

Eccentric resistance training and beta-hydroxy-beta-methylbutyrate free acid affects muscle PGC-1 alpha expression and serum irisin, nesfatin-1 and resistin in rats

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1 **Eccentric resistance training and β -Hydroxy- β -methylbutyrate free acid affects muscle PGC-1 α**
2 **expression and serum irisin, nesfatin-1 and resistin**

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15 **Running head:** Exercise and HMB-FA affects releasing peptides

16 **Summary Statement:** Eccentric resistance training and HMB-FA supplement may induce crosstalk
17 between releasing peptides from other tissues and increases maximal strength. Their combination has
18 greater effect compared to each intervention alone.

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39 **Abstract**

40 The hypothalamus controls metabolism and feeding behavior via several signals with other tissues. Exercise and
41 supplements can change hypothalamic signaling pathways, so the present study investigated the influence of
42 eccentric resistance training and β -Hydroxy- β -methylbutyrate free acid supplement on PGC-1 α expression, serum
43 irisin, nesfatin-1 and resistin concentrations. Thirty-two male rats (8 weeks old, 200 \pm 17 g body mass) were
44 randomized to control (CON), β -Hydroxy- β -methylbutyrate free acid (HMB) supplementation, eccentric resistance
45 training (ERT), and β -Hydroxy- β -methylbutyrate free acid supplementation plus eccentric resistance training
46 (HMB+ERT) groups. Training groups undertook eccentric resistance training (6 weeks, 3 times a week) and
47 supplement groups consumed HMB-FA orally (76 mg/kg/day). Twenty-four hours after the last training session, rats
48 were sacrificed after which serum and triceps brachii muscle were collected and sent to the laboratory for analyses.
49 Two-way ANOVA and Pearson correlation were employed (significant level: $P < 0.05$). The results showed that
50 eccentric resistance training increases skeletal muscle PGC-1 α gene expression, as well as serum levels of irisin and
51 nesfatin-1 ($P = 0.001$). Eccentric resistance training decreases serum concentration of resistin ($P = 0.001$). HMB-FA
52 supplement increases skeletal muscle PGC-1 α gene expression ($P = 0.002$), as well as serum concentration of irisin
53 and nesfatin-1 ($P = 0.001$). HMB-FA decreases the serum concentration of resistin ($P = 0.001$). Significant
54 correlations were observed between PGC-1 α gene expression and serum concentrations of irisin, nesfatin-1 and
55 resistin. Generally, HMB-FA with eccentric resistance training may induce crosstalk between releasing peptides
56 from other tissues and increases maximal strength. Their combination had a more substantial effect than each
57 intervention in isolation.

58

59 **Keywords:**

60 Exercise; HMB supplement; Maximal strength; PGC-1 α signaling pathway; Resistance training; Tissue crosstalk

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69 **Introduction**

70 Energy homeostasis is an important aspect of bioenergetics which can be defined as an equilibrium of energy intake
71 and energy expenditure (Lam and Ravussin 2016). The hypothalamus controls metabolism, feeding behavior
72 (Timper and Bruning 2017) and body mass via several pathways that affect appetite including Peroxisome
73 proliferator-activated receptor gamma coactivator (PGC-1 α) (Hu et al. 2016, Park and Ahima 2015). PGC-1 α is a
74 key signaling pathway in the metabolism of carbohydrate, lipids and the regulation of cellular energy (Liang and
75 Ward 2006). In addition, it stimulates mitochondrial biogenesis and promotes the remodeling of muscle tissue via
76 changes to fiber-type composition (Zhang et al. 2017). It is plausible that PGC-1 α affects irisin, nesfatin-1 and
77 resistin which are peptides involved in energy homeostasis (Shirvani and Arabzadeh 2018).

78 The myokine Irisin is predominantly produced by skeletal muscle after physical exercise, and creates crosstalk
79 between tissues. In particular, muscle-fat crosstalk changes the phenotype of white adipose tissue (converting white
80 fat into brown fat) and induces body mass loss (Fukushima et al. 2016). Irisin has been reported to activate
81 thermogenic programs in white adipose tissue and improve glycemia, which is dependent on PGC-1 α (Bostrom et al.
82 2012). Thus, elevated irisin has been posited to be a possible anti-obesity agent (Spiegelman 2013). Nesfatin-1 is an
83 anorexigenic protein likely to activate the melanocortin pathway and its involved in the regulation of blood glucose,
84 improves insulin sensitivity, energy homeostasis, and metabolism (Dore et al. 2017, Myers 2006, Oh et al. 2006).
85 Intracerebroventricular injection (ICV) of nesfatin-1 inhibited food intake in a dose-dependent manner results in a
86 decrease in total body fat and body mass loss, while anti-nesfatin-1 has increased the intake of food in male rats (Oh
87 et al. 2006). It was reported that nesfatin-1 promotes the differentiation of brown adipocytes through the PGC-1 α
88 (Wang et al. 2016). Hypothalamic resistin seems to be a key regulator of the brain-fat axis which regulates energy
89 homeostasis (Rodriguez et al. 2018). ICV infusion of resistin reduced epididymal fats and increased peripheral
90 insulin sensitivity (Park et al. 2008). Resistin modulates food intake, hypothalamic and peripheral lipid metabolism
91 (Nogueiras et al. 2010). It was reported that resistin regulates fatty acid β oxidation by suppressing expression of
92 PGC-1 α (He et al. 2018).

93 In the last decade, the use of supplements such as β -Hydroxy- β -methylbutyrate free acid (HMB) to promote fat loss
94 and muscle growth has increased. HMB is an active metabolite of the nutritionally essential branched-chain amino
95 acid (BCAA) leucine that has an anticatabolic role for muscle (reduces breakdown of muscle cell proteins) (He et al.
96 2016). There is evidence to support the inhibitory effects of HMB on dexamethasone-induced increase in protein

97 degradation and decrease in protein synthesis were regulated by p38/MAPK- and PI3K/Akt-dependent cell
98 signaling, respectively (Aversa et al. 2012). It was demonstrated that leucine-polyphenol combinations stimulate
99 irisin release and browning of adipose tissue (Brooke Baggett et al. 2013). To the authors knowledge, there has been
100 no study investigating the effects of HMB on nesfatin-1 and resistin. Overall, HMB is effective in the regulation of
101 many cellular processes such as protein synthesis and energy metabolism (Yin et al. 2010, Li et al. 2011, Duan et al.
102 2016, Wilson et al. 2013). HMB has numerous forms including HMB-FA and HMB-CA. HMB-FA is as dietary
103 supplement in the free acid form and has more bioavailability compared to HMB-CA, which is a monohydrated
104 calcium salt of the conjugate base (Wilson et al. 2013, Fuller et al. 2015). HMB supplementation has been shown to
105 increase muscle size (Wilson et al. 2012), and enhances force production during recovery from an injury that is
106 created by disuse-reloading (Alway et al. 2013).

107 Exercise has numerous influence on multiple gut peptides and consequently energy balance (Dorling et al. 2018).
108 Studies have investigated different modes of exercise training on PGC-1 α (P. C. Dinas et al. 2017, Jung and Kim
109 2014, Norheim et al. 2014), irisin (P. C. Dinas et al. 2017, Norheim et al. 2014, Samy et al. 2015), nesfatin-1 (Algul
110 et al. 2017, Ghanbari Niaki et al. 2013, Ghanbari-Niaki et al. 2010, Mogharnasi et al. 2018) and resistin (Cobbold
111 2018, Shafiee and Sharifi 2017, Garcia-Hermoso et al. 2017). The effects of HMB on these factors has not been
112 investigated widely. In addition, the combination of exercise and supplement may have different results than each
113 intervention alone. The aim of the present study was to investigate the influence of eccentric resistance training and β -
114 Hydroxy- β -methylbutyrate free acid supplement on PGC-1 α expression, serum irisin, nesfatin-1 and resistin
115 concentrations in rats.

116

117 **Material and methods**

118 *Permissions*

119 The present study was conducted with the written permission of the research deputy of Baqiyatallah University
120 (ethical code: IR.BMSU.REC.1394.82) and was in accordance with National Institutes of Health (NIH) publication.

121

122 *Animals and design*

123 Thirty-two male rats (Sprague Dawley family, 8 weeks old, 200 \pm 17 g weight) were used in this cross-sectional
124 study. Animals were kept in the Baqiyatallah University of Medical Science in the animal houses in special cages

125 where the floor was covered with clean wood chips. The temperature was 22 (± 2 °C), humidity between 45-50%
126 with a lighting-dark cycle of 12 hours light followed by 12 hours darkness. Special standard compressed food
127 (Behparvar of Karaj) for laboratory rats (crude protein: 19.50-20.50%, fat: 3.5-4.5%, fibre: 4-4.5%, calcium 0.95-
128 1%, phosphorus: 0.65-0.7%, salt: 0.5-0.55%, lysine 1.15%, methionine: 0.33%, threonine: 0.72, tryptophan: 0.25,
129 energy: 16.16-17 MJ/kg) was provided at regular times. The cages were fitted with urban filtered water in bottles of
130 500 ml. Rats were randomized into four groups (8 in each group) including control (CON), β -Hydroxy- β -
131 methylbutyrate free acid supplementation (HMB), eccentric resistance training (ERT), and HMB supplementation
132 plus eccentric resistance training (HMB+ERT). The training groups undertook eccentric resistance exercise training
133 on a ladder while control groups activity was limited to light intensity activity (i.e. walking around the cage).
134 Thirty minutes prior to the exercise training the HMB groups orally consumed freely force fed the supplement
135 (Beta-TOR, USA) at a dose of 76 mg/kg/day while non-supplement groups orally consumed a saline placebo. The
136 dosage equivalent in human studies is 3 to 6 g/day for an 80 kg person (Gallagher et al. 2000).

137

138 *One-repetition maximum measurement*

139 In the first session, one-repetition maximum (1RM) was considered as 50% of the rats body mass, as has been used
140 previously (Gil and Kim 2015). On completion, the final load of the first session was recorded as the 1RM for the
141 next session (Fig. 1).

142

143 *Training protocol*

144 Eccentric resistance exercise training was performed using a ladder (Manufactured by the Exercise Physiology
145 Research Center, Life Style Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran). The ladder was
146 made of wood with iron steps which had a height of 1.1 m, an inclination of 80 degrees and consisted of 26 steps in
147 total. The ladder was designed to make the rats descend the ladder while imposing a constant load. This protocol has
148 been used in previous research (Gil and Kim 2015). The rats performed 10 to 12 dynamic movements (repetitions)
149 during each landing so the intensity is different. Rats exercised on the ladder with a free load for a week,
150 standardized as a pre-training adaptation and to allow the rats to become accustomed to the exercise. After that, the
151 rats performed the ladder descent exercise with a weighted backpack. The exercise was loaded as follows: one
152 repetition of ladder exercise was conducted at 50%, 75%, 90%, 100% and 120% of 1RM, after which 30g was added

153 for each trial up to eight trials. Training ended before the 8th trial when rats showed signs of exhaustion, such as
154 unable to descend, or were hanging from the ladder. Eccentric resistance exercise was performed three times a week
155 for six weeks for a duration of 25 minutes per session.

156

157 ***Rat sacrifices, serum and triceps brachii muscle collection***

158 Exactly twenty-four hours post the last training session, rats were anesthetized with intraperitoneal administration of
159 a mixture of ketamine (supplied by Iranian company: Shiraz Iman Saba, Made in Holland, 30 – 50 mg/kg body
160 mass) and xylazine (supplied by Iranian company: Shiraz Iman Saba, Made in Holland, 3 – 5 mg/kg body mass).
161 Blood was collected into tubes and immediately processed for serum preparation during 10 min centrifugation at
162 $1000 \times g$. Serum was then stored at -80°C for future analysis. Triceps brachii muscle was excised, cleaned, divided
163 into three pieces, washed in ice-cold saline, and immediately frozen in liquid nitrogen and stored at -80°C until RNA
164 extraction.

165

166 ***Serum analysis***

167 Serum concentrations of irisin and nesfatin-1 were analyzed using ELISA (BioVendor Laboratory Medicine, Brno,
168 Czech Republic) standard operating procedures. The kit sensitivity for irisin and nesfatin-1 was 0.01 ng/ml and 14
169 ng/ml respectively. Irisin and nesfatin-1 kit inter and intra assay coefficients of variation were 10% and 8%
170 respectively. Serum resistin concentration was analyzed by ELISA (Biovendor Research and Diagnostic Products,
171 Czech Republic) standard operating procedures. The resistin kit sensitivity was 0.25 ng/ml. The inter and intra assay
172 coefficients of variation were 7% and 5% respectively.

173

174 ***Evaluation of gene expression***

175 RNA extraction was performed by RNA purification kits (AccuZol, Bioneer, Cat. No: k3090, Korea) and 85 to
176 95 mg of triceps brachii muscle was used for each sample. Complementary DNA (cDNA) making was performed by
177 cDNA synthesis kit (AccuPower RT PreMix) according to the manufacturer's instructions and oligo-(dt)₁₈ primers
178 (0.25 µg per reaction). Real-time PCR was performed by light Cyclor apparatus (Corbet Real time PCR machine,
179 Australia). QuantiFast SYBR Green PCR Kit (Cat. No. 204052; Qiagen, GmbH, Germany) in using 15 µL reaction
180 was used. The 15 µL reaction contained 0.5 µL single-strand cDNA, 7.5 µL Master Mix, 1 µL of the each forward

181 and reverse primers (5 pmol/μL), and 5 μL dH₂O. PGC1α sense primer was 5'-GACCCTCCTCACACCAAAC-3',
 182 and antisense primer was 5'-GCGACTGCGGTTGTGTATG-3' (Shi et al. 2013). The β-actin sense and antisense
 183 primers were 5'-TATCGGCAATGAGCGGTTCC-3' and 5'-CACTGTGTTGGCATAGAGG-3', respectively
 184 (Rahmati-Ahmadabad et al. 2017), which were used as normalizer gene.

185

186 **Statistical analysis**

187 Real-time PCR cyclic threshold (CT) was analyzed by the Pfaffl method (Pfaffl 2001). All data was stored and
 188 analyzed using SPSS software, (IBM, version 24). The Kolmogorov–Smirnov test was used to assess data
 189 distribution and Levene's test was used to assess the equality of variances. Repeated measures ANOVA was used to
 190 identify any difference in rats' body mass for the duration of the study as well as changes in 1RM. In order to infer
 191 differences between groups, two way ANOVA and Tukey Post *hoc* test was used. Correlations were calculated
 192 using Pearson Product Moment correlation. Due to the low sample size non parametric tests including the Friedman
 193 test and Spearman correlation were also conducted but this did not alter the interpretation of the findings so only the
 194 results of the parametric tests are presented. Effect size (ES) was reported to emphasize the size of the difference
 195 rather than confound the sample size. Significance was accepted if $P < 0.05$. Data are presented as mean ± standard
 196 deviation (SD) unless otherwise stated.

197

198 **Results**

199 There was no difference in body mass between groups ($F(5, 140) = 0.40, P = 0.84; ES = 0.01$) (Tab.1).

200

201 **Table. 1:** Rat body mass in control (CON), β-Hydroxy-β-methylbutyrate free acid supplementation (HMB), eccentric resistance
 202 training (ERT), and β-Hydroxy-β-methylbutyrate free acid supplementation plus eccentric resistance training (HMB+ERT)
 203 groups. N = 8 in each group.

204

Groups	Week 1 body mass (g)	Week 2 body mass (g)	Week 3 body mass (g)	Week 4 body mass (g)	Week 5 body mass (g)	Week 6 body mass (g)
CON	205.50±16.93	216.37±15.01	225.37±16.40	242.12±14.77	267.75±14.72	280.37±16.49
HMB	197.63±17.71	206.75±18.94	217.62±18.67	236.25±18.17	259.75±16.16	271.37±18.11
ERT	202.62±17.66	214.87±19.11	223.01±19.79	240.62±19.97	267.12±20.06	278.75±19.85
HMB+ERT	195.87±16.96	205.25±17.01	213.62±18.70	232.62±15.46	258.37±16.62	269.62±15.93

205

206 The mean weekly 1 RM of the exercise training groups initially (week 1, 2, 3) showed similar levels, as can be seen
207 in Fig. 1. 1RM was significantly higher in HMB+ERT compared ERT group in week 4 (998.68 ± 97.98 Vs $1113.62 \pm$
208 81.30 g, $F(1, 14) = 6.52$, $P = 0.02$; $ES = 0.31$), 5 (1795.38 ± 180.56 Vs 2033.89 ± 183.61 g, $F(1, 14) = 6.86$, $P = 0.02$;
209 $ES = 0.32$) and 6 (2150.56 ± 214.30 Vs 2433.63 ± 217.91 g, $F(1, 14) = 6.85$, $P = 0.02$; $ES = 0.33$) (Fig.1).

210

211 Training groups had higher tissue PGC1 α than non training groups ($F(1, 28) = 93.74$, $P = 0.001$; $ES = 0.77$) (Fig.
212 2A). PGC1 α gene expression was significantly higher in HMB groups than non-supplement groups ($F(1, 28) =$
213 11.59 , $P = 0.002$; $ES = 0.29$). Eccentric resistance training and HMB supplementation has the greatest PGC1 α gene
214 expression ($F(1, 28) = 5.52$, $P = 0.02$; $ES = 0.16$) (Fig. 2A).

215

216 For serum irisin, data analysis showed that there was a higher concentration in training groups compared to non-
217 training groups ($F(1, 28) = 104.78$, $P = 0.001$; $ES = 0.78$). (Fig. 2B). Results showed that serum irisin was
218 significantly higher in HMB groups than control ($F(1, 28) = 22.59$, $P = 0.001$; $ES = 0.44$). The highest irisin was for
219 HMB + ERT ($F(1, 28) = 4.53$, $P = 0.04$; $ES = 0.13$) (Fig. 2B).

220

221 For serum nesfatin-1, data analysis showed higher concentration in training groups compared to non-training groups
222 ($F(1, 28) = 31.46$, $P = 0.001$; $ES = 0.52$). (Fig. 2C). The results showed higher concentrations of serum nesfatin-1 in
223 HMB groups than non-supplement groups ($F(1, 28) = 34.76$, $P = 0.001$; $ES = 0.55$). The highest serum nesfatin-1
224 concentration was in the HMB + ERT group ($F(1, 28) = 18.87$, $P = 0.001$; $ES = 0.40$) (Fig. 2C).

225 For serum resistin, data analysis showed that there was a lower concentration in training groups compared to non-
226 training groups ($F(1, 28) = 63.44$, $P = 0.001$; $ES = 0.69$) (Fig. 2D). Results showed that serum resistin was
227 significantly lower in HMB groups than non-supplement groups ($F(1, 28) = 34.09$, $P = 0.001$; $ES = 0.54$). The lowest
228 serum resistin concentration was in HMB + ERT ($F(1, 28) = 18.01$, $P = 0.001$; $ES = 0.39$) (Fig. 2D).

229

230 Positive correlations between muscle PGC-1 α gene expression and plasma irisin and nesfatin-1 were observed but
231 there was a negative correlation with plasma resistin (Tab.2).

232

233

234

Table 2: Pearson's correlation coefficients of PGC-1 α mRNA to other variables.

Group	Variable	PGC-1 α gene expression		
		Serum Irisin	Serum Nesfatin-1	Serum Resistin
CON		$r = 0.10$ $P = 0.42$	$r = 0.21$ $P = 0.32$	$r = 0.18$ $P = 0.32$
HMB		$r = 0.54$ $P = 0.12$	$r = 0.48$ $P = 0.12$	$r = -0.54$ $P = 0.14$
ERT		$r = 0.63$ $P = 0.09$	$r = 0.60$ $P = 0.10$	$r = -0.86$ $P = 0.05$
HMB+ERT		$r = 0.95$ $P = 0.01^*$	$r = 0.85$ $P = 0.01^*$	$r = -0.89$ $P = 0.01^*$

235 * $P < 0.05$

236

237 **Discussion**

238 The findings of this study showed that eccentric resistance training resulted in greater skeletal muscle PGC-1 α
 239 relative gene expression, increases serum concentrations of irisin and nesfatin-1 and decreases serum concentrations
 240 of resistin compared to control. In addition, HMB supplement resulted in increased skeletal muscle PGC-1 α relative
 241 gene expression, increased serum concentrations of irisin and nesfatin-1, and decreased serum concentrations of
 242 resistin compared to control. The most important findings of the present study showed that a combination of
 243 eccentric resistance training and HMB supplement had a cumulative and greater effect on variables compared to
 244 exercise or HMB supplement alone.

245

246 There was a positive correlation between muscle PGC-1 α gene expression with serum irisin and nesfatin-1 and a
 247 negative correlation with serum resistin. Resistance training and HMB supplementation increases 1 RM whilst no
 248 significant changes occurred in rat body mass. It appears that eccentric resistance training with and without HMB
 249 supplement can affect signalling pathways via crosstalk between tissues to increase strength.

250

251 Different modes of exercise training can affect PGC-1 α gene expression, but resistance training has little effect on
 252 AMPK/PGC-1 α pathway (Jacobs et al. 2014). Resistance training increases the phosphorylation of the anabolic
 253 Akt/mTOR signaling pathway, as well as the activation of the translation initiation regulators p70 S6k, 4E-BP1, and
 254 eIF2B (Atherton et al. 2005). In contrast, aerobic endurance exercise increased phosphorylation of AMPK and
 255 protein levels of PGC-1 α (Atherton et al. 2005). However, in the present study, we observed enhanced PGC-1 α

256 gene expression in response to eccentric resistance training due to the similarities with aerobic endurance training as
257 both are able to act via the AMPK/PGC-1 α pathway.

258
259 Results of previous studies indicates that physical training can increase irisin. Daskalopoulou et al. (Daskalopoulou
260 et al. 2014) found plasma levels of irisin increased in response to increased exercise load by running on a treadmill
261 in active, young people. Also, Boström et al. (Bostrom et al. 2012) highlighted that irisin increased after three weeks
262 of aerobic training in rats and led to an increase in energy expenditure and improved glucose homeostasis. Huh et al.
263 (Huh et al. 2012) demonstrated that after 30 minutes of speed activity, concentrations of irisin increased
264 significantly. To the authors knowledge, this is the first study to report an increase in serum irisin concentration
265 following chronic eccentric resistance exercise. in rats. The results of this study are consistent with the results of
266 previous studies that investigate responses of other types of exercise training.

267
268 Plausible mechanisms for how exercise can increase irisin have been posited. Researchers have shown that exercise
269 increases PGC-1 α levels in skeletal muscle and increases the muscle-bearing FNDC5 membrane protein that results
270 in the production of irisin (Schnyder and Handschin 2015). AMPKs activation during exercise is one of the factors
271 for increasing PGC-1 α and irisin (Chavanelle et al. 2017). AMPKs activation leads to the phosphorylation of PGC-
272 1 α as FNDC5' s modifier and irisin secretion (Petros C. Dinas et al. 2017). Also, PGC-1 α activates PPAR γ . PPAR
273 γ is involved in energy metabolism and stimulates FNDC5 and irisin increase (Panati et al. 2016). It is highlighted
274 that there is a relation between irisin amounts and precursor of FNDC5 and PGC-1 α (Petros C. Dinas et al. 2017).
275 The results of the present study showed a significant and positive correlation between PGC-1 α gene expression and
276 plasma concentrations of irisin. The eccentric resistance training is likely to activate the PGC-1 α activating signals,
277 which may trigger a signal cascade to change the phenotype of the adipose tissue. Eccentric resistance training leads
278 to energy consumption and heat production by increasing muscular tissue ratio to fat tissue and increasing UCP1
279 (Chavanelle et al. 2017) thus increasing PGC-1 α , FNDC5, and irisin (Petros C. Dinas et al. 2017).

280
281 Production and secretion of irisin from the muscle is also mediated by SMAD3 (mothers against decapentaplegic
282 homolog 3). SMAD3 is a molecule that changes energy metabolism and regulates body mass. SMAD3 suppresses
283 FNDC5 and PGC-1 α in skeletal muscle and negatively regulate plasma irisin (Tiano et al. 2015). Exercise induces

284 phosphorylation of SMAD2 and Subsequently SMAD3 (Tiano et al. 2015). However, SMAD3 was not measured in
285 the present study so future research should investigate this possible mechanism for increasing irisin in response to
286 eccentric resistance training.

287
288 Ghanbari-Niaki et al. (2013) evaluated the effect of eight weeks of endurance training (five days a week for 60
289 minutes at a speed of 25 m/min with a zero gradient) on tissue nesfatin-1 gene expression and plasma levels of
290 nesfatin-1 (Ghanbari-Niaki et al. 2013). Their results indicated that training increased the expression and plasma
291 levels of nesfatin-1, which was related to plasma HDL concentration. Nesfatin is involved in the regulation of blood
292 glucose, improves insulin sensitivity, energy homeostasis, and metabolism (Dore et al. 2017). The effect of exercise
293 on nesfatin-1 has not been clearly recognized and not yet studied in response to eccentric resistance training.
294 However, there are possible mechanisms available. Studies have shown that nesfatin-1 are affected by various
295 factors (Li et al. 2014, Atici et al. 2017, Chaolu et al. 2011, Dore et al. 2017, J. F. Ge et al. 2015, Ayada et al. 2015).
296 For example, it has been shown that starvation in rats decreases serum nesfatin-1 levels up to 18%. But conversely,
297 it has been reported that nesfatin-1 concentrations returned to normal 1 to 12 hours after refeeding (Dore et al.
298 2017). In addition, some studies have shown that there is a direct relationship between nesfatin-1 and cortisol levels.
299 Central injection of nesfatin-1 increased adrenocorticotropins (Jin-Fang Ge et al. 2015). According to previous
300 studies, all of these factors are elevated as a result of eccentric resistance training protocols, which can be considered
301 as a possible cause for increasing nesfatin-1 as a result of this method compared to studies that have not seen any
302 changes. The adipose tissue also secretes various inflammatory cytokines that affect the expression and secretion of
303 adipokines. For example TNF- α has different effects on adiponectin, leptin and nesfatin-1. Studies have shown that
304 TNF- α , IL-6 and insulin increase the intracellular expression of nesfatin-1 in cultured fat cells (Ayada et al. 2015).
305 These findings show that the expression and secretion of nesfatin-1 are regulated from different pathways.

306
307 Some clinical studies have reported that there is a significant relationship between nesfatin-1 and insulin sensitivity
308 (Khalili et al. 2017). Therefore, it is likely that exercise alters the concentration of insulin and cortisol, influencing
309 blood glucose and nesfatin-1. These factors have not been examined in this study and warrant further investigation.

310

311 It has been shown that nesfatin-1 attenuated phosphorylation of S6K and S6 during brown adipocyte differentiation.
312 Nesfatin-1 via mTOR dependent mechanism promotes the differentiation of brown adipocytes. Activation of mTOR
313 induced by leucine or deletion of TSC1 decreased expression of brown adipocyte-related genes UCP1, UCP3,
314 PGC1 α and PRDM16, as well as COX8B and ATP5B. Both leucine and TSC1 deletion blocked nesfatin-1-induced
315 up-regulation of UCP1, PGC1 α , COX8B and ATP5B in differentiated brown adipocytes (Wang et al. 2016). Results
316 of the present study showed a significant and positive correlation between PGC-1 α gene expression and serum
317 level of nesfatin-1 which is likely because of mTOR activator elements that mentioned above.

318
319 Resistin, increases as a result of obesity due to a significant reduction in exercise and increase in energy intake
320 (Garcia-Hermoso et al. 2017). The present study also showed a significant and negative correlation between PGC-1 α
321 gene expression and serum levels of resistin. It possible that regular moderate-intensity physical training suppresses
322 the expression of dual specificity protein phosphatase 1 (DUSP1), increases the expression of PGC-1 α and reduces
323 the activities of JNK and ERK (Khadir et al. 2015). Khadir et al (2015) concluded that anti-inflammatory exercise
324 effects may be related to suppressing of NADPH oxidase, ERK1/2 and SAPK/JNK activities, and increases in SOD-
325 1 gene expression. In the presenst study we observed a decrease in resistin after eccentric resistance training and
326 possible regulation by PGC-1 α . Regarding the effects of HMB on PGC-1 α , He et al. (2016) suggested that dietary
327 supplementation with HMB increases the gene expression of PGC-1 α . They suggested that PGC-1 α plays a key role
328 in the transformation of skeletal muscle fiber type. As a nitrogen-free metabolite, HMB improves skeletal muscle
329 function, as well as the health of the body in both animals and humans (He et al. 2016).

330
331 The present study showed that HMB enhances the positive effects of resistance training on strength (1RM). Lee et
332 al. (2012) showed that leucine (0.5 mM) increases stimulates expression PGC-1 α by three- to fivefold in C2C12 cell
333 models (Li et al. 2012). Vaughan et al. (2013) reported that leucine (0.1–0.5 mM) dose-dependently enhanced PGC-
334 1 α expression in skeletal muscle cells (Vaughan et al. 2013). A few studies demonstrated the effects of HMB on
335 irisin. Baggett et al. (2013) investigate the synergistic effects of leucine and its metabolites with polyphenols on
336 irisin in myotubes and diet-induced obese mice. They demonstrate that leucine-polyphenol combinations stimulate
337 irisin and PGC-1 α (B. Baggett et al. 2013). To our knowledge, no research has examined the effects of HMB on
338 nesfatin-1 and resistin. The results of the present study showed that serum nesfatin-1 increases and serum resistin

339 decrease responses HMB supplement. The mechanism that HMB induced change in nesfatin-1 and resistin is not
340 understood and requires further research.

341 **Limitations**

342 Blood collection were not performed each week because of the associated costs, making it impossible to identify
343 how soon these changes may have occurred. The research was undertaken on a small sample of animals so effect
344 sizes have been included as well as the significance of both parametric and non parametric tests. Caution should be
345 exerted if generalizing the findings to humans.

346

347 **Conclusions**

348 The most important findings of present study showed that a combination of eccentric resistance training and HMB-
349 FA supplement has more effect on the primary outcomes measured compared to the exercise or supplement
350 intervention alone. Exercise and HMB supplement could increase PGC-1 α gene expression that may regulate the
351 other releasing tissues and change serum concentrations of irisin, nesfatin-1, and resistin. In general, we found that
352 eccentric resistance training with HMB supplementation could be affected by inter-tissue crosstalk that increases the
353 strength. Further research is needed to determine the effects of other peptides that would have allowed the authors to
354 make further inferences about cross talk.

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368

369 **Author contributions**

370 HSh designed this study. HSh and RM collected the materials and performed the experiments. SRA analyzed the
371 data. SRA and DRB wrote the manuscript. All authors read and approved the final version of the manuscript.

372

373 **Conflict of Interest**

374 The authors declare that they have no conflict of interest.

375

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377 None.

378 **Research involving human and animal participants**

379 Thirty-two male rats (Sprague Dawley family, 8 weeks old, 200±17 g weight) were used in this study. The present
380 study was conducted with the written permission of the research deputy of Baqiyatallah University (ethical code:
381 IR.BMSU.REC.1394.82) and was in accordance with National Institutes of Health (NIH) publication.

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Figure legends

Figure 1: The 1 RM of eccentric resistance training (ERT), and β -Hydroxy- β -methylbutyrate free acid supplementation plus eccentric resistance training (HMB+ERT) groups. N = 8 in each group.

Figure 2: The Real-time PCR of skeletal muscle tissue PGC-1 α relative mRNA expression (A), serum Irisin (ng/ml) (B), nesfatin-1 (ng/l) and resistin (ng/ml) (D) in control (CON), β -Hydroxy- β -methylbutyrate free acid supplementation (HMB), eccentric resistance training (ERT), and β -Hydroxy- β -methylbutyrate free acid supplementation plus eccentric resistance training (HMB+ERT) groups. N = 8 in each group

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