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RESEARCH ARTICLE

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

Hossein Shirvani[1], Saleh Rahmati-Ahmadabad[2,*], David Robert Broom[3] and Reza Mirnejad[4]

ABSTRACT

The hypothalamus controls metabolism and feeding behaviour via several signals with other tissues. Exercise and supplements can change hypothalamic signalling pathways, so the present study investigated the influence of eccentric resistance training and β-hydroxy-β-methylbutyrate free acid supplementation on PGC-1α expression, serum irisin, nesfatin-1 and resistin concentrations. Thirty-two male rats (8 weeks old, 200±17 g body mass) were randomly allocated to control, 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. Training groups undertook eccentric resistance training (6 weeks, 3 times a week) and supplement groups consumed β-hydroxy-β-methylbutyrate free acid (HMB-FA) orally (76 mg kg^{-1} day^{-1}). Twenty-four hours after the last training session, serum and triceps brachii muscle samples were collected and sent to the laboratory for analysis. Two-way ANOVA and Pearson correlation were employed (significance level: $P<0.05$). The results showed that eccentric resistance training increases skeletal muscle *PGC-1α* gene expression, as well as serum levels of irisin and nesfatin-1 ($P=0.001$). Eccentric resistance training decreased the serum concentration of resistin ($P=0.001$). HMB-FA supplementation increased skeletal muscle *PGC-1α* gene expression ($P=0.002$), as well as the serum concentration of irisin and nesfatin-1 ($P=0.001$), but decreased the serum concentration of resistin ($P=0.001$). Significant correlations were observed between *PGC-1α* gene expression and serum concentrations of irisin, nesfatin-1 and resistin. HMB-FA supplementation with eccentric resistance training may induce crosstalk between peptide release from other tissues and increases maximal muscle strength. The combination of the two interventions had a more substantial effect than each in isolation.

KEY WORDS: Exercise, HMB supplement, PGC-1α signalling pathway, Muscle strength, Hypothalamus, Energy homeostasis, Tissue crosstalk

[1]Exercise Physiology Research Center, Life Style Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran. [2]Department of Physical Education, Pardis branch, Islamic Azad University, Pardis, Iran. [3]Academy of Sport and Physical Activity, Faculty of Health and Wellbeing, Sheffield Hallam University, Sheffield S10 2BP, UK. [4]Molecular Biology Research Center, Systems Biology and Poisonings Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran.

*Author for correspondence (salehrahmati@pardisiau.ac.ir)

H.S., 0000-0002-0696-958X; S.R., 0000-0001-8751-1759; D.R.B., 0000-0002-0305-937X; R.M., 0000-0002-7297-3085

Received 17 December 2018; Accepted 5 May 2019

INTRODUCTION

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

The myokine irisin is predominantly produced by skeletal muscle after physical exercise, and creates crosstalk between tissues. In particular, muscle–fat crosstalk changes the phenotype of white adipose tissue (converting white fat into brown fat) and induces body mass loss (Fukushima et al., 2016). Irisin has been reported to activate thermogenic programmes in white adipose tissue and improve glycaemia, which is dependent on PGC-1α (Boström et al., 2012). Thus, elevated irisin has been posited to be a possible anti-obesity agent (Spiegelman, 2013). Nesfatin-1 is an anorexigenic protein that probably activates the melanocortin pathway; it is involved in the regulation of blood glucose, and improves insulin sensitivity, energy homeostasis and metabolism (Dore et al., 2017; Myers, 2006; Oh et al., 2006). Intracerebroventricular injection of nesfatin-1 inhibits food intake in a dose-dependent manner, resulting in a decrease in total body fat and body mass loss, while anti-nesfatin-1 increases the intake of food in male rats (Oh et al., 2006). It was reported that nesfatin-1 promotes the differentiation of brown adipocytes through PGC-1α (Wang et al., 2016). Hypothalamic resistin seems to be a key regulator of the brain–fat axis which regulates energy homeostasis (Rodríguez et al., 2018). Intracerebroventricular infusion of resistin reduces epididymal fats and increases peripheral insulin sensitivity (Park et al., 2008). Resistin modulates food intake, and hypothalamic and peripheral lipid metabolism (Nogueiras et al., 2010). It was reported that resistin regulates fatty acid beta oxidation by suppressing expression of PGC-1α (He et al., 2018).

In the last decade, the use of supplements such as β-hydroxy-β-methylbutyrate (HMB) to promote fat loss and muscle growth has increased. HMB is an active metabolite of the nutritionally essential branched-chain amino acid (BCAA) leucine, which has an anticatabolic role for muscle (reduces breakdown of muscle cell proteins) (He et al., 2016). Evidence suggests that the inhibitory effects of HMB on the dexamethasone-induced increase in protein degradation and decrease in protein synthesis are regulated by

Journal of Experimental Biology (2019) 222, jeb198424. doi:10.1242/jeb.198424

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

Exercise has numerous influences on multiple gut peptides and consequently energy balance (Dorling et al., 2018). Studies have investigated the effects of different modes of exercise training on PGC-1α (Dinas et al., 2017; Jung and Kim, 2014; Norheim et al., 2014), irisin (Dinas et al., 2017; Norheim et al., 2014; Samy et al., 2015), nesfatin-1 (Algul et al., 2017; Ghanbari Niaki et al., 2013b; Ghanbari-Niaki et al., 2010; Mogharnasi et al., 2018) and resistin (Cobbold, 2018; Shafiee and Sharifi, 2017; García-Hermoso et al., 2017). However, the effects of HMB on these factors have not been investigated widely. In addition, the combination of exercise and supplements may produce different results from each intervention alone. The aim of the present study was to investigate the influence of eccentric resistance training and HMB-FA supplementation on PGC-1α expression, and serum irisin, nesfatin-1 and resistin concentration in rats.

MATERIALS AND METHODS

Permissions

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

Animals and design

Thirty-two male rats (Sprague–Dawley family, 8 weeks old, 200±17 g body mass, mean±s.d.) were used in this cross-sectional study. Animals were kept in the Baqiyatallah University of Medical Science in the animal house in special cages in which the floor was covered with clean wood chips. The temperature was 22 ±2°C and the humidity was 45–50%, with a 12 h light:12 h dark cycle. Special standard compressed food (Behparvar Company, Karaj, Iran) for laboratory rats (crude protein 19.50–20.50%, fat 3.5–4.5%, fibre 4–4.5%, calcium 0.95–1%, phosphorus 0.65–0.7%, salt 0.5–0.55%, lysine 1.15%, methionine 0.33%, threonine 0.72%, tryptophan 0.25%, energy 16.16–17 mJ kg^{-1}) was provided at regular times. The cages were fitted with urban filtered water in 500 ml bottles. Rats were randomly allocated into four groups (N=8 in each group): control, 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). The training groups undertook eccentric resistance exercise training on a ladder while the non-exercise groups were limited to low intensity activity (i.e. walking around the cage).

Thirty minutes prior to exercise training, the HMB groups orally consumed the supplement (Beta-TOR, by intragastric gavage) at a dose of 76 mg kg^{-1} day^{-1} while the non-supplement groups orally consumed a saline placebo. The dosage equivalent in human studies is 3–6 g day^{-1} for an 80 kg person (Gallagher et al., 2000).

Training protocol

Eccentric resistance exercise training was performed using a ladder (manufactured by the Exercise Physiology Research Center, Life Style Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran). The ladder was made of wood with iron steps which had a height of 1.1 m, an inclination of 80 deg and consisted of 26 steps in total. The ladder was designed to make the rats descend the ladder while imposing a constant load. The rats performed 10–12 dynamic movements (repetitions) during each landing so the intensity differed. Rats exercised on the ladder with a free load for a week as a pre-training adaptation and to allow them to become accustomed to the exercise. Subsequently, the rats performed the ladder descent exercise with a weighted backpack. The exercise was loaded as follows: one repetition of ladder exercise was conducted at 50%, 75%, 90%, 100% and 120% of one-repetition maximum (1 RM, considered to be 50% of the rat's body mass), after which 30 g was added for each trial up to a maximum of eight trials. Training ended before the 8th trial if rats showed signs of exhaustion, such as being unable to descend or hanging from the ladder. On completion, the final load of the first session was recorded as the 1 RM for the next session (Fig. 1). Eccentric resistance exercise was performed 3 times a week for 6 weeks for a duration of 25 min per session.

Serum and triceps brachii muscle collection

Exactly 24 h after the last training session, rats were terminally anaesthetized by intraperitoneal administration of a mixture of ketamine (30–50 mg kg^{-1} body mass; Iman and Saba, Shiraz, Iran) and xylazine (3–5 mg kg^{-1} body mass; Iman and Saba). Blood was collected into tubes and immediately processed for serum preparation (10 min centrifugation at 1000 ***g***). Serum was then

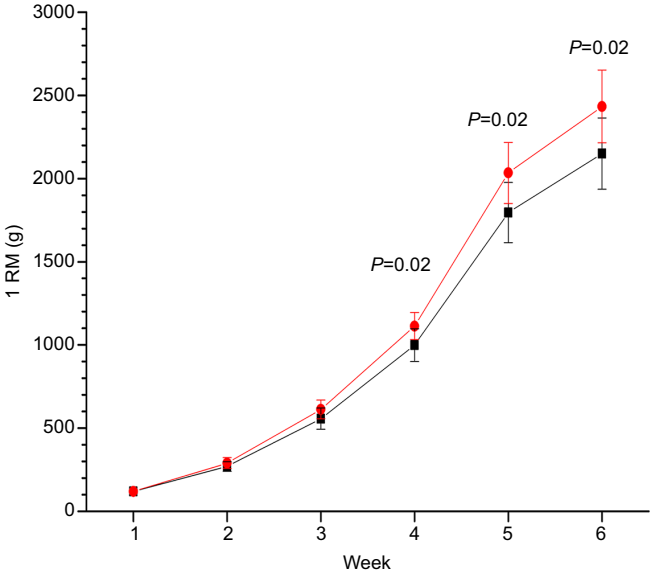

Fig. 1. One-repetition maximum (1 RM) values during the training protocol. Data show the 1 RM (see Materials and Methods) of eccentric resistance training (ERT; black), and β-hydroxy-β-methylbutyrate free acid supplementation plus eccentric resistance training (HMB+ERT; red) groups. Significant differences between groups are indicated. N=8 in each group.

Journal of Experimental Biology (2019) 222, jeb198424. doi:10.1242/jeb.198424

Table 1. Body mass of rats in the four experimental groups

	Body mass (g)					
Group	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Control	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

Experimental groups were as follows: control, 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). *N*=8 in each group.

stored at −80°C for later analysis. Triceps brachii muscle was excised, cleaned, divided into three pieces, washed in ice-cold saline, and immediately frozen in liquid nitrogen and stored at −80° C until RNA extraction.

Serum analysis

Serum concentrations of irisin, nesfatin-1 and resistin were analysed by ELISA (BioVendor Laboratory Medicine, Brno, Czech Republic) under standard operating procedures. The kit sensitivity for irisin, nesfatin-1 and resistin was 0.01, 14 and 0.25 ng ml^{-1}, respectively. Irisin and nesfatin-1 kit inter- and intra-assay coefficients of variation were 10% and 8%, respectively; resistin kit inter- and intra-assay coefficients of variation were 7% and 5%, respectively.

Evaluation of gene expression

RNA extraction was performed using the AccuZol RNA purification kit (cat. no. k3090, Bioneer, Daejeon, Republic of Korea) with 85–95 mg of triceps brachii muscle for each sample. cDNA was prepared using the AccuPower RT PreMix cDNA synthesis kit (Bioneeer), according to the manufacturer's instructions, with oligo-$(dt)_{18}$ primers (0.25 µg per reaction). Real-time PCR (qPCR) was performed using a Real Time PCR Machine (Corbett, Mortlake, NSW, Australia) with a QuantiFast SYBR Green PCR kit (cat. no. 204052, Qiagen GmbH, Hilden, Germany) in a 15 µl reaction volume, containing 0.5 µl single-strand cDNA, 7.5 µl Master Mix, 1 µl of the each forward and reverse primer (5 pmol $µl^{-1}$) and 5 µl dH_2O. PGC-1α sense primer was 5′-GACCCTCCTCACACCAAAC-3′ and antisense primer was 5′-GCGACTGCGGTTGTGTATG-3′ (Shi et al., 2013). *β-Actin* was used as a normalizer gene, with sense and antisense primers 5′-TATCGGCAATGAGCGGTTCC-3′ and 5′-CACTGTGTTGGC-ATAGAGG-3′, respectively (Rahmati-Ahmadabad et al., 2017).

Statistical analysis

qPCR cycle threshold (CT) was analysed by the Pfaffl (2001) method. All data were stored and analysed using SPSS software (IBM, version 24). The Kolmogorov–Smirnov test was used to assess data distribution and Levene's test was used to assess the equality of variances. Repeated-measures ANOVA was used to identify any differences in rat body mass for the duration of the study as well as changes in 1 RM. In order to infer differences between groups, two-way ANOVA and Tukey *post hoc* test was used. Correlations were calculated using Pearson product moment correlation. Because of the low sample size, non-parametric tests including the Freidman test and Spearman correlation were also conducted but this did not alter the interpretation of the findings so only the results of the parametric tests are presented. Effect size (ES) was reported to emphasize the size of the difference rather than confound the sample size. Significance was accepted if $P<0.05$. Data are presented as means±s.d. unless otherwise stated.

RESULTS

There was no difference in body mass between groups ($F_{5,140}=0.40$, $P=0.84$; ES=0.01) (Table 1). The mean weekly 1 RM of the exercise training groups initially (weeks 1–3) showed similar levels, as can be seen in Fig. 1. However, 1 RM was significantly higher in the HMB+ERT group versus the ERT group in week 4 (1113.62±81.30 versus 998.68±97.98 g, $F_{1,14}=6.52$, $P=0.02$; ES=0.31), week 5 (2033.89±183.61 versus 1795.38±180.56 g, $F_{1,14}=6.86$, $P=0.02$; ES=0.32) and week 6 (2433.63±217.91 versus 2150.56± 214.30 g, $F_{1,14}=6.85$, $P=0.02$; ES=0.33) (Fig. 1).

Training groups (ERT and HMB+ERT) had higher tissue PGC-1α mRNA expression than non-training groups ($F_{1,28}=93.74$, $P=0.001$; ES=0.77) (Fig. 2A). *PGC-1α* gene expression was significantly higher in HMB groups (HMB and HMB+ERT) than in the control group ($F_{1,28}=11.59$, $P=0.002$; ES=0.29). The ERT+HMB group had the greatest *PGC-1α* gene expression ($F_{1,28}=5.52$, $P=0.02$; ES=0.16) (Fig. 2A).

For serum irisin, data analysis showed that there was a higher concentration in training groups (ERT and HMB+ERT) versus non-training groups ($F_{1,28}=104.78$, $P=0.001$; ES=0.78). (Fig. 2B). Serum irisin concentration was significantly higher in HMB groups (HMB and HMB+ERT) than in the control group ($F_{1,28}=22.59$, $P=0.001$; ES=0.44). The highest irisin concentration was measured for the HMB+ERT group ($F_{1,28}=4.53$, $P=0.04$; ES=0.13) (Fig. 2B).

For serum nesfatin-1, data analysis showed a higher concentration in training groups (ERT and HMB+ERT) versus non-training groups ($F_{1,28}=31.46$, $P=0.001$; ES=0.52) (Fig. 2C). The results showed a higher concentration of serum nesfatin-1 in HMB groups (HMB and HMB+ERT) than in the control group ($F_{1,28}=34.76$, $P=0.001$; ES=0.55). The highest serum nesfatin-1 concentration was in the HMB+ERT group ($F_{1,28}=18.87$, $P=0.001$; ES=0.40) (Fig. 2C).

For serum resistin, data analysis showed that there was a lower concentration in training groups (ERT and HMB+ERT) versus non-training groups ($F_{1,28}=63.44$, $P=0.001$; ES=0.69) (Fig. 2D). The results showed that serum resistin concentration was significantly lower in HMB groups (HMB and HMB+ERT) than in the control group ($F_{1,28}=34.09$, $P=0.001$; ES=0.54). The lowest serum resistin

Table 2. Correlation between PGC-1α mRNA expression and serum concentration of irisin, nesfatin-1 and resistin

Group	Irisin	Nesfatin-1	Resistin
Control	*r*=0.10	*r*=0.21	*r*=0.18
	P=0.42	*P*=0.32	*P*=0.32
HMB	*r*=0.54	*r*=0.48	*r*=−0.54
	P=0.12	*P*=0.12	*P*=0.14
ERT	*r*=0.63	*r*=0.60	*r*=−0.86
	P=0.09	*P*=0.10	*P*=0.05
HMB+ERT	*r*=0.95	*r*=0.85	*r*=−0.89
	P=0.01*	*P*=0.01*	*P*=0.01*

Pearson's correlation coefficients; *$P<0.05$.

Journal of Experimental Biology (2019) 222, jeb198424. doi:10.1242/jeb.198424

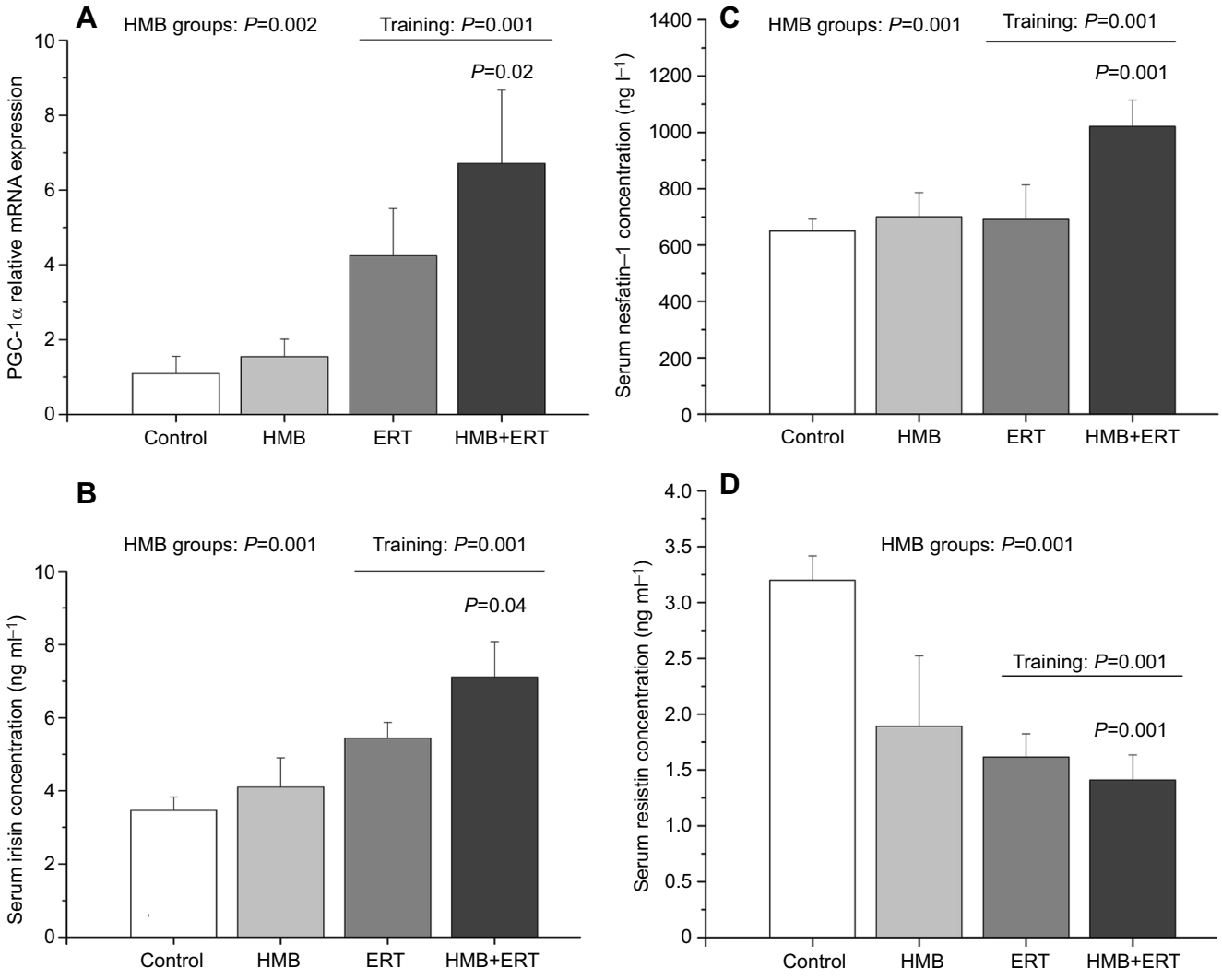

Fig. 2. PGC-1α mRNA expression and serum irisin, nesfatin-1 and resistin concentration in the four experimental groups. Skeletal muscle PGC-1α mRNA expression relative to that of β-actin (A), and serum irisin (B), nesfatin-1 (C) and resistin (D) concentration in the control, β-hydroxy-β-methylbutyrate free acid supplementation (HMB), eccentric resistance training (ERT), and β-hydroxy-β-methylbutyrate free acid supplementation plus eccentric resistance training (HMB+ERT) groups. Significant differences from control are indicated. *N*=8 in each group.

concentration was in the HMB+ERT group ($F_{1,28}$=18.01, P=0.001; ES=0.39) (Fig. 2D).

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

DISCUSSION

The findings of this study showed that eccentric resistance training resulted in greater skeletal muscle *PGC-1α* relative gene expression, increases in serum concentrations of irisin and nesfatin-1 and decreases in serum concentrations of resistin compared with control. In addition, HMB-FA supplementation resulted in increased skeletal muscle *PGC-1α* relative gene expression, increased serum concentrations of irisin and nesfatin-1, and decreased serum concentrations of resistin compared with control. The most important findings of the present study is that a combination of eccentric resistance training and HMB-FA supplementation had a cumulative and greater effect on variables compared with exercise or HMB-FA supplementation alone.

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

Different modes of exercise training can affect *PGC-1α* gene expression, but resistance training has little effect on the AMPK/PGC-1α pathway (Jacobs et al., 2014). Resistance training increases phosphorylation of the anabolic Akt/mTOR signalling pathway, as well as activation of the translation initiation regulators p70 S6k, 4E-BP1 and eIF2B (Atherton et al., 2005). In contrast, aerobic endurance exercise increases phosphorylation of AMPK and protein levels of PGC-1α (Atherton et al., 2005). However, in the present study, we observed enhanced *PGC-1α* gene expression in response to eccentric resistance training due to the similarities with aerobic endurance training as both are able to act via the AMPK/PGC-1α pathway.

The results of previous studies indicate that physical training can increase irisin concentration. Daskalopoulou et al. (2014) found plasma levels of irisin increased in response to increased exercise load in active, young people running on a treadmill. Also, Boström et al. (2012) highlighted that irisin increased after 3 weeks of aerobic training in rats and led to an increase in energy expenditure and improved glucose homeostasis. Huh et al. (2012) demonstrated that circulating irisin levels were significantly upregulated 30 min after acute exercise. To our knowledge, this is the first study to report an increase in serum irisin concentration following chronic eccentric resistance exercise in rats. The results of this study are consistent with the results of previous studies that investigated responses to other types of exercise training.

Plausible mechanisms for how exercise can increase irisin concentration have been posited. Researchers have shown that exercise increases PGC-1α levels in skeletal muscle and increases the muscle-bearing FNDC5 membrane protein that results in the production of irisin (Schnyder and Handschin, 2015). AMPK activation during exercise is one of the factors involved in increasing PGC-1α and irisin levels (Chavanelle et al., 2017). AMPK activation leads to the phosphorylation of PGC-1α as a modifier of FNDC5 and irisin secretion (Dinas et al., 2017). Also, PGC-1α activates PPARγ, which is involved in energy metabolism and stimulates increases in FNDC5 and irisin (Panati et al., 2016). A relationship between irisin levels and the precursor of FNDC5 and PGC-1α has been highlighted (Dinas et al., 2017). The results of the present study showed a significant and positive correlation between *PGC-1α* gene expression and plasma concentrations of irisin. The eccentric resistance training is likely to activate the PGC-1α activating signals, which may trigger a signal cascade to change the phenotype of the adipose tissue. Eccentric resistance training leads to energy consumption and heat production by increasing muscular tissue to fat tissue ratio and increasing UCP1 (Chavanelle et al., 2017), thus increasing PGC-1α, FNDC5 and irisin (Dinas et al., 2017).

The production and secretion of irisin from the muscle is also mediated by SMAD3 (mothers against decapentaplegic homolog 3). SMAD3 is a molecule that changes energy metabolism and regulates body mass. SMAD3 suppresses FNDC5 and PGC-1α in skeletal muscle and negatively regulates plasma irisin (Tiano et al., 2015). Exercise induces phosphorylation of SMAD2 and subsequently SMAD3 (Tiano et al., 2015). However, SMAD3 was not measured in the present study so future research should investigate this possible mechanism for increasing irisin in response to eccentric resistance training.

Ghanbari-Niaki et al. (2013a) evaluated the effect of 8 weeks of endurance training (5 days a week for 60 min at a speed of 25 m min^{-1} with a zero gradient) on tissue nesfatin-1 gene expression and plasma levels of nesfatin-1. Their results indicated that training increased the expression and plasma levels of nesfatin-1, which was related to plasma high-density lipoprotein concentration. Nesfatin is involved in the regulation of energy homeostasis and metabolism, and improves insulin sensitivity (Dore et al., 2017). The effect of exercise on nesfatin-1 has not been clearly recognized or studied in response to eccentric resistance training. However, there are possible mechanisms by which this could occur. Studies have shown that nesfatin-1 concentration is affected by various factors (Atici et al., 2017; Ayada et al., 2015; Chaolu et al., 2011; Dore et al., 2017; Li et al., 2014). For example, it has been shown that starvation in rats decreases serum nesfatin-1 levels up to 18% (Stengel et al., 2009). However, it has been reported that nesfatin-1 concentrations returned to normal 1–12 h after refeeding (Dore et al., 2017). In addition, some studies have shown that there is a direct relationship between nesfatin-1 and cortisol levels. Central injection of nesfatin-1 increased adrenocorticotropin levels (Ge et al., 2015). According to previous studies, cortisol and adrenocorticotropin are elevated as a result of eccentric resistance training protocols. These changes could be considered a possible cause of the subsequent increase in nesfatin-1. Adipose tissue also secretes various inflammatory cytokines that affect the expression and secretion of adipokines. For example TNF-α has different effects on adiponectin, leptin and nesfatin-1 (Hector et al., 2007; Medina et al., 1999; Ayada et al., 2015; Ramanjaneya et al., 2010). Studies have shown that TNF-α, IL-6 and insulin increase the intracellular expression of nesfatin-1 in cultured fat cells (Ayada et al., 2015). These findings show that the expression and secretion of nesfatin-1 are regulated by different pathways.

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

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

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

The present study showed that HMB enhances the positive effects of resistance training on strength (1 RM). Li et al. (2012) showed that leucine (0.5 mmol l^{-1}) increases expression of PGC-1α 3- to 5-fold in C2C12 cell models. Vaughan et al. (2013) reported that leucine (0.1–0.5 mmol l^{-1}) dose-dependently enhanced PGC-1α expression in skeletal muscle cells. Just one study demonstrated the effects of HMB on irisin (Baggett et al. 2013). Baggett et al. (2013) investigated the synergistic effects of leucine and its metabolites with polyphenols on irisin in myotubes and diet-induced obese

mice. They demonstrated that leucine–polyphenol combinations stimulate irisin and PGC-1α (Baggett et al., 2013). To our knowledge, no previous research has examined the effects of HMB on nesfatin-1 and resistin. The results of the present study showed that serum nesfatin-1 increases and serum resistin decreases responses to HMB-FA supplementation. The mechanism of HMB-induced change in nesfatin-1 and resistin is not understood and requires further research.

Limitations

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

Conclusions

The most important findings of the present study are that a combination of eccentric resistance training and HMB-FA supplementation has a greater effect on the primary outcome compared with exercise or supplement intervention alone. Exercise and HMB-FA supplementation could increase *PGC-1α* gene expression, which may regulate other peptide-releasing tissues and change serum concentrations of irisin, nesfatin-1 and resistin. We found that HMB-FA supplementation with eccentric resistance training may induce crosstalk between releasing peptides from other tissues and increases maximal muscle strength. Further research is needed to determine the effects of other peptides to enable us to make further inferences about crosstalk.

Acknowledgements

The authors appreciate the helpful comments of A. Ghanbari-Niaki (Exercise Biochemistry Division, Faculty of Sport Sciences, University of Mazandaran, Babolsar, Mazandaran, Iran).

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: H.S., S.R.; Methodology: H.S., S.R.; Software: H.S., S.R., R.M.; Validation: S.R., D.R.B.; Formal analysis: S.R., D.R.B., R.M.; Investigation: H.S., S.R., D.R.B.; Resources: S.R.; Data curation: S.R., R.M.; Writing - original draft: H.S., S.R.; Writing - review & editing: S.R., D.R.B., R.M.; Visualization: S.R.; Supervision: S.R.; Project administration: H.S.; Funding acquisition: H.S., R.M.

Funding

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

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