Changes in UPR-PERK pathway and muscle hypertrophy following resistance training and creatine supplementation in rats

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Abstract

The unfolded protein response (UPR) plays a pivotal role in some exercise training induced-physiological adaptation. Our aim was to evaluate the changes in the protein kinase R-like endoplasmic reticulum kinase (PERK) arm of the UPR and hypertrophy signaling pathway following 8 weeks of resistance training and creatine (Cr) supplementation in rats. Thirty-two adult male Wistar rats (8 weeks old) were randomly divided into 4 groups of 8: untrained + placebo (UN + P), resistance training + placebo (RT + P), untrained + Cr (UN + Cr) and resistance training + Cr (RT + Cr). Trained animals were submitted to the ladder-climbing exercise training 5 days per week for a total of 8 weeks. Cr supplementation groups received creatine diluted with 1.5 ml of 5% dextrose orally. The flexor hallucis longus (FHL) muscle was extracted 48 hours after the last training session and used for western blotting. After training period, the RT + Cr and RT + P groups presented a significant increase in phosphorylated and phosphorylated /total ratio hypertrophy indices, phosphorylated and phosphorylated/total ratio PERK pathway proteins and other downstream proteins of the PERK cascade compared with their untrained counterparts ($P < 0.05$). The increase in hypertrophy indices were higher but PERK pathway proteins were lower in the RT-Cr group than in the RT + P group ($P < 0.05$). There was no significant difference between the untrained groups ($P > 0.05$). Our study suggests that resistance training in addition to Cr supplementation modifies PERK pathway response and improves skeletal muscle hypertrophy.

Key Points

- Creatine has a synergistic effect on hypertrophy caused by resistance training.
- Resistance training increases phosphorylates PERK proteins and its downstream pathway.
- Creatine supplementation during resistance training modifies PERK pathway response.

Introduction

The endoplasmic reticulum (ER) is an intracellular complex organelle involved in trafficking of proteins, modification, proper folding and calcium homeostasis [17]. Many pathophysiological conditions disturb protein synthesis and folding capacity which can lead to ER stress. Following ER stress, cells initiate the UPR to restore normal function and remove stress conditions in the ER lumen [29]. The UPR is characterized by three signaling cascade pathways: the PERK, the inositol-requiring protein 1 (IRE1) and the activating transcription factor 6 (ATF6) [34]. This signaling pathway regulates different functions such as antioxidant response [11], mitochondrial biogenesis [18], glucose metabolism [23] and maintenance of muscle mass.

Under accumulation of misfolded/unfolded protein conditions, chaperone-binding protein/glucose-regulated protein 78 (BiP/GRP78) disassociates from PERK and becomes activated [30]. Under these conditions, oligomerization and auto-phosphorylation PERK lead to phosphorylates eukaryotic translation initiation factor 2 α (eIF2 α) attenuating mRNA translation and subsequently increasing downstream

factors such as activating transcription factor 4 (ATF4) and effecting expression of stress-responsive genes (C/EBP homologous protein (CHOP)) and DNA damage-inducible protein 34 (GADD34) [24].

Growing evidence suggests that PERK pathway has an important role in skeletal muscle mass, amino acid metabolism, energy expenditure and antioxidant response [23, 12]. PERK also acts as a calcium sensor and plays an essential role in regulating muscle contractions [21]. Recent observations have demonstrated that the targeted ablation of PERK decreases skeletal muscle hypertrophy and reduces muscle cross-sectional area (CSA) [12]. Acute exercise triggers the UPR in skeletal muscle [35], but is affected with chronic exercise [19]. However, there are contradictory findings regarding UPR response to exercise training [6, 28]. It has been observed that after 8 weeks of resistance training, the rate of PERK phosphorylation and ATF4 expression became elevated in elderly populations [9] while PERK/eIF2 α was unchanged after acute resistance exercise [26].

The mTOR has a great effect on regulating protein synthesis and is involved in muscle hypertrophy caused by resistance training [25]. It has been reported that amino acid transporter expression following resistance exercise might be linked to mTOR signaling and eIF2 α phosphorylation [8]. On the other hand, Cr supplementation in addition to resistance training increases the diameter of skeletal muscle fibers by stimulating the expression of insulin-like growth factor-1 (IGF-1) and subsequently activating the AKT-mTOR pathway [10]. Given that both PERK and mTOR are involved in protein synthesis, skeletal muscle mass [23] and satellite cell homeostasis [36], there is limited information regarding the effect of resistance training-induced hypertrophy on PERK pathway.

The main focus of our study was to examine the effect of 8 weeks of ladder climbing exercise alone or in combination with creatine supplementation on PERK pathway signaling (BiP, PERK, p-PERK, eIF2 α , p-eIF2 α , ATF4 and CHOP) and hypertrophy indices (mTOR, p-mTOR and AKT, p-AKT) in male wistar rats. In fact, the goal was to answer the question of whether such resistance training would cause skeletal muscle hypertrophy. If yes, did the PERK pathway contribute to the hypertrophy? And finally, can creatine supplementation enhance the potential effectiveness of this pathway?

Materials And Methods

Animals and experimental design

In this study, thirty- two adult male Wistar rats (eight-week-olds) were obtained from the Razi Vaccine and Serum Institute in Karaj, Iran. After two weeks of familiarization with the environment, the subjects were randomly divided into four groups (each group of eight rats) consisting of untrained + Placebo (UN + P), resistance training + Placebo (RT + P), untrained + Cr (UN + Cr) and resistance training + Cr (RT + Cr). All the rats were kept in standard cages made from polycarbonate and under controlled conditions with a 12:12-h light-dark cycle and room temperature maintained at 22°C. Furthermore, all rats were provided with water and food ad libitum. The rats had free access to food and water during the entire experimental

period. Changes in body weight were measured weekly. The study design was approved by the animal research ethics committee of Kurdistan University of Medical Sciences (IR.MUK.REC.1397.5010).

Resistance exercise

In the present study, climbing a vertical ladder (110 cm high × 18 cm wide, 2 cm grid steps, 80 degrees of inclination) was considered the resistance training protocol. At the top of the ladder, the rats reached a resting chamber (L×W×H = 20 cm × 20 cm × 20 cm). Previous studies have shown that each rat is able to climb this device 8 to 12 times [16]. Before the start of the main protocol, the rats were introduced to the ladder for 5 days. For this reason, each animal was placed in different parts of the ladder (base, middle, and upper stair) to practice climbing the ladder without extra load. Touching the rat's tail initiated the ladder climb without any electrical stimulation. After the adaptation period, the rats of the training groups performed maximal carrying load (MCL) test. A cylinder containing weights (metal balls) fixed to the base of each rat tail by a clip was used to create resistance. At the bottom of the climbing apparatus, the rats had to climb up with a load of 50% of their body weights. The overload (30 g) in each set was gradually increased over time. This process continued until the rats could not climb up the ladder after 3 consecutive trials. The last load to be carried up to the top of the ladder was recorded as MCL for that session.

The resistance training protocol was performed for 8 weeks, 5 days per week. The number of times that the rats climbed the ladders was 10 times per session and 120 seconds rest intervals was set between attempts. During training sessions, rats climbed a ladder with 50%, 75%, 90% and 100% of their individual MCL (each one twice), then 30 grams were added at each attempt to complete the 10 sets (if a rat was not able to climb the stairs, the climb was performed with the prior weight). The final highest load successfully carried at the end of the training was considered as the MCL for the next training session [16].

Creatine supplementation

The doses of Cr supplementation was performed according to the recommendations of the International Society of Sports Nutrition [3]. Cr supplementation began one week before the start of the main resistance training program (loading phase, 0.3 g/kg/day). Subjects then received 0.05 g/kg/day Cr (in powder form with a purity of 99.9%, Ultimate Sports Nutrition (USN), USA) at the maintenance phase for 8 weeks. In the present study, supplementation recipient groups (RT + Cr, UT + Cr) received Cr monohydrate diluted with 1.5 ml of dextrose solution (5%) using a syringe and by dripping the solution in the mouth. Placebo recipient groups (RT + P, UT + P) consumed the same amount of dextrose solution (1.5 ml) during this period. In the current study, an oral solution was used for supplementation which had a good taste (by adding dextrose). Therefore, without harming the rats, the prescribed dose was fully received. During the intervention period, the rats were weighed weekly to determine their supplemental dose.

Tissue sampling

48 hours after the last training session and following 12 hours of fasting, the rats were weighed and then anesthetized using ketamine (80 mg/kg of body mass) and xylazine (12 mg/kg of body mass). While waiting for the effects of anesthesia to appear, the skin that surrounds the lower right and left legs were shaved and removed carefully to show the leg muscles. The next step involved the Achilles tendon being cut to determine the flexor hallucis longus (FHL) muscle (posterior and lateral surface of the body of the fibula). Then, the proximal and distal FHL muscle tendons were dissected and cut swiftly. Immediately after muscle separation, the weight of the FHL muscle was achieved with a precision analytical scale (Sartorius group (acculab) Germany, ATILON model, readability 0.0001 g). The ratio of FHL muscle weight to total body weight was also calculated. Finally, the left FHL muscle was fixed in 10% formalin solution to evaluate changes in muscle cross section, and the right FHL was frozen in liquid nitrogen and stored at -80°C until western blotting analysis.

Histological analysis of the FHL muscle

The FHL muscle was fixed in 10% formalin solution for 2 days, after which graded ethanol was used for dehydration and clearing. In the embedding phase, specimens were embedded in paraffin to produce paraffin blocks. These blocks were cut at 5 µm thickness using a microtome (Reichert-Jung, Model 2800 E Frigocut) and attached onto a slide glass. The slides were stained with hematoxylin and eosin (H&E) for analyzing the CSA of the FHL muscle. Photographs were taken using an optical microscope (Olympus IX81) equipped with a Ha- mamatsu EM-CCD (Model C9100). The CSA of the FHL muscle tissue was assessed by Image J software.

Western blotting

FHL muscle tissues (100 mg) were homogenized in ice-cold RIPA lysis buffer (50mM Tris, pH 8, 150mM sodium chloride, 1.0% NP-40, 1% sodium dodecyl sulfate (SDS), 1% ROCHE). Homogenized samples were centrifuged at 12000 × g for 15 minutes at 4°C. The supernatant was collected and the protein concentration measured (wavelength 595 nm) by a Bio-Rad protein-determining kit based on the Bradford method [2], and kept at a temperature of -20° C. The homogenized samples obtained were mixed in a ratio of 1:1 with a 2X loading buffer (50 mM tris - hydrogen chloride, pH = 6.8%, sodium dodecyl sulfate, 10% glycerol, 5% beta-mercaptoethanol and% 0.005 Bromophenyl blue). The samples were boiled for 5 minutes until all proteins were completely denatured. The proteins were isolated by SDS gel (10%) electrophoresis, then transferred to a nitrocellulose membrane, and blocked on the shaker for one hour with a combination of bovine serum albumin (BSA) 1%, tween 20 in phosphate-buffered saline (PBS) 0.1%. After washing in PBS-T solution, the membrane was incubated within a solution containing diluted anti-rabbit antibody in BSA-PBS-T solution. The antibodies used for immunoblotting were eIF2α (sc-517214; 1:500), p-eIF2α (sc-101670; 1:500), PERK (sc-377400; 1:500), p-PERK (sc-32577; 1:500), mTOR (sc-1550-R; 1:500), p-mTOR (sc-293133; 1:500), AKT (sc-135829; 1:500), p-AKT (sc-52940; 1:500) ATF4 (sc-390063; 1:500), and β-Actin (sc-130657) from the Santa Cruz Biotechnology company, and CHOP (ab-11419; 1:500) and BiP (ab-11419; 1:500) from Abcam company. The nitrocellulose membrane was removed from the solution and washed with PBST (three times each time for 5 minutes). Then, it was

incubated for 1 hour with secondary antibody (anti rabbit/anti mouse depending on the primary antibody). The chemiluminescence chemical reaction and x-ray radiography film (Fujii) were used for visualization. Protein measurement densitometry analysis was carried out using Image J software (National Institutes of Health, Bethesda, Maryland, USA). The target genes band were normalized against β -Actin as loading control.

Statistical Analysis

To assess normality of data distribution and equality of variances, the Shapiro–Wilk and Levene tests were used, respectively. Analysis of variance (ANOVA) with repeated measure [within-subject effects (time), between-subject effects (group), interaction between the two types of effects (group $\times$ time)] and Bonferroni post-hoc test were used to determine intra-group and inter-group changes in body weight and Maximal carrying load (MCL) variables. In relation to other research variables, one-way ANOVA and Tukey's post hoc tests were used to compare differences between groups (in the post test). SPSS software package (version 23) was used for data analysis and the statistically significant difference was set at $p < 0.05$.

Results

Body weight

Before intervention (in the pre-test), there was no significant difference between groups regarding body weight ($P = 0.099$). There was a significant main effect of time ($P = 0.001$), group $\times$ time interaction ($P = 0.001$) and a main effect of group ($P = 0.001$) for body weight. Between groups comparisons showed that body weight was higher in the RT + Cr group compared with the RT + P group ($P = 0.003$), and in the RT + Cr group compared with the UN + Cr group ($P = 0.002$) after the 8-week intervention. There was also a significant difference between the two untrained groups ($P = 0.002$). Overall, body weight in all four groups increased after 8 weeks compared to the pretest (in all cases, $P = 0.001$, Fig. 1a).

FHL muscle weight and FHL /body weight ratio

There were significant differences in weight of the FHL muscle ($P = 0.001$) and FHL/body weight ($P = 0.001$) between the groups. The FHL and FHL/body weight of the RT + Cr group were significantly higher than the other groups. The RT + P group also presented higher FHL and FHL/body weight compared with non-trained groups. Moreover, UN + P showed lower FHL and FHL/body weight compared with the UN + Cr group ($P < 0.001$, Fig. 1b).

Maximal carrying load (MCL)

Before intervention (in the pre-test), there were no differences between groups regarding body weight ($P = 0.524$). There was a significant main effect of time ($P = 0.001$), group $\times$ time interaction ($P = 0.001$) and main effect of group ($P = 0.001$). This variable was higher in the RT + Cr group compared with the RT + P

group at 8 weeks ($P = 0.001$). However, MCL in both groups increased after 8 weeks compared to the pretest (in both, $P = 0.001$, Fig. 1c).

Cross-sectional area (CSA) of FHL muscle fibers

A representative microscope image of the H&E stain used to analyze the change in FHL muscle fibers is shown in Fig. 2a. CSA of the FHL muscle was markedly higher in trained groups compared with untrained groups ($P = 0.001$). In addition, the CSA increase in the RT + Cr group was higher than that in the RT + P group ($P = 0.003$). There were also no statistically significant differences between UN + Cr and UN + P groups ($P = 0.905$, Fig. 2b). A greater rightward shift in the frequency distribution of CSA of the RT + Cr and RT + P groups was observed compared with UN + Cr and UN + P. The rightward shift was more intense in the RT + Cr group compared with the RT + P group (Fig. 2C).

Hypertrophy signaling proteins

After 8 weeks of resistance training, compared with RT + P group, the RT + Cr group presented higher p-mTOR ($P = 0.042$) and p-mTOR/mTOR ratio ($P = 0.005$) (Fig. 3b) as well as p-AKT ($P = 0.022$) and p-AKT/AKT ratio ($P = 0.001$) (Fig. 3c) in FHL muscle. However, the indices mentioned above were higher in the RT + Cr group than the UN + Cr group and in the RT + P group compared to the UN + P (in all cases, $P = 0.001$). Furthermore, there were no differences between UN + Cr and UN + P groups (p-mTOR ($P = 0.128$), p-mTOR/mTOR ratio ($P = 0.347$), p-AKT ($P = 0.264$), and p-AKT/AKT ratio ($P = 0.085$). No significant difference was observed between all groups in relation to mTOR ($P = 0.072$) and AKT ($P = 0.065$).

PERK pathway

At the end of 8 weeks of resistance training, RT + Cr group, compared with RT + P group, presented lower p-PERK, p-PERK /PERK ratio (Fig. 4b), p-eIF2 α , p-eIF2 α / eIF2 α ratio (Fig. 4c), BiP, ATF4 and CHOP (Fig. 4d) in the FHL muscle (in all cases, $P = 0.001$), but no differences were observed in relation to PERK ($P = 0.19$) and eIF2 α ($P = 0.12$) content. However, for all the variables mentioned above, a significant increase was observed in the RT + Cr group compared to the UN + Cr group ($P = 0.001$). Moreover, same results were observed in the RT + P group compared to the UN + P group ($P = 0.001$). There were no statistical differences between UN + Cr and UN + P groups (BiP; $P = 0.931$, PERK; $P = 0.209$, p-PERK; $P = 0.953$, p-PERK/PERK ratio; $P = 0.998$ eIF2 α ; $P = 0.920$, p-eIF2 α ; $P = 0.805$, p-eIF2 α /eIF2 α ratio; $P = 0.756$ ATF4; $P = 0.997$ and CHOP; $P = 0.964$).

Discussion

The findings of the present study indicate that 8 weeks of resistance training alone and in combination with Cr supplementation increase the phosphorylation of elements involved in the protein synthesis and skeletal muscle hypertrophy signaling pathway (p-mTOR, p-AKT). These changes were more pronounced in the group that had been given Cr supplementation in addition to resistance training. However, no significant difference was observed in total amounts of these proteins. Furthermore, the cross-sectional

area of FHL muscle myofibers increased in trained groups and this increase was greater in RT + Cr group compared to the RT + P group.

In support of the present findings, it has previously been reported that Cr supplementation leads to skeletal muscle hypertrophy through increasing the expression of insulin-like growth factor- 1 (IGF-1) [4]. In line with the current results, Ferretti et al. [10] reported that Cr supplementation combined with 8 week ladder climbing training increased the phosphorylated AKT and phospho-AKT/total AKT, but had no effect on the mTOR and phospho-mTOR/total mTOR. Aguiar et al. [1] investigated the effect of Cr supplementation and 5 weeks of a resistance training program (5 d/wk) on muscle hypertrophy in an animal model. They found that Cr supplementation had no additional effect on muscle hypertrophy which contradict the findings of the present study. The discrepancy between the findings is probably due to the resistance training protocol (jumping into a 38-cm deep vat of water), its duration (5 d/wk) and type of muscle. As noted, our findings indicate that increase of FHL muscle mass and MCL as a result of resistance ladder climbing training improves with Cr supplementation. These effects are due to enhanced cross-sectional area of the FHL muscle and increased p-mTOR and p-AKT levels. Cr supplementation provides the conditions for higher resistance training overload [32], which in turn promotes more satellite cell proliferation [14] and activates the signaling pathway involved in protein and glycogen synthesis [15]. Cunha et al. [5] investigated the effect of Cr on PI3K/AKT signaling pathway and its downstream intracellular targets in mice. Their findings showed that Cr increased the phosphorylation of AKT, which in turn causes phosphorylation and activation of mTOR and ultimately controls protein translation. Resistance training stimulates protein synthesis and muscle hypertrophy by increasing AKT-mTOR-p70S6K phosphorylation [27]. In this regard, Kwon et al. [20] reported that 8 weeks ladder climbing exercises increased AKT phosphorylation, p-mTOR and the ratio of the p-mTOR to total mTOR in wistar rats. However, the decrease in p-AKT/AKT ratio is due to the greater increase of total AKT compared to p-AKT. In the present study, we observed that both resistance training and Cr supplementation can stimulate the markers of muscle hypertrophy. As mentioned, the findings of the present study also indicate that p-mTOR, p-mTOR/mTOR ratio, p-AKT and p-AKT/AKT ratio increased in both RT + Cr and RT + P groups compared to the control groups which indicates activation of the AKT/mTOR pathway [10, 33]. It is noteworthy that Cr supplementation has synergistic effects and these changes were greater in the RT + Cr group compared to the RT + P group that represents further stimulation of hypertrophy signaling due to Cr supplementation.

In the current study, the level of BiP, p-PERK, p-eIF2 α , CHOP and ATF4 increased in the training groups compared with their control group counterparts. Interestingly, these changes were greater in the RT + Cr group than in the RT + P group. In agreement with the current findings, Wu et al. [35] stated that one session of exhaustive treadmill running increases UPR components such as BiP, GADD34, ATF4, CHOP, and sXBP1. Similarly, Pereira et al. [28] found that 8 weeks downhill running increased the p-IRE1, p-PERK and p-eIF2 α in both extensor digitorum longus (EDL) and soleus muscles. However, up-regulation of the levels of BiP, p-PERK and p-eIF2 α occurred only in the soleus muscle following uphill running, and running on a slope-free surface. Memme et al. [22] examined the electrical stimulation of tibialis anterior (TA) and extensor digitorum longus (EDL) muscles for 7 days in Sprague-Dawley rats. They found that ATF4,

downstream signaling of PERK pathway, increased on 7th day, while BiP did not change significantly during those 7 days. In another study, Hentilä et al. [13] did not observe a significant change in PERK protein content one hour after acute resistance exercise, but following 48 hours of recovery, BiP, PERK and ATF4 proteins increased in young and older men. In addition, CHOP mRNA levels rose one hour after acute exercise, but returned to baseline after 48 hours. Unlike acute exercise, the chronic resistance training did not change PERK pathway at 4–5 days after the end of the training period. They suggested that ER stress might depend on recovery time after exercise training. In the present study, 48 hours after the last training session, the FHL muscle was extracted and it was observed that the protein content of PERK pathway increased significantly in the trained groups compared with untrained groups. However, these changes were less in the RT + Cr group compared to the RT + P group. This is probably due to the effect of Cr on improving recovery acceleration.

In a study by Kim et al. [19] the expression levels of ATF3/4, BiP, and PGC-1 α varied in the high-intensity training group compared to the lower-intensity training group. In other research, Kim et al. [18] studied the effects of 12 weeks of aerobic (treadmill running) and resistance training (ladder climbing) on ER stress in the cardiac muscle of obese rats. The phospho-PERK/PERK ratio decreased in both trained groups compared to the control group. This suggests that exercise-induced UPR activation depends on the modality and duration of exercise as well as the health status of the subjects [19, 13]. In resistance exercises, the main source of energy supply is the anaerobic system and the mechanical pressure applied to the skeletal muscles affects the protein folding in ER [13]. On the other hand, a rise in UPR due to resistance exercise might be a response to maintaining muscle homeostasis [35] rather than maintaining muscle mass [13].

Gallot et al. [12] recently examined the role of the PERK signaling pathway on regulation of skeletal muscle mass and function in adult mice. They reported that the targeted removal of PERK decreases skeletal muscle mass, muscle strength and contractile function. It has also been observed that the ablation of PERK causes a slow-to-fast fiber-type transition, myofiber atrophy, disruption of protein turnover, and increases the expression of atrophy-related molecules. UPR is sensitive to energy changes and the availability of substrate [31], and this is more pronounced in muscle contractions [35]. Therefore, according to the importance of energy availability during exercise [4] and the role of Cr in regenerating energy sources, Cr supplementation could play a role in modulating the PERK response.

The eIF2 α phosphorylation induces glutathione (GSH) synthesis and improves antioxidant capacity [23], while intracellular levels of GSH declines during intense exercise [7]. Thus, phosphorylation of the PERK-eIF2 α pathway is a mechanism for adaptation to exercise training. Therefore, in addition to the energetic effects [4], Cr supplementation improves the antioxidant capacity (up-regulate GSH) [7]. In the present study, the decrease in PERK pathway activation in the RT + Cr group compared to RT + P group is probably due to the effects of Cr on enhancing antioxidants capacity.

In conclusion, the results of this study provide novel data concerning the role of Cr supplementation during resistance exercise in PERK pathway. We observed that 8 weeks of ladder climbing training in

addition to Cr supplementation improves muscle hypertrophy and modifies PERK pathway response. This is probably due to the reconstruction of energy resources, the acceleration of recovery and enhancement of antioxidant capacity.

Declarations

Compliance with ethical standards

Animals used in the current research were treated in accordance with the National Institutes of Health Guide for the care and use of laboratory animals, and the study design was reviewed and approved by the Animal Research Ethics Committee of Kurdistan University of Medical Sciences (IR.MUK.REC.1397.5010).

Conflict of interest

None of the authors declare any conflict of interest related to this research.

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Author contributions

Hersh N and Dariush SH-V performed the experiments, analyzed the data, and drafted the manuscript. Dariush SH-V designed the research. Mohammad Raman M conducted the Histological assays and participated in the writing of the manuscript. The authors declare that all data were generated in-house and that no paper mill was used.

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Figures

Figure 2

a) Histological H&E staining images of cross-sectional areas of the FHL muscle. Muscle fibers (F), Endomysium (arrows), Perimysium (P). b) FHL muscle cross-sectional area following 8 weeks intervention. c) Frequency distribution of the FHL muscle fibers according to CSA ranges: 0 – 5500 µm². The data presented as the mean ± SEM. *** P < 0.001 vs. UN+Cr, ++ P < 0.01 vs. RT+P, ^^^ P < 0.001 vs. UN+P.

Figure 3

Comparison between groups regarding hypertrophy indices after 8 weeks intervention. a) Western blot analysis of protein expression. b) mTOR, phospho mTOR (p-mTOR) and p-mTOR/mTOR ratio. c) AKT, phospho AKT (p-AKT), p-AKT/AKT ratio. β -Actin were probed as an internal control. The data presented as the mean $\pm$ SEM. *** $P < 0.001$ vs. UN+Cr, + $P < 0.05$ vs. RT+P, ^^ $P < 0.001$ vs. UN+P.

Figure 4

Comparison between groups regarding PERK indices after 8 weeks' intervention. a) Western blot analysis of protein expression. b) PERK, phospho PERK (p-PERK), p-PERK/PERK ratio. c) eIF2α, phospho eIF2α (p-eIF2α) and eIF2α/p-eIF2α ratio d) BiP, CHOP, ATF4. β-Actin were probed as an internal control. The data presented as the mean ± SEM. *** P < 0.001 vs. UN+Cr, +++ P < 0.001 and ++ P < 0.01 vs. RT+P, ^^^ P < 0.001 vs. UN+P.