Research Article

Effects of prolonged whey protein supplementation and resistance training on the gene expression of IGF-1 and gastrocnemius muscle weight in young male Wistar rats

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Abstract

This study aimed to investigate the combined effect of prolonged whey protein supplementation and resistance training on the expression of the insulin-like growth factor-1 (IGF-1) gene, the weight of the Gastrocnemius muscle, and One-repetition maximum in young male Wistar rats. In this study, twenty-one young male Wistar rats, aged eight weeks and weighing between 200 to 250 grams, were randomly assigned to four groups: training (T), supplementary training (ST), Sham (Sh), and control (C). The resistance training program was conducted for six weeks, five days a week, with the training intensity increasing from 50 to 100% of the rats' body weight. The rats received the whey supplement via the gavage method based on their body weight, using whey production by ON company. Forty-eight hours after the final training session, the quadriceps muscles of the rats were extracted and the expression level of the IGF-1 gene was evaluated using the Real-Time PCR method. Statistical analysis was conducted using a one-way variance test and Scheffe's test. The results showed that the training-supplement group exhibited a significant increase in IGF-1 expression compared to the sham group ($P<0.05$). Moreover, the weight of the gastrocnemius muscle of rats and also the One-repetition maximum in the training-supplement group significantly increased compared to the training, sham, and control groups ($P<0.05$). The findings suggest that the concomitant use of resistance training and whey protein supplementation has a synergistic effect on IGF-1 gene expression in skeletal muscle, which may contribute to enhanced muscle hypertrophy and 1RM.

Key Words: **IGF-1, Hypertrophy, Whey protein, Resistance training**

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Introduction

Skeletal muscle is the body's most extensive organ and can increase in size in response to exercise or dietary supplementation (Morisasa, Yoshida, et al. 2022). Skeletal muscle plays a crucial role in body movement, physical activity, energy metabolism, and the production of numerous myokines (Lin, Li et al. 2021). The process of muscle hypertrophy is contingent upon the incorporation of protein into the muscle tissue. Resistance training triggers signaling pathways that are involved in the regulation of muscle protein synthesis, transcription, and satellite cell activity. Hypertrophy is characterized by an enlargement in the mass and volume of cells or muscle fibers, without an increase in their number. This process occurs as a result of the incorporation of protein into the muscle fibers. Skeletal muscle hypertrophy is essential for athletic performance, as well as in various pathological conditions, and is crucial for maintaining motor independence in individuals. The concurrent utilization of protein intake and resistance training is the most effective means of inducing skeletal muscle hypertrophy and regeneration. Furthermore, to promote muscle hypertrophy, it is necessary to maintain a positive protein balance (Deldicque 2020).

In response to heavy resistance exercises, both local and systemic acute responses occur in the expression pattern of genes, protein production, and muscle metabolism. These responses are crucial in creating adaptations over an extended training period. Resistance training is one of the methods that stimulates IGF-1 gene expression in skeletal muscle. The PI3K/Akt pathway is the primary cellular cascade responsible for regulating skeletal muscle growth. The Akt molecule, also known as protein kinase B (PKB) or messenger molecule, functions as both an effector of anabolic signals and a dominant inhibitor of catabolic signals. Akt signals to mTOR, which activates various downstream effectors to exert anabolic effects. One of mTOR's primary targets is P70S6K, which plays a crucial role in muscle hypertrophy. IGF-1, as an upstream molecule, has the potential to be an effector of the PI3K/Akt pathway, ultimately leading to protein synthesis in the ribosome

ribosome by phosphorylating P70S6K (Vitale, Pellegrino, et al. 2019, Gholipour, Seifabadi et al. 2020). Research has shown that resistance training increases IGF-1 mRNA levels in skeletal muscle. Furthermore, IGF-1 has been demonstrated to directly stimulate muscle protein synthesis, resulting in skeletal muscle hypertrophy (Askari, Bijeh et al. 2017). Studies have demonstrated that IGF-1 is a powerful inhibitor of atrophic genes and is even more effective than testosterone in this regard (Lin, Li et al. 2021). The up-regulation of the IGF-1 gene expression is a critical factor in promoting muscle hypertrophy (Gorzi, Jazaei et al. 2019).

Studies have shown that engaging in sports exercises can significantly increase the expression of the IGF-1Ec gene in Wistar rats (Nasrollahi, Gaeini et al. 2020). Moreover, an eight-week resistance training program has been found to elevate Akt expression and reduce FOXO3 expression in rat muscle tissue (Rostamian Dolatshanlou, Cheragh-Birjandi et al. 2023). Resistance training is one of the methods that can increase IGF-1Ec gene expression and promote muscle protein synthesis when essential amino acids are present. It is widely regarded as the most effective way to increase muscle mass, as the increased absorption of amino acids enhances muscle protein production through the activity of the IGF-1/Akt/m-TOR pathway (Wackerhage, Smith et al. 2017).

Whey protein, derived from milk, is widely regarded as the most effective protein compound due to its high absorption rate and superior essential amino acid (EAA) content, including a significant proportion of branched-chain amino acids (BCAAs). The body cannot synthesize EAAs in adequate amounts, and their deficiency can hinder cellular protein synthesis and muscle hypertrophy. Therefore, consuming sufficient EAAs is crucial (Lam, Khan et al. 2019). Whey protein supplements provide a greater availability of amino acids in the bloodstream than meals containing eggs or beef protein, with a more significant amount accessible within an hour of consumption (Li and Liu 2019, Gwin, Church et al. 2020). Long-term supplementation with whey protein or amino acids has been shown to upregulate IGF-1 gene expression in skeletal muscle, resulting in increased muscle fiber cross-sectional area and strength. The amino acid profile of whey protein is similar to that of skeletal muscle, providing a high percentage of EAAs for cellular protein synthesis (West, Abou Sawan et al. 2017). Further studies have found that high doses of whey protein can increase local IGF-1 levels and stimulate tissue growth in rats, especially when combined with resistance training (Jang, Kim et al. 2021). Thus, this study aims to assess the impact of resistance training with and without whey protein supplementation on IGF-1 gene expression and gastrocnemius muscle weight in young male Wistar rats.

Materials and Methods

Animals

The present study employed an experimental pre/post-test design to evaluate the expression of the IGF-1 gene and the weight of the gastrocnemius muscle. A total of 21 healthy young male Wistar rats, aged eight weeks and weighing approximately 200-250 grams, were selected as the sample from Royan Research Institute. All rat maintenance and dissection procedures were conducted following the ethics committee at the Institute of Physical Education (Ethics ID: SSRI.REC-2205-1628) in the animal house of the Faculty of Sports Sciences at Shahid Rajaei University. The rats were randomly assigned to four groups: two experimental groups subjected to resistance training (n=6) and resistance training with whey protein supplementation (n=6), a control group (n=3), and a sham group (n=6) that underwent the stress protocol without exercising or receiving supplements. The rats were housed in a room with a temperature of 23±2°C and a relative humidity of 50±5%, with a 12-hour light-dark cycle.

Resistance training protocol

The resistance training protocol involved young male Wistar rats climbing a one-meter ladder with 34 steps spaced two centimeters apart and inclined at an angle of 85 degrees. The first two weeks of the protocol were for adaptation to the environment and familiarization with resistance training. The main training period lasted for six weeks with five sessions per week. Each session consisted of three sets, with each set comprising five repetitions, and a rest period of one minute between repetitions and two minutes between sets. Weights were attached to two-thirds of the proximal end of the rats' tails using adhesive tape to add resistance. In the first week, each rat's weight was set at 50% of its body weight, and the weight was increased by 10% each week following the overload principle. The weight reached approximately 100% of the rats' body weight at the end of the training period, and increasing the weight each week was dependent on completing the previous week's training period. The control group underwent eight weeks without any stress, while the sham group only climbed up and down the ladder for ten minutes and received distilled water via gavage (Aram, Jun-Young et al. 2015, Gholipour, Seifabadi et al. 2020).

Whey supplementation

In this study, whey protein isolate from Optimum Nutrition (ON) was used as a supplement. It was dissolved in distilled water and administered through gastric gavage to the rats in the supplement group, 30 minutes after resistance training (Ahmadi-Kani Golzar, Fathi et al. 2017). The recommended dosage of whey protein for humans is around 20 grams per serving when combined with a regular diet and exercise plan. To ascertain the appropriate dose for the rats, a human equivalent dose was calculated based on

body surface area and adjusted for the difference in surface area between humans and rats. The dose of whey protein for the rats was estimated to be 2.05 grams per kilogram of body weight, based on the human equivalent dose.

Samples collection

Forty-eight hours after the final training session and following a 12-hour fast, the rats were dissected alternately from different groups. Anesthesia was induced by intraperitoneal injection of a combination of Ketamine (90 mg/kg body weight) and Xylazine (10 mg/kg body weight), and the Gastrocnemius muscle of the left leg was extracted. The muscle tissues were weighed precisely with an accuracy scale of 0.01 g and rinsed in disposable sterile plates with sodium chloride washing serum (0.9%) and PBS solution. The tissues were immediately frozen in a nitrogen tank and transported to the molecular cell laboratory of the Faculty of Veterinary Medicine, University of Tehran, for quantifying IGF-1 gene expression using the Real-Time PCR method.

Gene Expression

RNA extraction

RNA was extracted from homogenized tissue by adding 400 microliters of lysing solution to 200 microliters of tissue in a microtube and vortexing for 20 seconds. The mixture was combined with 300 microliters of precipitation solution and swirled 10 times before transferring to a column-containing microtube and centrifuging at 13,000 rpm for one minute. After adding washing buffers I and II and centrifuging, the column was transferred to a nucleotide-free microtube and centrifuged for two minutes at 13,000 rpm until the remaining ethanol was dried. To elute RNA, 50 microliters of RNase-free distilled water was added to the column and incubated at 55°C for five minutes before centrifuging at 13,000 rpm for one minute. RNA purity was checked using a nanodrop device, and the remaining RNA was stored at -70°C for cDNA preparation.

Preparation of cDNA

For cDNA synthesis, the Synaclon cDNA synthesis kit (Cat. No: RT5201) and sterile, nuclease-free microtubes were used. Eight microliters of extracted RNA and one microliter of random hexamer were added to a 0.2 ml microtube, and the final volume was adjusted to 10 microliters with distilled water. The microtube was incubated at 65°C for five minutes and then placed on ice for two minutes. The microtube was then briefly centrifuged to ensure that all materials were in the bottom of the tube and not attached to the walls.

To proceed with cDNA synthesis, a separate solution was prepared consisting of 2 microliters of 10X M-Mulv buffer, 0.5 microliters of M-Mulv Reverse Transcriptase enzyme, 2 microliters of 10 mM dNTP Mix, 0.5 microliters of protective enzy-

Table 1. Sequence of primers

Genes	Primer sequences
IGF-F	TACTTCAACAAGCCCACAGG
IGF-R	CTCATCCACAATGCCTGTCT
GAPDH-1	GACATGCCGCCTGGAGAAAC
GAPDH-2	AGCCCAGGATGCCCTTTAGT

-me Rnase inhibitor, and DEPC-treated water to bring the final volume to 10 µl. This solution was added to the microtube containing the RNA and random hexamer, mixed well, and then centrifuged. The microtube was incubated at 42°C for one hour for cDNA synthesis. The enzyme was inactivated by heating the mixture at 85°C for five minutes.

Measurement of IGF-1

The statistical analysis of the real-time PCR data was conducted using the comparative CT method, also known as the $2^{\wedge(-\Delta\Delta CT)}$ method, which involves calculating the ΔCT value by subtracting the CT value of the reference gene from the CT value of the target gene. The ΔΔCT value was then determined by subtracting the ΔCT value of the control group from the ΔCT value of the experimental group, and the fold change in gene expression was calculated as $2^{\wedge(-\Delta\Delta CT)}$. To determine the statistical significance of the results, various methods such as t-tests or ANOVA were used depending on the number of groups and the type of data. It was important to ensure that the data met the assumptions of the statistical test used, such as normal distribution and equal variance. In addition to statistical analysis, the quality of the experimental data was evaluated by checking for outliers and assessing the reproducibility of the results. Appropriate controls were included in the experiment to ensure the accuracy of the results. The sequence of primers is presented in Table 1.

Statistical analysis

After collecting the primary data, the normality of the data distribution was verified by conducting the Shapiro-Wilk test. Once normality was established, statistical analysis was conducted to compare the data between groups. To accomplish this, a one-way ANOVA test was implemented. Due to unequal group sizes, Scheffe's post hoc test was used. The correlated t-test was used to examine changes in the stabilizing variable. Statistical calculations were performed using SPSS version 26 software with a predetermined significance level of $P \geq 0.05$.

Results

Figure 1 displays the results of muscle weight gain, revealing a significant increase in the weight of the Gastrocnemius muscle of rats in the supplemental training group compared to the training, sham, and control groups (P=0.01).

This suggest that the training-supplement group experienced the

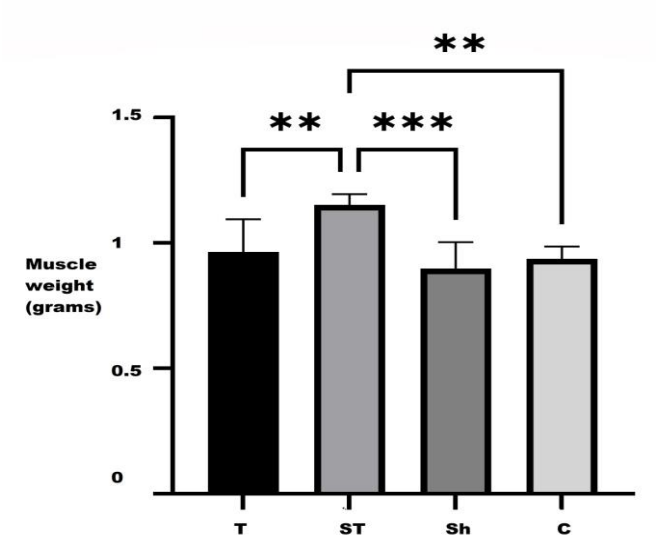

Figure 1. Muscle weight of rats at different groups of studies. Data were show as mean ± SD. *Shows significant difference at P ≥ 0.05

most pronounced muscle growth. Also, Figure 2 shows differences in One-Repetition Maximum between the groups.

Figure 2 shows that both experimental groups increased their 1RM from pre-test to post-test, indicating that the strength was improved. However, the group that received training plus Whey supplement had higher 1RM values than the group that received training only. This suggests that the supplement had an additional effect on enhancing strength.

Figure 3 represents the results of IGF-1 gene expression, indicating a significant increase (P=0.01) in the expression level of the IGF-1 gene in the quadriceps muscle of rats in the training-supplement group compared to the training and Sham groups.

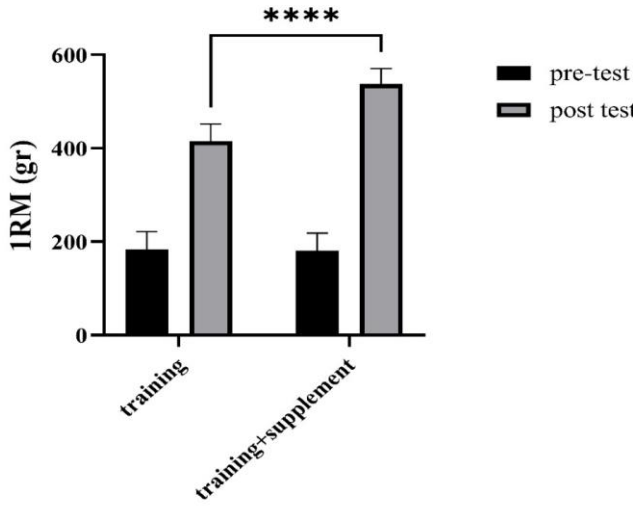

Figure 2. Figure 2. Difference in One- Repetition Maximum. Data were show as mean ± SD. *Shows significant difference at P ≥ 0.05

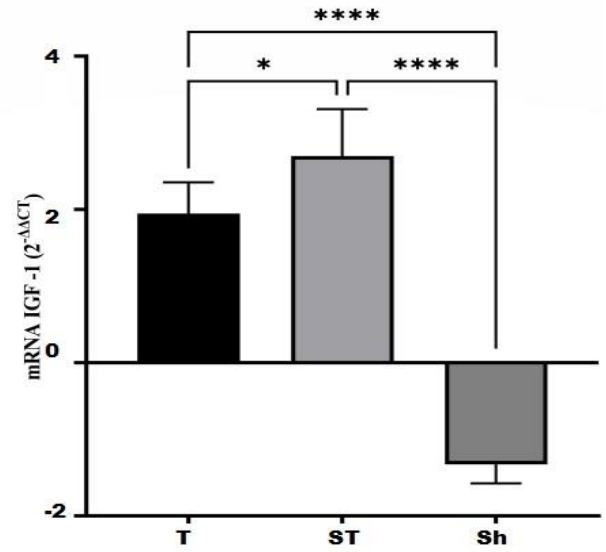

Figure 3. IGF-1 gene expression. Data were show as mean ± SD. *Shows significant difference at P ≥ 0.05

Discussion

The study's results indicate that both resistance training and resistance training with whey protein supplements have a notable impact on IGF-1 gene expression. The percentage change between the two values was calculated, revealing that IGF-1 gene expression was 38.6% higher in the supplemental resistance training group compared to the resistance training group. These findings suggest that the inclusion of whey protein supplement in resistance training could potentially result in a more substantial increase in IGF-1 gene expression, which is essential in promoting muscle growth and also leads to an increase in 1RM.

The gene expression of IGF-1 is considered one of the indicators of muscle hypertrophy (Gorzi, Jazaei et al. 2019). Many anabolic messenger pathways have been identified that can overlap with each other. These pathways include PI3K/Akt/m-TOR pathway, MAPK pathway, calcium-dependent pathways, etc. In the meantime, the PI3K/Akt/mTOR pathway is considered the main cellular cascade of skeletal muscle growth regulation, and IGF-1 increases muscle protein synthesis by activating this pathway. IGF-1 activates PI3K and then leads to the phosphorylation and activation of Akt which directly phosphorylates the TSC complex and allows Rheb to be activated in the mTOR-bound form. The main target of m-TOR is p70S6K, which plays a very important role in starting mRNA translation and muscle protein synthesis. In addition, mTOR exerts its anabolic effects by inhibiting 4E-BP1, which is a negative regulator for eIF4E protein. e-IF4E is also a powerful mediator in mRNA translation and protein synthesis. Akt messenger molecule inhibits catabolic activities independently of mTOR. Akt phosphorylates FOXO proteins, which are a subgroup of the family of forkhead transcription factor

and cause muscle atrophy. Deactivation of FOXO proteins inhibits MuRF-1 and atrogin-1 ubiquitin ligases, which reduces muscle protein breakdown (Coffey and Hawley 2017).

IGF-1 phosphorylates PI3K and Akt kinases after binding to the insulin receptor. Akt also activates the important protein m-TOR. so that the e-IF4E complex becomes available to mRNA and the synthesis of new proteins begins in the ribosome (Kashef and Khaje Bahrami 2019). On the other hand, IGF-1 stimulates the 5-alpha reductase enzyme, the synthesis of adrenal and gonadal androgens, and the transmission of the androgen receptor signal. GH/IGF-1 axis plays an important role in ACTH-dependent production of DHEAS by the human adrenal gland. It increases the sensitivity of the adrenal gland to ACTH and induces the expression and activity of key enzymes of adrenal androgen biosynthesis. In addition to the effect of LH hormone, IGF-1 itself also stimulates the proliferation of testicular Leydig cell precursors and is an essential local mediator for testicular DNA synthesis and steroidogenesis. Therefore, the IGF system is important for Leydig cell differentiation and androgen biosynthesis. Finally, the testosterone hormone itself causes an increase in the amount of IGF-1 Ec and then stimulates the PI3K/Akt/m-TOR pathway (Sakuma 2013).

IGF-1 plays a key role in muscle growth by activating m-TOR. The Ec isoform of IGF-1, acting as a myokine, regulates muscle growth through several mechanisms, such as directly stimulating muscle protein synthesis by phosphorylating p70S6K, suppressing FOXO nuclear localization and transcription activities to inhibit protein degradation, and modulating the response of satellite cells to exercise-induced muscle injury to affect hypertrophy adaptations. By regulating the expression of many genes in skeletal muscle, IGF-1 stimulates protein synthesis and increases the diameter of skeletal muscle fibers. GH and its actions control physical growth, and the GH/IGF-1 axis is crucial for normal growth. Local production of IGF-1 in skeletal muscle is believed to be linked to local muscle hypertrophy from exercise, while GH regulates IGF-1 gene expression in the liver. The anabolic effects of GH are primarily due to its reinforcing effect on IGF-1, meaning that the effects of GH on tissues are caused by IGF-1. Fluctuating GH secretion promotes growth mainly through the expression of the IGF-1 gene. Various factors stimulate GH secretion, such as an increase in amino acids in the blood, hypoglycemia, exercise, shock, excitement, and ghrelin, a hormone secreted from the stomach before eating (Hall and Hall 2020). Consuming amino acids either orally or intravenously can increase GH secretion and result in increased muscle strength and hypertrophy (Chromiak and Antonio 2002). GH secretion is influenced by various factors, including an increase in blood amino acids. GH stimulates the production of somatomedins, which are small proteins produced by various tissues, including skeletal muscle and liver. Somatomedin C or IGF-1 has a significant impact on hypertrophy and muscle growth (Hall and Hall 2020). Increasing amino acid absorption can promote muscle protein production through the activity of the IGF-1/Akt/m-TOR pathway (Wackerhage, Smith et al. 2017). Studies have demonstrated that consuming whey protein supplements or using amino acids for an extended period at a high dose can increase the expression of the IGF-1 gene in skeletal muscle, resulting in increased cross-sectional area (Csapo, Gumpenberger et al. 2020) of muscle fibers and muscle strength (Mori and Tokuda 2018, Lednev, Kravchenko et al. 2020). Injecting IGF-1 locally in the muscle can change all bulking activities, including satellite cell proliferation, gene expression, and protein production. Therefore, increasing the expression of the IGF-1 gene is crucial for muscle bulking (Gorzi, Jazaei et al. 2019).

Resistance exercise has been shown to significantly increase IGF-1 mRNA expression. Anabolism primarily occurs through amino acids, which the body cannot synthesize and must be obtained through the diet. Deprivation of even one amino acid can disrupt the process of cellular protein synthesis by inhibiting the initial stage of mRNA translation. An increase in amino acids in plasma and muscle cells triggers an anabolic response (Schoenfeld 2020). Among the many well-known supplements, whey protein, derived from milk (20% early-absorbed whey protein and 80% late-absorbed casein protein), has a high biological value and leads to faster absorption of essential amino acids in the bloodstream (Schoenfeld 2020). All 20 amino acids participate in building body proteins, and eight of these amino acids are considered essential because the body cannot produce them, so they must be obtained through the diet, just like vitamins and minerals. Deprivation of any essential amino acid can disrupt cellular protein synthesis by inhibiting the initial stage of mRNA translation (Tiidus, Tupling et al. 2012, Schoenfeld 2020). Whey protein contains all essential amino acids required for muscle protein synthesis, with a higher leucine content than other proteins, making it more effective in increasing IGF-1 expression and being considered an essential factor in skeletal muscle hypertrophy (Teixeira, Santos et al. 2019).

Kandil et al. (2020) found that whey protein supplements have therapeutic properties against secretory cells in the pituitary gland. They proved that whey protein supplement is beneficial for the secretory function of somatotrope cells (GH secretion) and gonadotrope cells (LH/FSH secretion), reducing the effects of muscle atrophy (Kandil, Sabry et al. 2020). The anabolic effect of GH is primarily realized through its reinforcing effect on IGF-1, meaning that the anabolic effects of GH are caused by IGF-1, not the direct effects of GH on tissues. The effects of fluctuating GH secretion in promoting growth are prolonged largely through the expression of the IGF-1 gene. Several factors stimulate GH secretion, such as an increase in amino acids in the blood, hypog-

-lycemia, exercise, shock, excitement, and ghrelin (a hormone secreted from the stomach before eating) (Hall and Hall 2020). Consuming quality protein such as whey provides all the essential amino acids necessary for growth and can increase GH secretion. GH is considered an enhancer of IGF-1 gene expression in skeletal muscle, and IGF-1 activates the PI3K/Akt/m-TOR pathway, which is known as the main pathway in muscle hypertrophy (Schoenfeld 2020). Some found that inducing a high dose of whey protein in rats increased local IGF-1 levels in the colon and growth plate significantly, proving the effectiveness of whey protein in tissue growth through the expression of IGF-1 (Jang, Kim et al. 2021). Dietary protein and amino acids are crucial nutritional factors for regulating the expression and secretion of IGF-1, but the intracellular mechanism that increases IGF-1 gene expression is unclear. The level of IGF-1 mRNA and protein, as well as the phosphorylation of mTOR, increase with an increase in the concentration of essential amino acids (Wan, Wang et al. 2017).

Conclusions

In general, resistance training and dietary supplementation, especially with whey protein, can impact the metabolic regulation of insulin-like growth factor 1 (IGF-1), leading to an acceleration in muscle growth and size and 1RM enhancement. The regulation of IGF-1 is complex and involves several factors, including GH secretion, amino acid availability, and activation of the PI3K/Akt/mTOR pathway. It is important to note that the effects of resistance training and dietary supplementation on IGF-1 regulation are influenced by various factors, such as age, sex, and training status. Nonetheless, the evidence suggests that incorporating resistance training and whey protein supplementation into a well-balanced diet can have positive effects on muscle growth and size by impacting the metabolic regulation of IGF-1.

What is already known on this subject?

Some of the main points from this article include:

• Resistance training can stimulate GH secretion, which in turn increases IGF-1 production.

• Whey protein supplementation can provide essential amino acids that are necessary for IGF-1 synthesis and activation.

• Resistance training and whey protein supplementation can activate the PI3K/Akt/mTOR pathway, which is a signaling cascade that mediates the anabolic effects of IGF-1 on muscle protein synthesis and hypertrophy.

• The effects of resistance training and whey protein supplementation on IGF-1 regulation may be influenced by various factors, such as age, sex, and training status.

• Resistance training and whey protein supplementation can have positive effects on muscle growth and size by impacting the metabolic regulation of IGF-1.

What this study adds?

The present study was the first research that investigated the combined effect of prolonged whey protein supplementation and resistance training on the expression of insulin-like growth factor-1 (IGF-1) gene and the weight of the Gastrocnemius muscle in young male Wistar rats. In addition, the results showed that the selected interventions can affect the regulation of insulin-like growth factor 1, and accelerate muscle growth and size.

Organ Cross-Talk Tips:

- IGF-1 growth factor has cross-talk with gastrocnemius muscle weight, and this interaction is influenced by whey protein supplementation
- IGF-1 growth factor has a cross-talk with gastrocnemius muscle weight through resistance training.

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Compliance with ethical standards

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

Ethical approval This study was approved by the Ethical Standards of the Institutional and/or National Research Committee (SSRI.REC-2205-1628).

Informed consent Animal study.

Author contributions

Conceptualization: F.K.S, F.Sh, M.S.; Methodology: F.K.S, F.Sh, M.S.; Software: F.K.S, F.Sh, M.S.; Validation: F.K.S, F.Sh, M.S.; Formal analysis: F.K.S, F.Sh, M.S.; Investigation: F.K.S, F.Sh, M.S.; Resources: F.K.S, F.Sh, M.S.; Data curation: F.K.S, F.Sh, M.S.; Writing - original draft: F.K.S, F.Sh, M.S.; Writing – review & editing: F.K.S, M.S.; Visualization: F.K.S, F.Sh, M.S.; Supervision: F.K.S.; Project administration: F.K.S, F.Sh, M.S.; Funding acquisition: F.K.S.

References

Ahmadi-Kani Golzar, F., R. Fathi and S. Mahjoub (2017). "The effects of endurance training and whey protein supplementation on inflammation and insulin resistance in the rats fed with high-fat diet."

Scientific Journal of Kurdistan University of Medical Sciences 22(4): 80-90. doi: https://doi.org/10.22102/22.4.80

Aram, Y., H. Jun-Young, C. Soon-Mi, H. Dong-Woo, K. Ki-Jeong and L. Suk-Ho (2015). "Comparison of multiple sets and progressive single set of resistance training on muscle mass and strength in rat." Exercise Science 24(3): 267-274. doi: https://doi.org/10.15857/ksep.2015.24.3.267

Askari, B., N. Bijeh and A. R. Lamir (2017). "Effects of 8 Weeks of Resistance Training and IGF-1 Injection on Biochemical Markers of Cancer and Colorectal Structures in Rats." Medical Laboratory Journal 11(6). doi: https://doi.org/10.29252/mlj.11.6.S1

Chromiak, J. A. and J. Antonio (2002). "Use of amino acids as growth hormone-releasing agents by athletes." Nutrition 18(7-8): 657-661. doi: https://doi.org/10.1016/S0899-9007(02)00807-9

Coffey, V. G. and J. A. Hawley (2017). "Concurrent exercise training: do opposites distract?" The Journal of physiology 595(9): 2883-2896. doi: https://doi.org/10.1113/JP272881

Csapo, R., M. Gumpenberger and B. Wessner (2020). "Skeletal muscle extracellular matrix–what do we know about its composition, regulation, and physiological roles? A narrative review." Frontiers in physiology 11: 253. doi: https://doi.org/10.3389/fphys.2020.00253

Deldicque, L. (2020). Protein intake and exercise-induced skeletal muscle hypertrophy: An Update, MDPI. 12: 2023. doi: https://doi.org/10 .3390/nu12072023

Gholipour, M., M. Seifabadi and M. R. Asad (2020). "Comparing the effects of endurance and resistance trainings on gene expression involved in protein synthesis and degradation signaling pathways of Wistar rat soleus muscle." Tehran University of Medical Sciences Journal 77(11): 668-677. doi: http://tumj.tums.ac.ir/article-1-10197-en.html

Gorzi, A., R. Jazaei, A. Rahmani and A. Bahari (2019). "The effects of different rest interval durations between resistance exercise sets on gene expression of CGRP and IGF-1 of muscle in male wistar rats." Research in Sport Medicine and Technology 17(18): 23-33. doi: https://doi.org/10.29252/jsmt.17.18.23

Gwin, J. A., D. D. Church, R. R. Wolfe, A. A. Ferrando and S. M. Pasiakos (2020). "Muscle protein synthesis and whole-body protein turnover responses to ingesting essential amino acids, intact protein, and protein-containing mixed meals with considerations for energy deficit." Nutrients 12(8): 2457. doi: https://doi.org/10.3390/nu12082457

Hall, J. E. and M. E. Hall (2020). Guyton and Hall textbook of medical physiology e-Book, Elsevier Health Sciences. ISBN: 9780323597128

Jang, J. H., S. Kim, H. J. Lee, H. J. Suh and K. Jo (2021). "Stimulating effect of whey protein hydrolysate on bone growth in MC3T3-E1 cells and a rat model." Food & Function 12(11): 5109-5117. doi: https://doi.org/10.1039/D1FO00338A

Kandil, N., D. A. Sabry, N. E. Ashry and H. I. El-Sayyad (2020). "Therapeutic Potential of Whey against Aging Related Cytological Damage of Adenohypophysis of Rat." sch. j. agric. life sci 3(8): 304-310. doi: https://doi.org/10.36349/EASJALS.2020.v03i09.002

Kashef, M. and S. Khaje Bahrami (2019). "The effect of 4 and 6 weeks of resistance training on serum levels of myonectin and IGF-1 in sedentary young men." Razi Journal of Medical Sciences 25(10): 31-37. doi: https://doi.org/20.1001.1.22287043.1397.25.10.1.2

Lam, F.-C., T. M. Khan, H. Faidah, A. Haseeb and A. H. Khan (2019). "Effectiveness of whey protein supplements on the serum levels of amino acid, creatinine kinase and myoglobin of athletes: a systematic review and meta-analysis." Systematic reviews 8(1): 1-12. doi: https://apastyle.apa.org/style-grammar-guidelines/references/dois-urls

Lednev, E. M., I. V. Kravchenko, V. A. Furalyov, E. A. Lysenko, I. S. Lemesheva, A. A. Grushin, V. E. Dubrov, O. L. Vinogradova and D. V. Popov (2020). "Effect of amino acids on IGF1 gene expression in human myotubes and skeletal muscle." Growth Hormone & IGF Research 53: 101323. doi: https://doi.org/10 .1016/j .ghir .2020 .101323

Li, M. and F. Liu (2019). "Effect of whey protein supplementation during resistance training sessions on body mass and muscular strength: a meta-analysis." Food & function 10(5): 2766-2773. doi: https://doi.org/10.1039/c8fo02497j

Lin, Y.-A., Y.-R. Li, Y.-C. Chang, M.-C. Hsu and S.-T. Chen (2021). "Activation of IGF-1 pathway and suppression of atrophy related genes are involved in Epimedium extract (icariin) promoted C2C12 myotube hypertrophy." Scientific reports 11(1): 10790. doi: https://doi.org/10.1038/s41598-021-90281-w

Mori, H. and Y. Tokuda (2018). "Effect of whey protein supplementation after resistance exercise on the muscle mass and physical function of healthy older women: A randomized controlled trial." Geriatrics & gerontology international 18(9): 1398-1404. doi: https://doi.org/10 .1111/ggi .13449

Morisasa, M., E. Yoshida, M. Fujitani, K. Kimura, K. Uchida, T. Kishida, T. Mori and N. Goto-Inoue (2022). "Fish protein promotes skeletal muscle hypertrophy via the Akt/mTOR signaling pathways." Journal of Nutritional Science and Vitaminology 68(1): 23-31. doi: https://doi.org/10 .3177/jnsv .68 .23

Nasrollahi, H., A. A. Gaeini, S. Biglari and A. Ghardashi Afousi (2020). "Changes of insulin-like growth factor I gene expression in gastrocnemius muscle of male Wistar rats after a period of high-intensity interval training." Daneshvar Medicine 25(5): 31-38.

Rostamian Dolatshanlou, S., S. Cheragh-Birjandi and A. Yaghoubi (2023). "The Effects of Different Training on Gene Expression Involved in Muscle Autophagy in Elderly Rats." Journal of Isfahan Medical School 40(699): 1016-1022. doi: https://doi.org/10.48305/JIMS.V40.I699.1016

Sakuma, K. (2013). "Basic biology and current understanding of skeletal muscle." Nova Biomedical. ISBN9781628083675

Schoenfeld, B. J. (2020). Science and development of muscle hypertrophy, Human Kinetics. doi: https://doi.org/10 .5040/9781492597668

Teixeira, F. J., H. O. Santos, S. L. Howell and G. D. Pimentel (2019). "Whey protein in cancer therapy: A narrative review." Pharmacological research 144: 245-256. doi: https://doi.org/10 .1016/j .phrs .2019 .04 .025

Tiidus, P. M., A. R. Tupling and M. E. Houston (2012). Biochemistry primer for exercise science, Human kinetics. doi: https://doi.org/10 .5040/9781492597668

Vitale, G., G. Pellegrino, M. Vollery and L. J. Hofland (2019). "Role of IGF-1 system in the modulation of longevity: controversies and new insights from a centenarians' perspective." Frontiers in endocrinology 10: 27. doi: https://doi.org/10 .3389/fendo .2019 .00027

Wackerhage, H., J. Smith and D. Wisneiwski (2017). "Molecular exercise physiology." Oxford textbook of childrenVs sport and exercise medicine: 430-440. doi: https://doi.org/10 .1093/med/9780198757672 .003 .0034

Wan, X., S. Wang, J. Xu, L. Zhuang, K. Xing, M. Zhang, X. Zhu, L. Wang, P. Gao and Q. Xi (2017). "Dietary protein-induced hepatic IGF-1 secretion mediated by PPARγ activation." PLoS One 12(3): e0173174. doi: https://doi.org/10.1371/journal.pone.0173174

West, D. W., S. Abou Sawan, M. Mazzulla, E. Williamson and D. R. Moore (2017). "Whey protein supplementation enhances whole body protein metabolism and performance recovery after resistance exercise: a double-blind crossover study." Nutrients 9(7): 735. doi: https://doi.org/10 .3390/nu9070735