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# PPAR $\gamma$ /Pgc-1 $\alpha$ -Fndc5 pathway up-regulation in gastrocnemius and heart muscle of exercised, branched chain amino acid diet fed mice

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## Abstract

**Background:** Previous studies have revealed the inductive effect of branched-chain amino acids (BCAAs) catabolism on fatty acid oxidation and metabolism, especially in muscle cells. In the present investigation, we have attempted to address whether a combination of BCAAs supplement consumption with aerobic exercise could elaborate the expression of *PPAR $\gamma$* , *Pgc-1 $\alpha$*  and *Fndc5* genes in gastrocnemius muscle and heart tissue of male C57BL/6 mice.

**Methods:** Thirty-six young male mice with an average weight of  $18 \pm 2$  g were selected. Mice were randomly assigned to 6 groups: 20 mg/mL of BCAAs consumption with simultaneous exercise-training, 60 mg/mL of BCAAs consumption with simultaneous exercise-training, exercise-trained with no BCAAs consumption group, 20 mg/mL BCAAs without exercise-training, 60 mg/mL BCAAs without exercise-training, and untrained mice without BCAAs consumption.

**Results:** The findings showed a combination of 20 mg/mL BCAAs with aerobic exercise significantly increased *Fndc5*, *PPAR $\gamma$* , *Pgc-1 $\alpha$*  gene expression in skeletal muscles although, circulating Irisin levels remained unchanged ( $p < 0.05$ ). Interestingly, plasma urea and lactate levels were significantly increased in 60 mg/mL BCAAs administrated mice which performed exercised ( $p < 0.05$ ). Two-way analysis of variance (ANOVA) was used to examine significant difference between groups and sedentary group.

**Conclusions:** Results showed inductive effect of 20 mg/mL BCAAs on expression levels of *Fndc5*, *PPAR $\gamma$* , *Pgc-1 $\alpha$*  in gastrocnemius muscle similar with counterparts in heart tissue. Of note, higher serum irisin levels were detected after 20 mg/mL BCAAs supplementation coincided with the exercise.

**Keywords:** Aerobic exercise, Branched-chain amino acids, Catabolism, *Fndc5*, Irisin, *Pgc-1 $\alpha$* , *PPAR $\gamma$*

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## Background

Branched Chain Amino Acids (BCAAs), including Leucine, Isoleucine, and Valine, are essential amino acids that body is unable to synthesize and needs to be provided by the diet. BCAAs play a significant role in energy homeostasis as they are important for the maintenance of skeletal muscle [1]. Restorative effects of BCAAs on muscle soreness and fatigue are already well recognized [2, 3]. A great proportion of BCAAs metabolism occurs in skeletal muscle, where metabolism is regulated. In muscle, BCAAs stimulate mammalian target of rapamycin complex 1 (mTORC1) and protein synthesis and inhibit protein catabolism [4–6]. Moreover, BCAAs improve aerobic capacity and threshold of anaerobic activities in human. Previous studies have shown that consumption of BCAAs increases lipid metabolism and reduces concentration of skeletal muscle triglycerides. Interestingly, dietary BCAAs supplementation has intensified lifetime via promotion of mitochondrial biogenesis and enhancement in *Sirtuin 1* (*Sirt1*) expression besides a reduction in oxidative damage [7–11]. Therefore BCAAs can be beneficial for energy expenditure and health status.

Recent studies revealed substantial role of fibronectin type III domain containing 5 (FNDC5) and its secretory type, irisin, in energy metabolism and regulation [12, 13]. *FNDC5* expression is induced by exercise [12, 13]. Huh et al. have shown that plasma irisin and expression of *FNDC5* are increased in response to acute exercise [15]. Of significant worth is the positive association between oxidative components and ROS with irisin content in trained mice [16].

Another key player in regulation of metabolism is peroxisome proliferator-activated receptor gamma (*PPAR* $\gamma$ )/peroxisome proliferator-activated receptor gamma coactivator 1-alpha (*Pgc-1* $\alpha$ ) axis. *Pgc-1* $\alpha$  stimulates the expression of nuclear respiratory factor (NRF), which leads to expression of mitochondrial transcription factor A (TFAM) and mitochondrial biogenesis. *Pgc-1* $\alpha$  stimulates and promotes mitochondrial biogenesis and capillary density as well as exercise capacity [10, 17, 18]. Consequently, aerobic exercise, also could increase *PGC-1* $\alpha$  expression [19]. Therefore it seems that there is a positive feedback on exercise capacity by *PGC-1* $\alpha$ .

Another key factor for promotion of mitochondrial biogenesis, oxidative capacity and preventing of the mitochondrial dysfunction in skeletal muscle is *Sirt1* [20].

Also, up-regulation of mitochondrial biogenesis and increase in *PGC-1* $\alpha$  expression have been shown in a previous study [10]. Moreover, Aydin et al. indicated cardiac muscle produces more irisin than skeletal muscle [21]. Furthermore, BCAAs supplementation has been indicated to lead an increment of performance in rats subjected to moderate-intensity training [3]. Thus, the aim of present study is determination of BCAAs

supplementation effects in association with aerobic training on plasma irisin and expression of gastrocnemius muscle *Fndc5*, *PPAR* $\gamma$ , *Pgc-1* $\alpha$ , *Sirt1* in male C57BL/6 mice.

## Methods

### Bioinformatics studies

A list of BCAAs and FNDC5 related proteins were extracted by an intensive literature review and text mining from a period of 2013–2017 (list is not shown). This list was subjected to the STRING V10.5 (<https://string-db.org/>) web site [22]. In order to obtain the maximal data of FNDC5 and related proteins network, a low confidence level ( $> 0.150$ ) was set. Among the visual network, which was obtained, *PPAR* $\gamma$ , *Pgc-1* $\alpha$ , *Sirt1* were shown to be interacted with FNDC5 straightly. At the next step, STRING default confidential level was set to  $> 0.400$  (mid confidence level) in order to define the interactions of *PPAR* $\gamma$ , *Pgc-1* $\alpha$ , *Sirt1* with other proteins. We also assessed similarity of studied protein network between human and mouse via STRING V10.5.

### Ethical issue

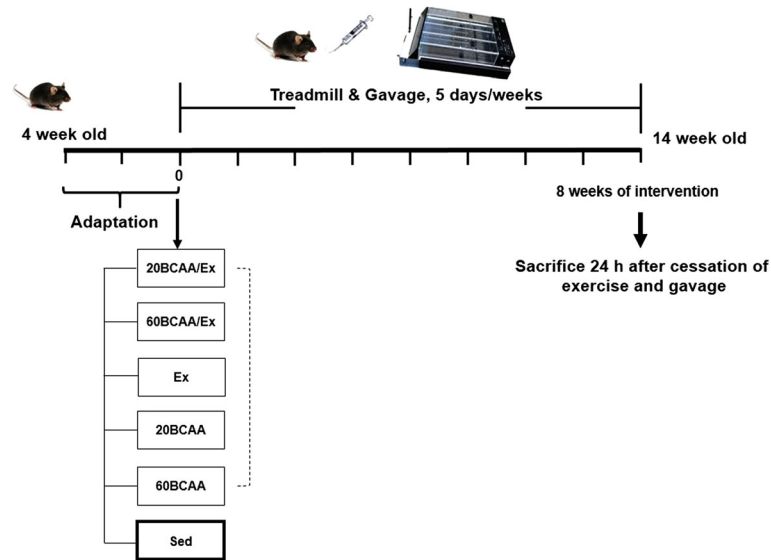
Approval to perform experiments on mice was obtained by the ethics committee of Royan Institute (IR.ACECR.ROYAN.REC.1396.19).

### Animals and protocols

Four-week old wild type male C57BL/6 mice, were provided by Royan Institute (Tehran, Iran). Mice were housed in a temperature controlled room ( $24 \pm 3$  °C) with a humidity of 65% ( $\pm 5\%$ ) and 12 h light/12 h dark cycle (lights from 08:00 am to 8:00 pm). Mice were allowed to acclimate for two weeks prior to the start of the experiment. Mice at their 6th weeks of age with an approximate weight of  $18 \pm 2$  g, were randomly divided into six groups ( $N = 6$ ). The groups were: (20BCAA/Ex): supplemented with 20 mg/mL of BCAAs with simultaneous exercise-training; (60BCAA/Ex): supplemented with 60 mg/mL of BCAAs with simultaneous exercise-training; (Ex): nonsupplemented, undergoing exercise; (20BCAA): supplemented with 20 mg/mL BCAAs; (60BCAA): supplemented with 60 mg/mL BCAAs (Sed): untrained mice without BCAAs intake (Fig. 1).

### BCAAs supplementation and food intake

Mice were fed with free access to standard food (13% (w/w) fat, 30% (w/w) protein, 57% (w/w) carbohydrate with a total 2900 kcal) and tap water. BCAAs (Bio nutrition, USA) were dissolved in distilled water and applied by gavage (500  $\mu$ L) once per day for 8 weeks (20 mg/mL/day for 20BCAA group and 60 mg/mL/day for 60BCAA group). The reason for selecting 20 and 60 mg/mL of BCAAs was that 20 mg/mL of BCAAs was



**Fig. 1** Schematic representation of protocol used for BCAAs supplementation and exercise. As depicted, acclimation was performed for two weeks. Then, mice were randomly divided to training ( $n = 6$ ) and sedantary (Sed;  $n = 6$ ) groups. Training groups were EX as exercised group. 20BCAA and 60BCAA groups were mice supplemented with 20 and 60 mg/mL of BCAAs respectively. 20BCAA/EX and 60BCAA/EX were exercised groups supplemented with 20 and 60 mg/mL of BCAAs respectively. BCAAs supplementation was performed by oral gavage (500  $\mu$ L) 5 days per week. On the other hand mice were exercised by placing on the motor-driven treadmill as described in materials and methods for 5 days per week. Exercise and BCAAs supplementation was carried out as an intervention for 8 weeks. One day after the final session of intervention (gavage or exercise), mice were sacrificed for further experiments

already indicated to contain beneficial effects [9, 23]. Therefore, 60 mg/mL of BCAAs (three times more concentrated dose) was considered as an overdose.

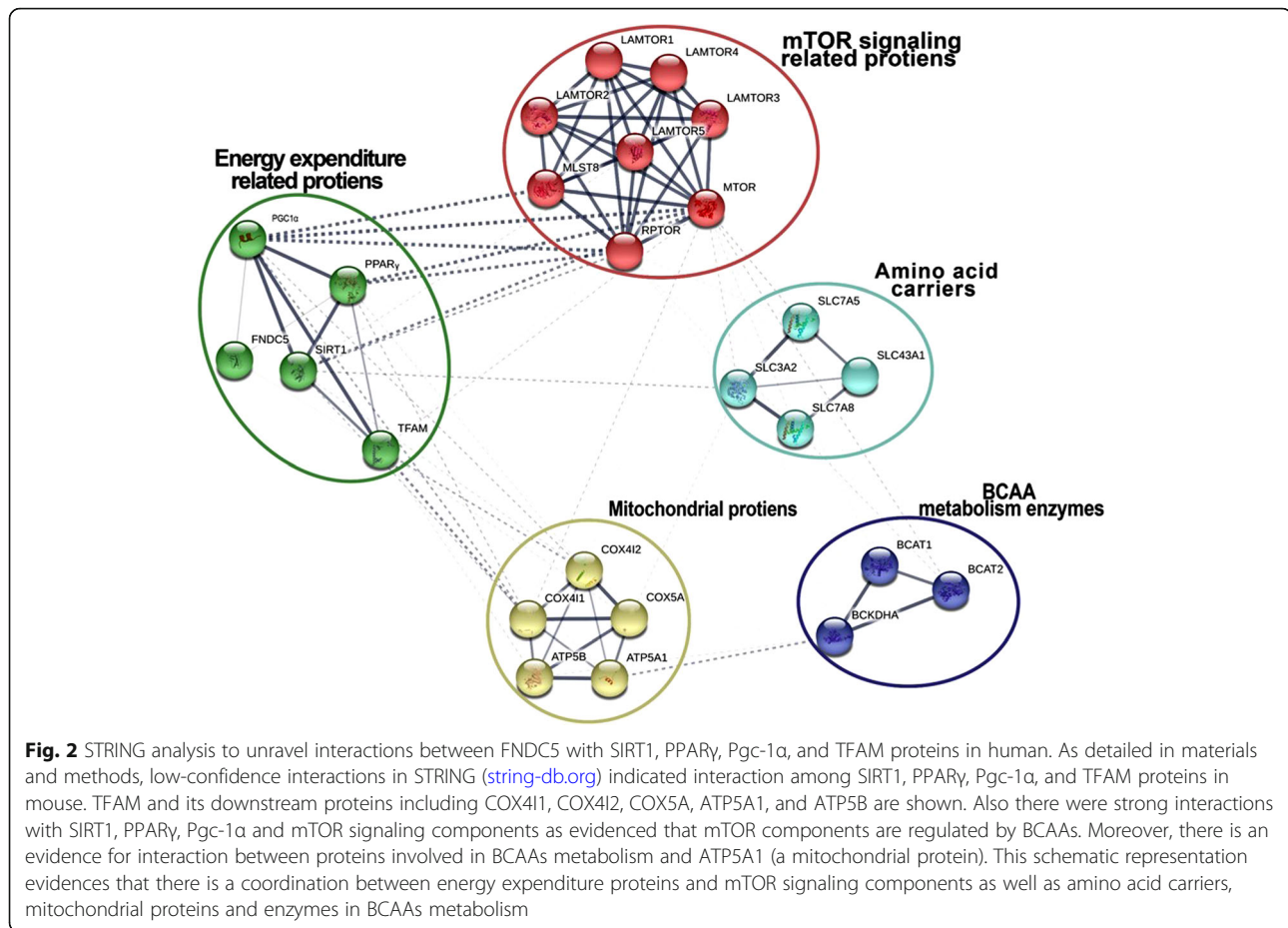
**Exercise training protocol, tolerance, and motor coordination tests**

Endurance exercise training (Ex) and exhaustion tests were performed on a motorized treadmill. Ex was performed briefly as a type of moderate-high intensity exercise on treadmill for 8 weeks (5 days/week). After being acclimated to treadmill exercises over two weeks, running speed and duration of exercise were progressively increased to reach  $\sim 70\%$   $VO_2$  max (25 m/min). Whole exercise training was 45 min. The grade of treadmill slope

was set to 0% and the speed of treadmill was set to increase in a rate of 3 m/min, initiating from 10 m/min to the final speed of 25 m/min ( $\sim 70\%$   $VO_2$  max) [3, 19, 24]. Fixed speed rotarod was implemented to measure motor coordination and balance. Each mouse was received three trials per day for two consecutive days. The mice were trained at an initial constant speed of 8 rpm for 5 min. Then the speed was increased to 30 rpm for 5 min and the latency to fall was recorded. Time score was measured as the mean of the best performances over the three trials at the second day [10]. After 8 weeks of exercise and oral consumption of BCAAs, mice were fasted for 7 h before euthanasia. Mice were euthanized under the combined administration of xylazine (10 mg/kg

**Table 1** Primer list

Gene	Forward primer (5'-3')	Reverse primer (5'-3')	Annealing temperature ( $^{\circ}$ C)	Accession no.
<i>Gapdh</i>	TGCCGCCTGGAGAAACC	TGAAGTCGCAGGAGACAACC	58.6	NM_008084.2
<i>Pgc-1a</i>	CCCTGCCATTGTAAAGACC	TGCTGCTGTTCTGTTTC	52	NM_008904.2
<i>Fndc5</i>	TCATTGTTGTGGTCCTCTTC	GCTCGTTGTCCTTGATGATA	60	NM_027402.3
<i>PPAR<math>\gamma</math></i>	TGAGACCAACAGCCTGAC	GTTCACCGCTTCTTCAAATC	58	NM_001127330.1
<i>ATP5a1</i>	TCCGCTTACATTCCAACA	ACACAGACAAACCCACAT	54	NM_007505.2
<i>ATP5b</i>	GGTAGCGTTGGTATATGG	CTCCTGGTCTCTGAAGTA	54	NM_016774.3
<i>Cox4i1</i>	ATGGGAGTGTGTGAAGAGT	CATCAGGCAAGGGGTAGT	54	NM_009941.3
<i>Tfam</i>	CTTCAACCACCACCACT	AATCTCTAAGCCTCTCAATACAA	56	NM_009360.4
<i>Sirt1</i>	GGCAGTAACAGTGACAGT	CTCTCCGTATCATCTTCCAA	61	NM_001159589.2



body weight per mouse) and ketamine (80 mg/kg body weight per mouse) (Fig. 1).

#### Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from gastrocnemius muscle and heart using TRIzol reagent (Sigma, USA). Samples were treated with DNaseI (TaKaRa) to remove contaminating genomic DNA. cDNA synthesis was performed with 1  $\mu$ g of total RNA by cDNA synthesis kit according to the manufacturer's instruction (TaKaRa). qRT-PCR was carried out with CYBR green (TaKaRa, Japan) using Corbet rotor gene 6000 (Qiagen, Australia). Assessment of gene expression was performed according to  $\Delta\Delta CT$  method. Expression level of genes was reported relative to *glyceraldehyde-3-phosphate dehydrogenase* (*Gapdh*) expression level as already was implemented in similar studies [25–27].

Primers were ordered from micro-gene (South Korea) and their sequences are listed in Table 1.

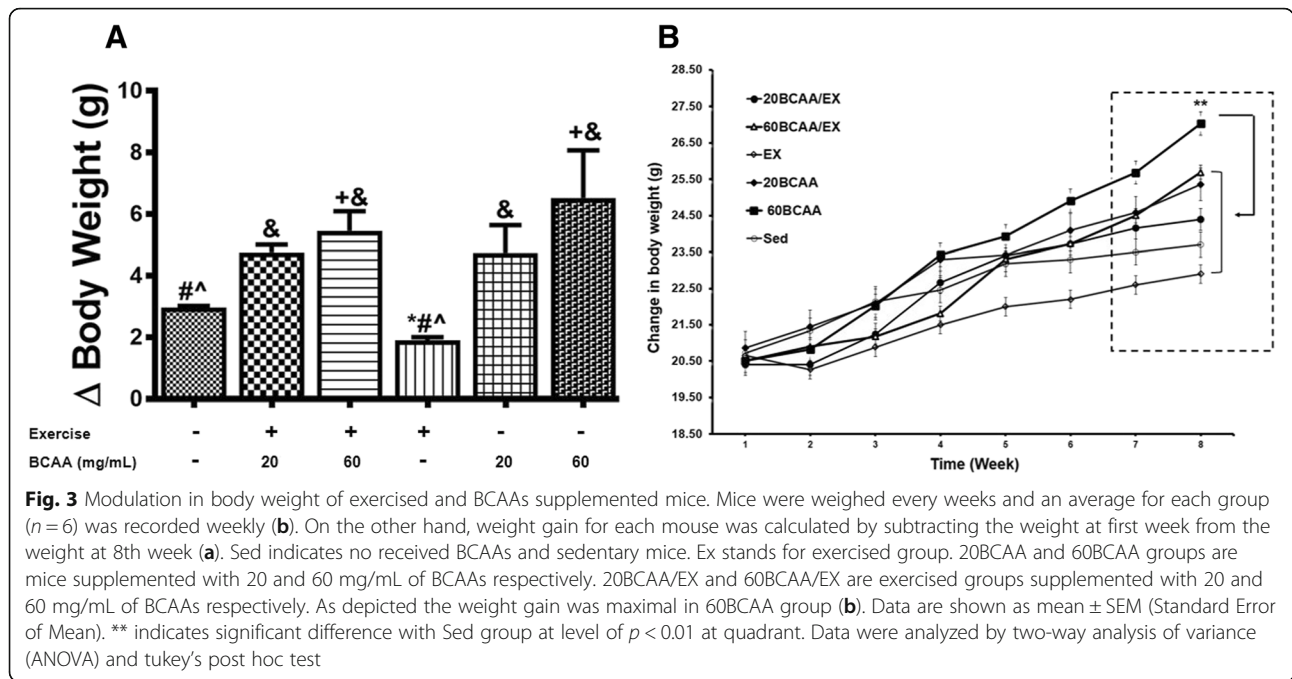
#### Protein extraction and western blotting

Tissues were lysed using TRI reagent (Thermo Scientific, 15,596–018), according to the manufacturer protocol. Equal amounts of each protein sample (30  $\mu$ g) were

separated by SDS- PAGE and transferred to PVDF membranes (Bio Rad, 162–0176). After blocking the membranes with 10% skim milk (Millipore, 115,363), membranes were incubated with different primary antibodies for 2 h at room temperature. Primary antibodies were rabbit anti-FNDC5 antibody (1:2000, Abcam, AB174833) and mouse anti GAPDH antibody (1:5000, Santa Cruz). Then, membranes were incubated for 1 h at room temperature with an appropriate secondary antibody: horseradish peroxidase (HRP)-conjugated goat antimouse IgG (1:5000, Dako, P0447), or HRP-conjugated goat anti-rabbit IgG (1:16000, Santa Cruz, SC2301). HRP-conjugated IgG bound to each protein band was visualized by an Amersham ECL Advance Western Blotting Detection Kit (GE Healthcare). The intensity of each band was quantified by Image J software.

#### Plasma measurement of irisin, urea and lactate

Blood was collected from the right ventricle of each mouse and drawn into an EDTA containing tube. To separate plasma, blood containing tubes were centrifuged for 15 min at 1600 $\times$ g at 4  $^{\circ}$ C. All samples were frozen in liquid nitrogen and stored at  $-80$   $^{\circ}$ C until



further use. Plasma irisin levels were measured using a commercially available enzyme-linked immunosorbent assay ELISA Kit (EK-067-29, Phoenix, USA). The assay kit was highly sensitive to irisin in mice and detection range of this kit was 0.1–1000 ng/mL. Meanwhile, plasma urea concentration (Pars Azmon, Iran) and lactate (Biorex Fars, Iran) were measured according to the manufacturer's instructions.

**Statistical analysis**

Statistical analysis was performed using GraphPad Prism Software (Version 7.0a Graph Pad Software Inc., La Jolla, CA). Kolmogorov–Smirnov test was used for normalizing of distribution, and variables were normally distributed. Results are presented as mean  $\pm$  standard error of mean (SEM). Data of RT-qPCR and western blotting were obtained on triplicate data sets for each sample and analyzed by two-way analysis of variance (ANOVA) with tukey's post hoc test due to multiple comparisons. Differences at  $p < 0.05$  were considered to be significant in all analyses.

**Results**

**Bioinformatics analyses indicated a connection ring of FNDC5 with SIRT1, PPAR $\gamma$ , Pgc-1 $\alpha$ , and TFAM**

STRING low confidence level showed a text mining-based interaction between FNDC5 with SIRT1, PPAR $\gamma$ , Pgc-1 $\alpha$ , and TFAM (Fig. 2). Next, analyses of high-confidence interactions indicated a strong interaction among SIRT1, PPAR $\gamma$ , Pgc-1 $\alpha$ , and TFAM proteins (Data not shown). As

TFAM is in connection with mitochondrial DNA (mtDNA) replication and transcription activity, its downstream proteins including COX4I1, COX4I2, COX5A, ATP5A1 and ATP5B are also involved. Of important, there is a high similarity in protein sequences between human and mouse for a number of aforementioned proteins emphasizing similar text mining-based interactions between these proteins in human (Additional file 1: Figure S1) [22].

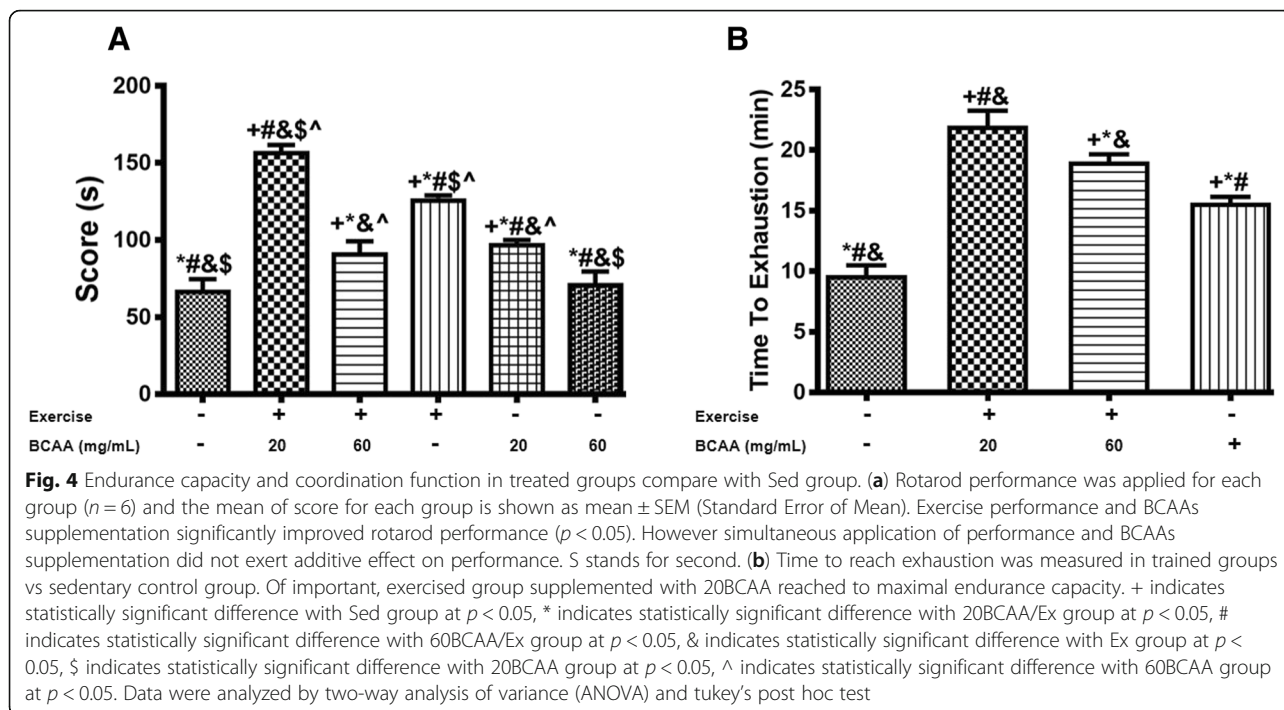
**Animal characteristics measurements**

Body weight (Fig. 3a, b) and gastrocnemius muscle weight (Table 2) were significantly increased in 60BCAA group.

**Table 2** Heart and gastrocnemius muscle weight of mice

BCAAs (mg/mL)	Exercise	Heart Weight (g)	Gastrocnemius Weight (g)
–	–	0.14 $\pm$ 0.01 <sup>*#&amp;</sup>	0.15 $\pm$ 0.01 <sup>&amp;S^A</sup>
20	+	0.20 $\pm$ 0.02 <sup>+S^A</sup>	0.16 $\pm$ 0.01 <sup>S^A</sup>
60	+	0.22 $\pm$ 0.03 <sup>+&amp;S^A</sup>	0.17 $\pm$ 0.26 <sup>S^A</sup>
–	+	0.18 $\pm$ 0.02 <sup>+#</sup>	0.18 $\pm$ 0.01 <sup>+^A</sup>
20	–	0.15 $\pm$ 0.01 <sup>*#</sup>	0.21 $\pm$ 0.01 <sup>+*#</sup>
60	–	0.15 $\pm$ 0.01 <sup>*#</sup>	0.23 $\pm$ 0.02 <sup>+*#&amp;</sup>

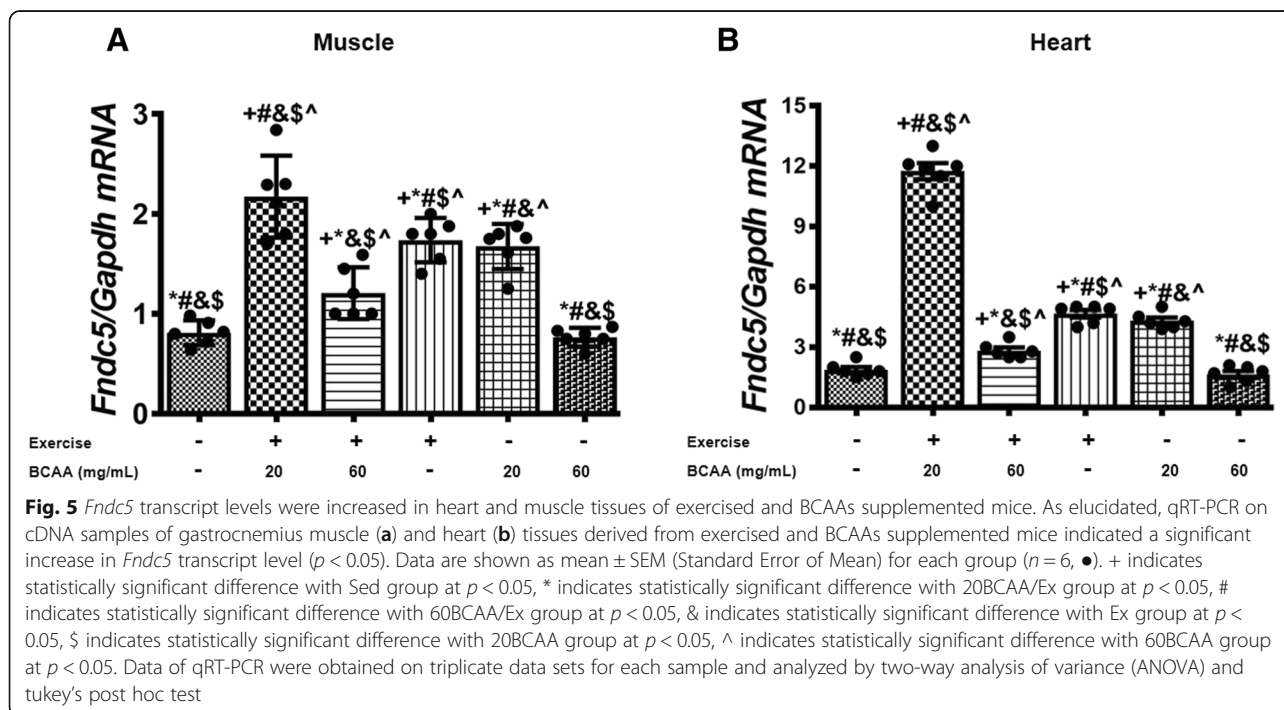
Values are expressed as mean  $\pm$  SEM. + indicates statistically significant difference with Sedentary group at  $p < 0.05$ , \* indicates statistically significant difference with 20BCAA/Ex group at  $p < 0.05$ , # indicates statistically significant difference with 60BCAA/Ex group at  $p < 0.05$ , & indicates statistically significant difference with Ex group at  $p < 0.05$ , \$ indicates statistically significant difference with 20BCAA group at  $p < 0.05$ , ^ indicates statistically significant difference with 60BCAA group at  $p < 0.05$

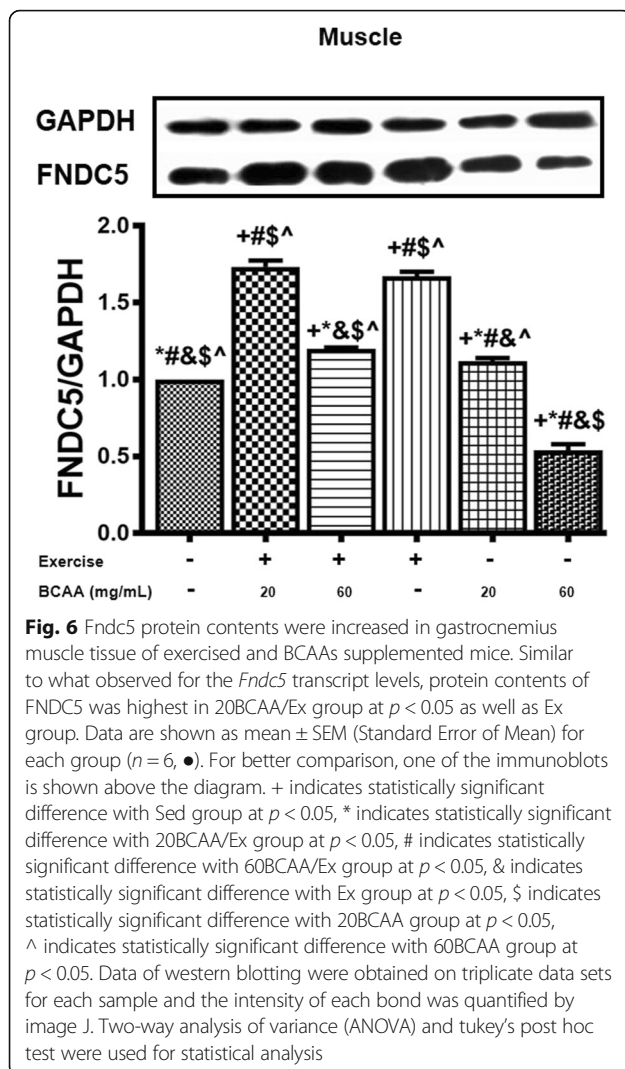


Upon eight weeks performance of tolerance and motor coordination test, 20BCAA/Ex group, got the highest score (Fig. 4a). Also by taking exhausting test, we indicated no improve in endurance capacity for 60BCAA/Ex group compared with 20BCAA/Ex group (Fig. 4b).

**Effect of different concentrations of BCAA and aerobic exercise on *Fndc5***

Data revealed that *Fndc5* expression was significantly increased in both, 20BCAA/Ex, and 60 BCAA/Ex comparing to Sed group (untrained without BCAAs intake -)





in heart and gastrocnemius muscles (Fig. 5a, b). Interestingly, supplementation with 60 mg/mL BCAAs was not able to increase *Fndc5* expression, while at lower concentration of BCAA (20 mg/mL) upregulated the expression of *Fndc5* significantly (Fig. 5a, b). Furthermore, the highest increase in *Fndc5* expression was accomplished when a combination of exercise was performed with 20 mg/mL BCAAs supplementation (20BCAA/Ex group) (Fig. 5a, b). In the gastrocnemius skeletal muscle, protein levels of FNDc5 were significantly higher in Ex and 20BCAA/Ex groups as compared to Sed group. Similar to the *Fndc5* mRNA, protein content of FNDc5 was not up-regulated in 60BCAA group (Fig. 6).

#### BCAAs supplementation and aerobic exercise effects on the expression of genes involved in mitochondrial biogenesis

Additionally, we assessed the expression of mitochondrial genes in gastrocnemius and heart muscles. As expected,

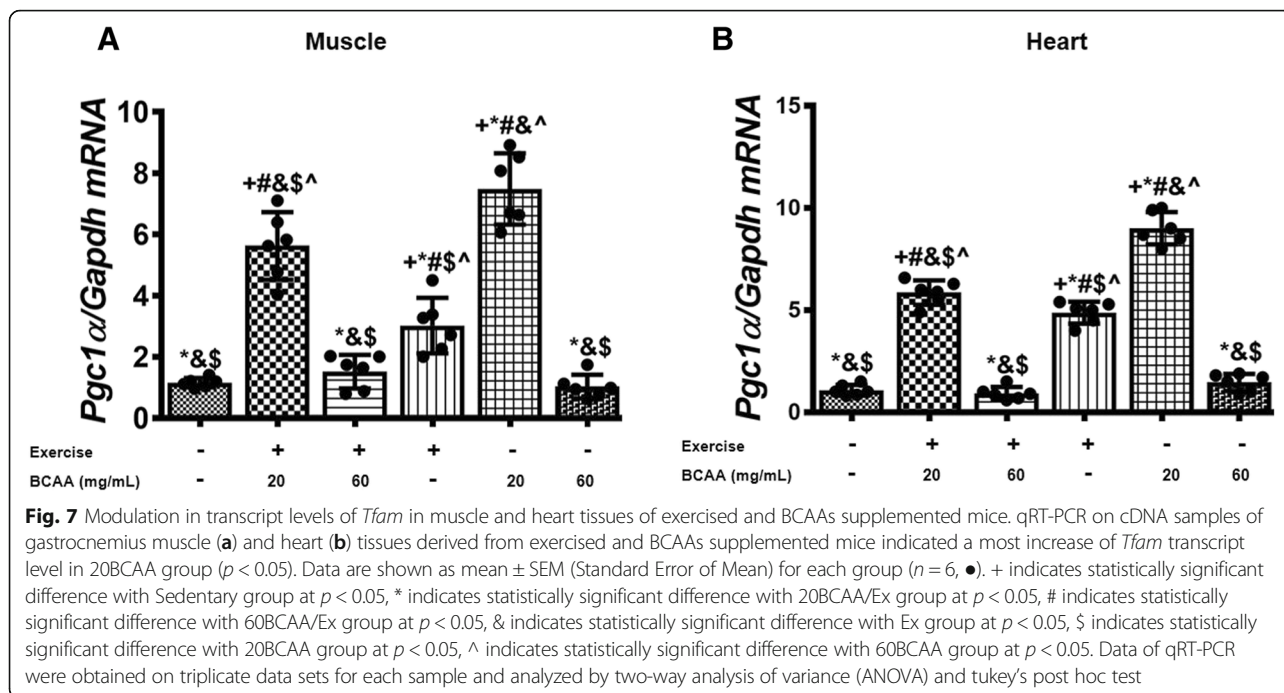
our data indicated that BCAAs increased the expression of mtDNA transcription factor A (*Tfam*), *Cox4i1*, a and b subunits of the mitochondrial  $H^+$ -ATP synthase (*a-F1-ATPase*, *b-F1-ATPase*) in both of gastrocnemius and heart muscles (Figs. 7, 8, 9, 10). However, the maximum enhancement was yielded when BCAAs at concentration of 20 mg/mL (20BCAA) was supplemented. Of important the effect of exercise was not significant with BCAAs supplementation (Figs. 7, 8, 9, 10). A similar trend of modulation in transcripts levels of aforementioned genes was obtained in heart tissue of the mice (Figs. 7, 8, 9, 10). As these genes are under regulation of *Pgc-1 $\alpha$* , we assessed transcript level of *Pgc-1 $\alpha$*  in both gastrocnemius and heart tissues. Again, the same trend was observed for *Pgc-1 $\alpha$*  expression in both heart and muscle tissues (Fig. 11). Exercise (Ex) increased transcript levels of *Pgc-1 $\alpha$*  (Fig. 11), whereas maximal increase in *Pgc-1 $\alpha$*  mRNA was yielded when 20 mg/mL BCAAs supplemented (20BCAA) (Fig. 11). Importantly, no significant modulation in *Pgc-1 $\alpha$*  transcript levels was observed when higher amount of BCAAs (60 mg/mL) [60BCAA] was implemented (Fig. 11). Data revealed that increased expression of *Pgc-1 $\alpha$*  due to the administration of 20 mg/mL BCAAs (20BCAA) was more than exercise (Ex) (Fig. 11). Notably, we found that *Sirt1* mRNA was increased in 20BCAA/Ex group, the same as *Fndc5* mRNA in gastrocnemius and heart muscle (Fig. 12). To our knowledge, *Pgc-1 $\alpha$*  is known as the co-activator of *PPAR $\gamma$* . Data indicated that *PPAR $\gamma$*  gene expression was significantly increased in exercise group (Ex) (Fig. 13). Interestingly, administration of 60 mg/mL BCAAs during the exercise (60BCAA/Ex) decreased transcript levels of *PPAR $\gamma$*  (Fig. 13). However supplementation of 20 mg/mL BCAAs with exercise (20BCAA/Ex) significantly increased the expression of *PPAR $\gamma$*  (Fig. 13). Moreover, high amount of BCAAs (60 mg/mL) [60BCAA] administration reversed the expression of *PPAR $\gamma$*  (Fig. 13).

#### Correlation analysis of circulating irisin with muscle and heart *Fndc5* mRNAs

The impact of exercise on plasma levels of irisin was later investigated by ELISA. As depicted, ELISA results revealed that plasma concentration of irisin significantly was changed in mice 20BCAA/Ex and EX groups compared to Sed group (Fig. 14a). Correlation of irisin levels with *Fndc5* revealed that circulating irisin had positive associations ( $r = 0.65$  and  $0.7$  for muscle and heart respectively) with the expression of this gene (Fig. 14b, c). Interestingly, increased amount of plasma irisin was related to the increased expression level of *Fndc5* in 20BCAA/Ex group.

#### Plasma profile of lactate and urea in exercised and BCAAs supplemented mice

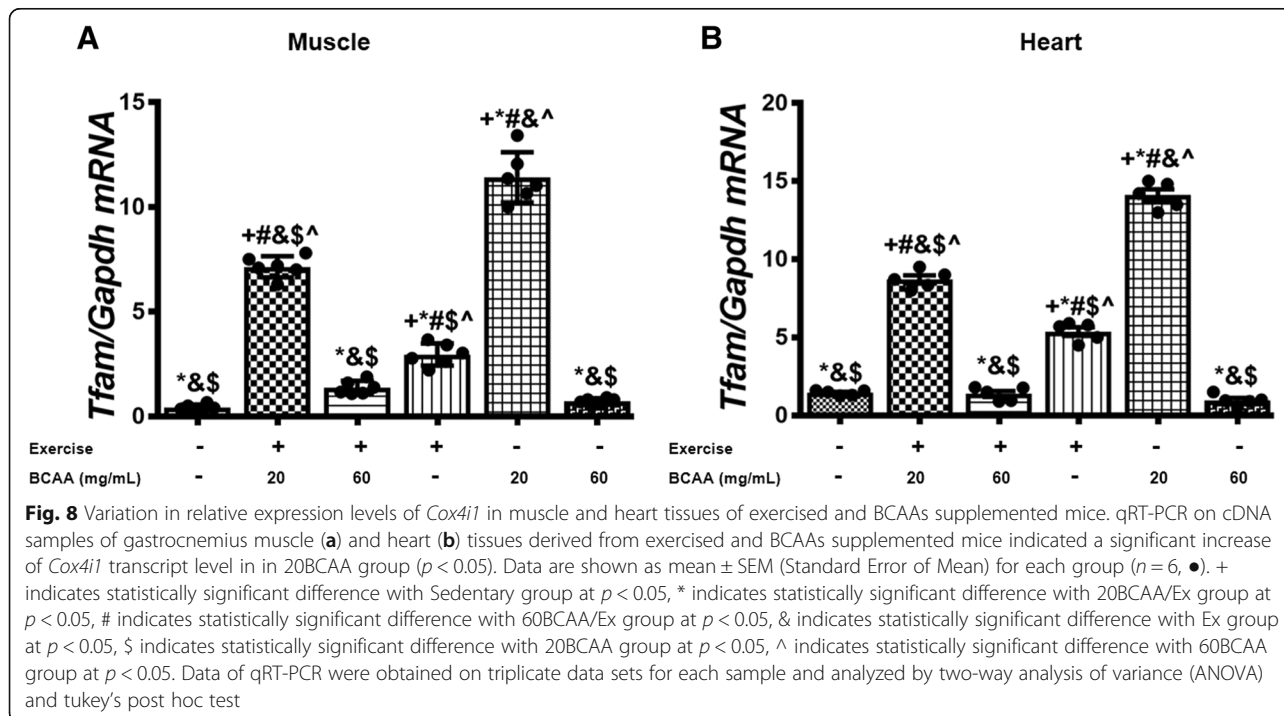
Meanwhile, lactate and urea content in plasma increased significantly in 60BCAA/Ex group (Fig. 15). Importantly,



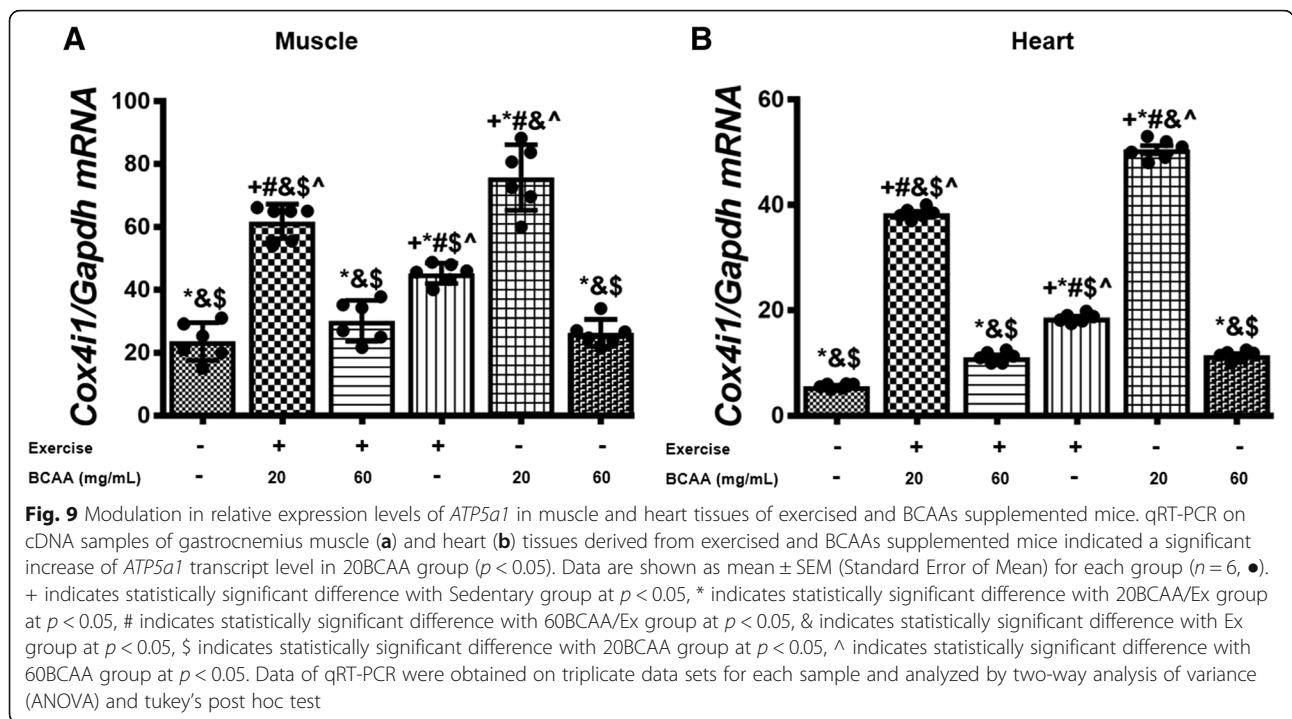
we did not observe any significant change in aforementioned parameters in 20BCAA/Ex group. Consistently, in 20BCAA/Ex group whole endurance capacity in treadmill tests was improved (Fig. 4b) as well as better performance on motor coordination test (Fig. 4a).

### Discussion

Our results indicated that BCAAs supplementation increased body weight, heart and gastrocnemius muscle weight. As it was earlier reported, BCAAs supplementation promotes protein synthesis and inhibits protein

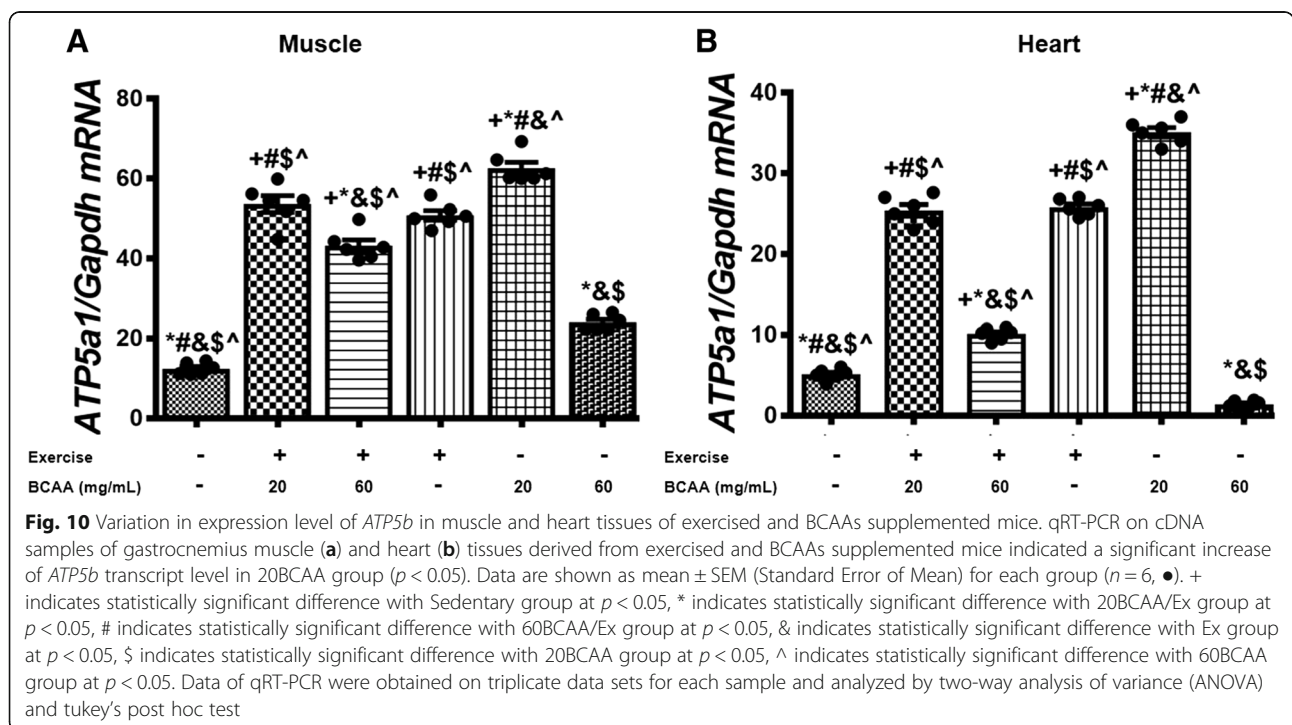


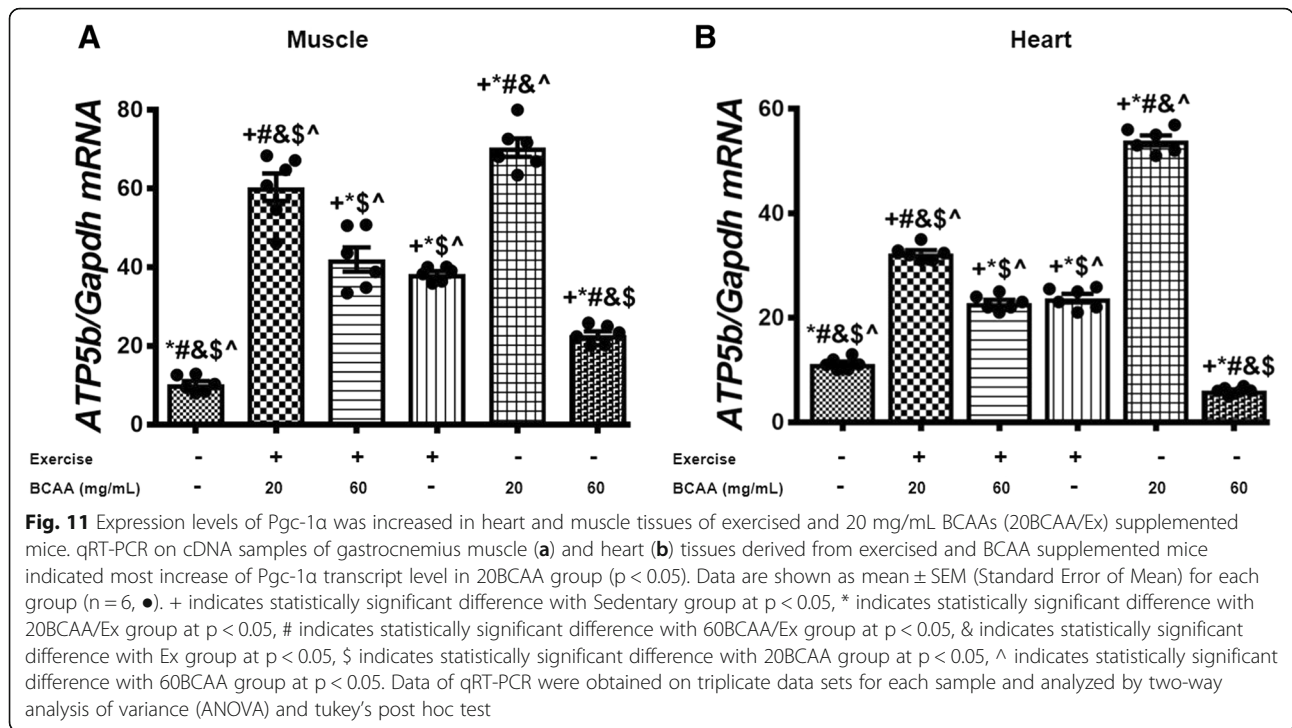




degradation via mTOR pathway, therefore increases muscle and heart weight [6] Also, importance of cardiac BCAAs catabolism and functional impact on cardiac development and physiology is well recognized [28]. D'Antona et al. indicated that BCAAs supplementation increases mitochondrial biogenesis through *Pgc-1 $\alpha$*  and

*Sirt1* expression in primary cardiac and skeletal myocytes, which is accompanied by enhanced physical endurance They also demonstrated BCAAs supplemented mice was accompanied by enhanced mitochondrial biogenesis and function in cardiac and skeletal muscles but not in liver or fat. They showed that BCAAs

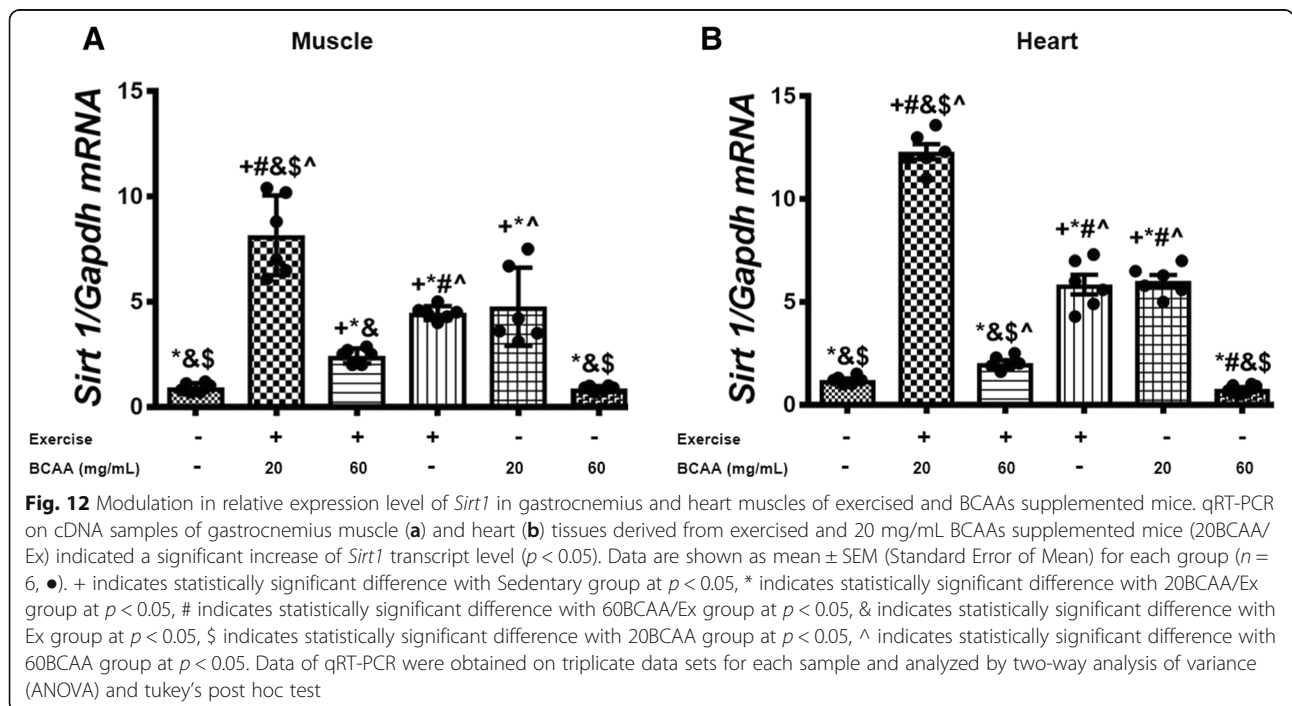


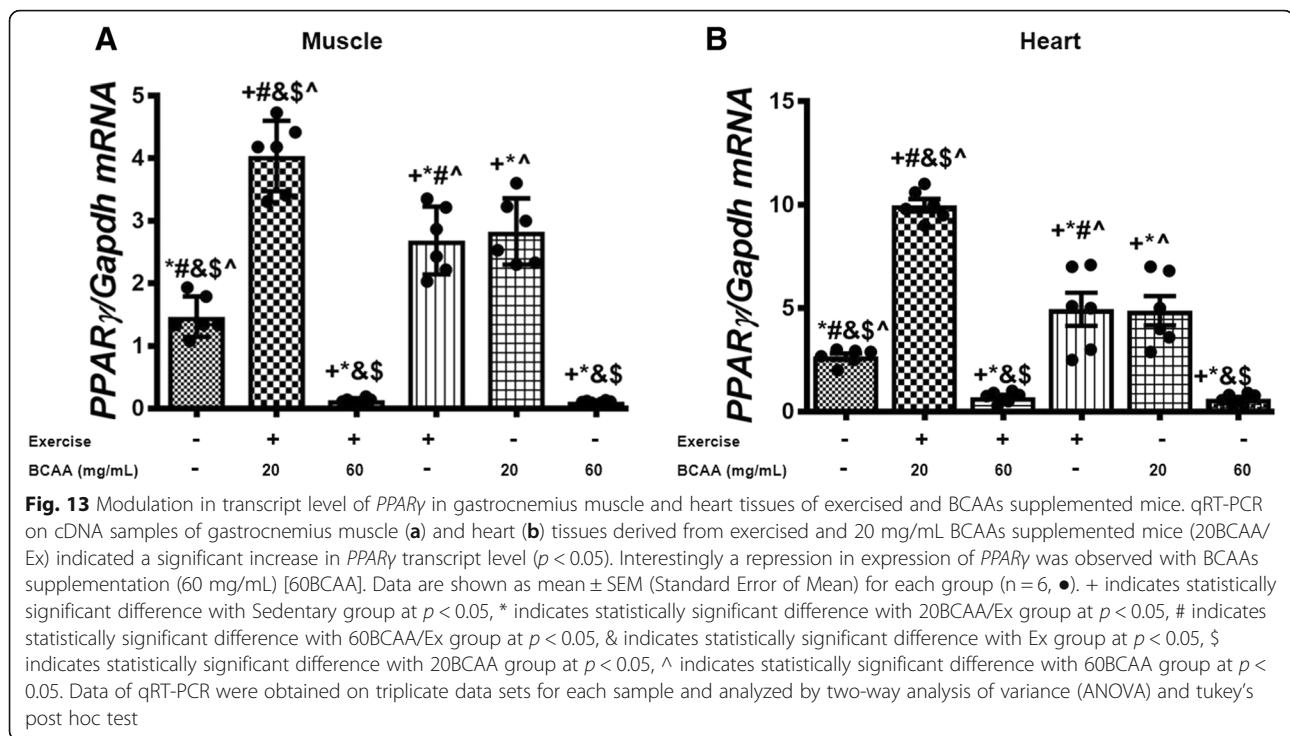


activated mTOR signaling and could enhance mitochondrial biogenesis [10].

Physical exercise is a well-recognized factor that could increase *Fndc5* gene expression [14, 21, 30]. Our results confirmed such concept as exercise increased transcript level of muscle and heart *Fndc5*. Interestingly BCAAs (20 mg/mL) supplementation (20BCAA) was beneficial

to increase the transcript level of *Fndc5*. However, higher amount of BCAAs (60 mg/mL) did not change *Fndc5* transcript level even though the expression of mitochondrial genes decreased. This is possibly due to triggering an increase in energy expenditure [30]. Furthermore, elevated circulating BCAAs levels have been correlated with severity of insulin resistance. It is





hypothesized that elevated circulating BCAAs observed in insulin resistance may result from dysregulated BCAAs degradation. Moreover BCAAs metabolism results in an accumulation of toxic BCAAs metabolites, then in turn trigger the mitochondrial dysfunction and stress signaling associated with insulin resistance despite of increment in mitochondrial content and enhancement of oxidative capacity in skeletal muscle [29, 31]. Detailed molecular mechanism of BCAAs overdose on *Fndc5* gene expression in the skeletal and heart muscles remains to be obscure.

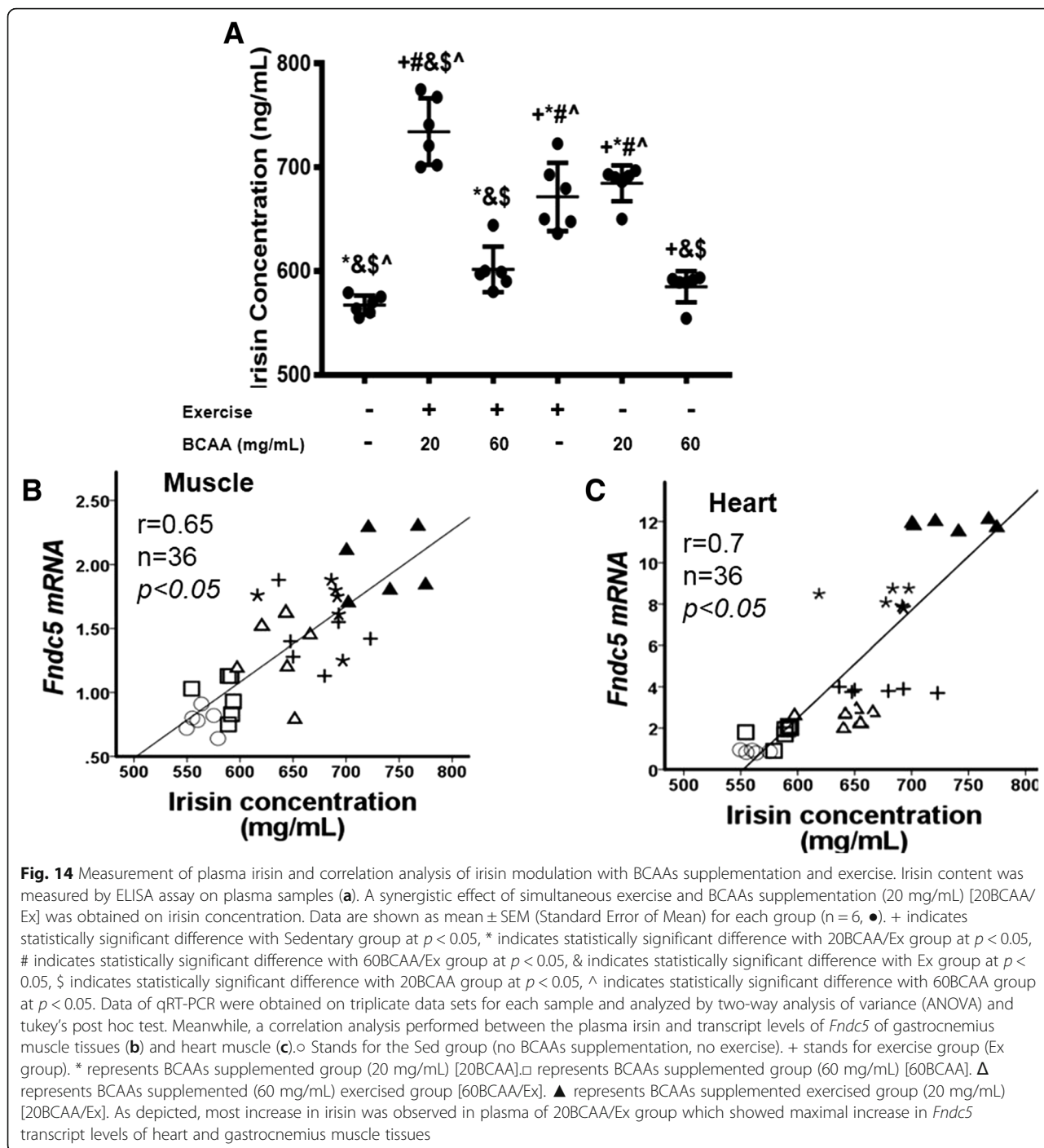
Additionally, the synergetic effect of exercise interaction with 20 mg/mL BCAAs supplementation (20BCAA/Ex) on induction of *Fndc5* transcription was evident in our data. We found this interaction had positive effect on *Fndc5* expression and metabolism. Also, we indicated protein content of FNDC5 decreased in 60BCAA as compared with sedentary group. Interestingly, we observed the protein level of FNDC5 in the skeletal muscle was higher in exercised mice in contrast with 20BCAA group. Presumably, this incompatibility between mRNA and protein levels of FNDC5 in the mice is related to the activity of some regulators which affect protein translation [32, 33].

Our findings were in good agreement with Aydin et al. which reported a positive association with metabolism and *Fndc5* gene expression [21]. Unlike our findings, Pekkala et al. indicated no significant up-regulation in *Fndc5* of skeletal muscle in trained individuals [34]. This discrepancy may be mainly due to the type of exercise or

simply reflecting differences between human and mice metabolism. The secretory type of *Fndc5* is irisin, which affects total body energy expenditure, as well as browning of WAT (white adipose tissue) [14]. There are several controversy results about *Fndc5* and irisin levels in plasma [13, 14, 16, 21, 35]. Our data showed irisin level was increased in 20BCAA/Ex group. Therefore, we assume that, irisin is increased in response to exercise and 20 mg/mL of dietary BCAAs.

*Pgc-1 $\alpha$* , is a co-activator of various nuclear receptors as well as transcription factors. Previous study has indicated that *Pgc-1 $\alpha$*  is involved in regulation of energy metabolism, as well as thermogenesis, in skeletal muscle. In skeletal muscle, *Pgc-1 $\alpha$*  regulates not only the mitochondrial function but also metabolic activity of the cell [36, 37]. Tadaishi et al. revealed that, BCAAs metabolism mainly occurs in mitochondria of skeletal muscle and is induced by *Pgc-1 $\alpha$*  [36]. However, higher amount of BCAAs (60 mg/mL) reversed such inductive responses, possibly by increased BCAAs catabolic flux which triggers insulin resistance in myoblast [38].

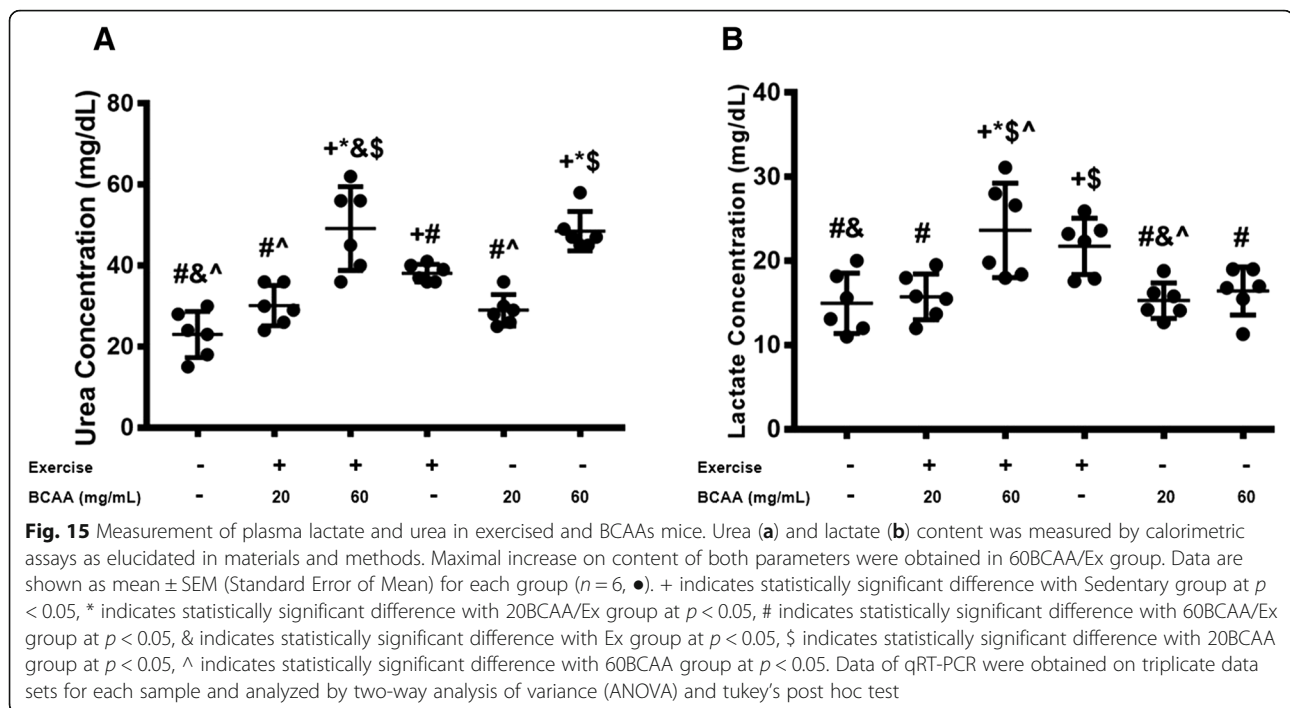
In this study we have examined the effect of exercise and BCAAs supplementation on transcript levels of *Sirt 1*, *PPAR $\gamma$* , *Pgc-1 $\alpha$* , *Tfam*, *Cox4i1*, *ATP5a1*, *ATP5b* and *Fndc5* involved in mitochondrial biogenesis and for the first time we have shown that endurance exercise could increase *ATP5a* and *ATP5b* mRNA expression. In present study, we have demonstrated that *Sirt 1* and *Fndc5* mRNA had a similar trend and they had a positive correlation with each other. *PPAR $\gamma$*  expression was



observed in trained mice, emphasizing that exercise could enhance *PPAR $\gamma$*  gene expression [38]. Our results are in agreement with the previous study which indicated *Sirt1* up-regulation and life span extension, and increasing in mitochondrial biogenesis, and ROS production decrease in the skeletal muscle of male mice [10]. One of the limitation of our study was lack of data about no mitochondrial related functional evidences in either tissues, such as mitochondrial number, respiration

capacity,  $\beta$ -oxidation to support the physiological observation of BCAAs overload. This is critical issue which should be investigated in further experiments.

According to our knowledge endurance exercise increases the capacity of skeletal muscle for aerobic metabolism through an increase in mitochondrial capacity to generate ATP via oxidative phosphorylation [39], presumably through upregulation of *Pgc-1 $\alpha$*  and *PPAR $\gamma$*  expression [40]. Furthermore, BCAAs supplementation may improve



muscle fiber size, physical endurance and it may increase mitochondrial activity as already shown that treatment with BCAAs in mice caused significant increase in *Pgc-1 $\alpha$* , *Tfam* and *Sirt1* expression in skeletal muscle. Furthermore, BCAAs supplementation is responsible for antioxidant defense and reduction of ROS [10]. Enhancement of *Pgc-1 $\alpha$*  expression triggers the interaction of *Pgc-1 $\alpha$*  with PPAR $\gamma$ , which elaborates the oxidative capacity of mitochondria. On the other hand, *Pgc-1 $\alpha$*  mediates BCAAs metabolism in skeletal muscle through increasing the transcript levels of *branched chain amino acid transaminase 1 (Bcat1)*, *Bcat2* and *branched-chain alpha-keto acid dehydrogenase (Bckdh)* [41]. Increment in BCAAs catabolism leads to an increase of cellular Acetyl-CoA, Acetoacetate, and Succinyl-CoA which reduces glucose oxidation rate and lactate production [36, 39]. Final response of the myoblast to these events is the up-regulation of *Fndc5* through unidentified cascade.

## Conclusion

Our present study suggests that consuming 20 mg/mL BCAAs with exercise (20BCAA/Ex) had positive effect on expression level of gastrocnemius muscle *Fndc5*, PPAR $\gamma$ , *Pgc-1 $\alpha$*  similar with heart tissue. In addition, Irisin levels were increased at most level after 20 mg/mL BCAAs supplementation which coincided with the exercise (20BCAA/Ex). However, overloading of higher amount of BCAA may have adverse these effects on transcript levels of *Fndc5*. However, physiological aspects of this finding needs to be confirmed in further studies.

## Additional file

**Additional file 1: Figure S1.** Comparative study on amino acid content of proteins in human and mouse. As shown, the assessed proteins were the same as were obtained by STRING analysis (Fig. 2). Dark color indicates more homology in amino acid residues and bright red color represents more dissimilarity between human and mouse proteins. Of interest most similarity was obtained for PPAR $\gamma$ , and FNDC5 between mouse and human. (JPG 86 kb)

## Abbreviations

ANOVA: Analysis of variance; BBB: Blood-brain barrier; BCAAs: Branched chain amino acids; Bcat: Branched chain amino acid transaminase; Bckdh: Branched-chain alpha-keto acid dehydrogenase; Ex: Endurance exercise training; FNDC5: Fibronectin type III domain containing 5; Gapdh: Glyceraldehyde-3-phosphate dehydrogenase; mtDNA: Mitochondrial DNA; mTORC1: Mammalian target of rapamycin complex 1; NRF: Nuclear respiratory factor; *Pgc-1 $\alpha$* : Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PPAR $\gamma$ : Peroxisome proliferator-activated receptor gamma; qRT-PCR: Quantitative real-time PCR; SEM: Standard error of mean; Sirt1: Sirtuin 1; TFAM: Mitochondrial transcription factor A; TG: Triglyceride; WAT: White adipose tissue

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## Availability of data and materials

All of the raw data and the rest of materials are remained in Royan Institute for Biotechnology and are available upon request.

## Authors' contributions

Design of study was done by NA, KG, FT and MHNE and experiments was performed by NA. Analyses and data mining were performed by NA, IN, FR

and ST technical assistances were performed by ZS, FR and ST. Interpretation of the obtained information was done by NA, KG, FT and MHNE. The manuscript was written by NA and KG and was approved by FT and MHNE. All authors read and approved the final manuscript.

#### Ethics approval

Approval of mouse usage in this study was obtained by the Ethics committee of Royan Institute.

#### Consent for publication

All authors support submission to this journal.

#### Competing interests

The authors declare that they have no competing interests.

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