein 1 in epididymal adipose tissue. SC, sedentary control; SS, sedentary supplemented; RTC, resistance training control; RTS, resistance training supplemented. IL, interleukin; UCP-1, mitochondrial uncoupling protein 1.

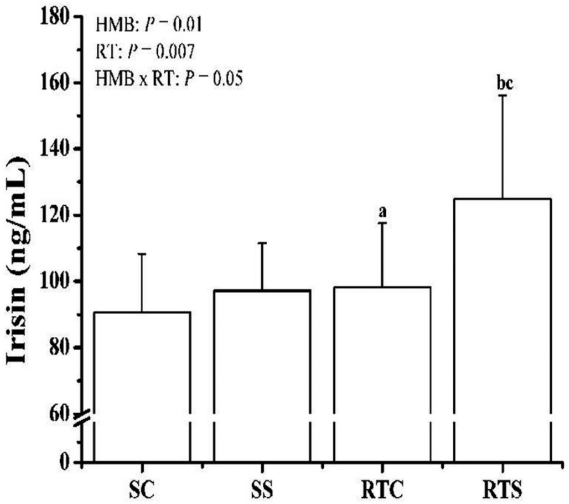

FIG. 6. Concentration of irisin in epididymal adipose tissue. SC, sedentary control; SS, sedentary supplemented; RTC, resistance training control; RTS, resistance training supplemented. $^{a}p < 0.05$ vs. SC; $^{b}p < 0.05$ vs. SS; $^{c}p < 0.05$ vs. RTC.

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).

Plasma lipid profile

Plasma concentration of VLDL-c was lower in training groups (RTC and RTS) compared to sedentary groups ($F_{1,44} = 130.72$, $p = 0.001$; ES = 0.748) (Table 2). The supplemented groups (SS and RTS) had lower plasma VLDL-c concentration than control groups ($F_{1,44} = 7.99$, $p = 0.007$; ES = 0.154). Plasma non-HDL-c concentration was lower in training groups (RTC and RTS) than in sedentary groups ($F_{1,44} = 32.98$, $p = 0.001$; ES = 0.428). Plasma concentration of triglycerides was lower in training groups (RTC and RTS) compared

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.

to sedentary groups ($F_{1,44}$ = 130.72, p = 0.001; ES = 0.748). The supplemented groups (SS and RTS) exhibited lower plasma triglyceride concentration than control groups ($F_{1,44}$ = 7.99, p = 0.007; ES = 0.154). Plasma total cholesterol was lower in training groups (RTC and RTS) than in sedentary groups ($F_{1,44}$ = 81.44, p = 0.001; ES = 0.649).

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 the lack of 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 that 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 fibre-type muscle (i.e. soleus) than in fast-glycolytic fibre-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 PGC-1α [31, 32]. Additionally, HMB associated with polyphenols was suggested to activate sirtuin signalling and the 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 acid oxidation and thermogenesis through other mechanisms apart from 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 phosphorylation 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 triglyceride concentrations were ~17% greater in the RTS than in the 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 they reinforce the usefulness of combining CaHMB supplementation and RT to promote attributes associated with good health.

CONCLUSIONS

The supplementation with CaHMB improved the benefits of RT in reducing body fat and was associated with increased muscular genic expression of IL-6 and FNDC-5, and irisin concentration in WAT, despite the lack of change in body protein and strength.

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Conflict of Interest

Juliano Magalhães Guedes, Maria do Carmo Gouveia Pelúzio, John A. Rathmacher, Tiago Ferreira Leal, Miguel Araújo Carneiro Júnior, Diego Milhomem de Carvalho, Leandro Licursi de Oliveira and Antônio José Natali declare that they have no conflict of interest.

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