

Evaluating the value of p70^{s6k} and mTOR signaling pathway in monitoring exercise-induced central fatigue in rats

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ABSTRACT

Exercise leads to muscle fatigue and decreased muscle strength in response to contraction activity, and besides, it causes central fatigue. In the current study, we evaluated the value of p70^{s6k} and mTOR signaling pathways in monitoring exercise-induced central fatigue in rats. For this purpose, 12 male rats were divided into control (n=6) and intervention (n=6) groups. The intervention group performed five sessions of climbing a one-meter ladder with a weight hanging on the tail for eight weeks. The weekly load increase was based on the mice's body weight, so it reached 30% in the first week to 200% in the eighth week. In order to evaluate central fatigue, the sedation score system was used. Forty-eight hours after the last training session, a blood sample was prepared, the expression level of related proteins was measured by the ELISA method, and the one-way ANOVA method was used for statistical analysis. This study showed that central fatigue did not significantly affect the total mTOR protein content (F=0.720, P=0.421). However, the level of phosphorylated mTOR in the intervention group had a significant difference compared to the control group (F=684.893, P=0.001, Eta²=0.988). There was a significant effect for total p70^{s6k} content (F=5.84, P=0.04, Eta²=0.42). Also, for phosphorylated p70^{s6k}, there was a significant difference between the mentioned groups (F=7.262, P=0.027, Eta²=0.476). In General, it was shown in this study that central fatigue is directly related to the increase in p70^{s6k} production and phosphorylation of p70^{s6k} and mTOR. Therefore, these two proteins can probably be evaluated for monitoring exercise-induced central fatigue, although we need more evaluations.

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Introduction

Intense training and subsequent recovery form the basis of improving athletic performance. A little tiredness, mild depression, weakness, anxiety, irritability, and difficulty concentrating or sleeping are normal for athletes who participate in intense training or competitions (1). Athletes may experience persistent muscle pain, decreased coordination, decreased libido, and frequent upper respiratory tract infections. This state is called overreaching, and its experience is considered a part of intense training. These symptoms and the loss of performance capacity in an athlete are quickly resolved if they benefit from a period of light activity (2). If overreaching goes on for too long, fatigue occurs, and the mentioned symptoms and loss of exercise capacity can last for several weeks to several months (3).

Fatigue means the inability to maintain the necessary energy during an activity, which leads to decreased performance (3). This phenomenon is in the form of a decrease in the ability of receptive contraction or receptive stimulation of the muscle or a decrease in the ability of voluntary activation through spinal and supraspinal factors. There are many theories and countless types of research about

the causes of fatigue (1). According to the beginning of the motor commands from the cerebral cortex and its end in the muscle contraction filaments, according to the location of this imbalance, fatigue is divided into two types, central and peripheral. Environmental fatigue occurs in parts far from the neuromuscular connection (after the neuromuscular junction) (2). If this problem occurs in parts close to the body (before the neuromuscular connection), it is related to the neuromuscular connection. It is called central fatigue. This means that during a monotonous activity, a person reaches a place where he is forced to struggle more to maintain his strength. Or in other words, the intensity of cortical commands increases (4). The cause of these changes can be peripheral (decreasing the ability to produce force in the muscle) or central (reducing the power of the central nervous system to create nerve impulses) (1, 3).

Possible sites involved in the development of exercise-induced central fatigue include axonal action potential junctions where axonal branching sites may be closed and lead to loss of muscle cell activity. Also, the muscle reflex may affect the afferent motor nerves (failure to transmit the message from the nerve to the muscle and stop the contraction) (5). Stimulation of type III and IV neurons leads to decreased neuron excitability and inhibition of

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motor cortex (brain) output. The excitability of the cells in the brain's motor cortex may induce magnetic stimulation during the change in performance of motor tasks. The serotonergic synaptic effect may increase the feeling of fatigue (6). This condition may be caused by the increase in the influx of tryptophan, the precursor of serotonin, to the brain due to the decrease in the concentration of open chain amino acids (BCAAs) in the blood. The release of cytokines caused by exercise IL-5 causes fatigue, and IL-1 induces pathological behavior (5).

Since the increase in protein synthesis is the leading cause of skeletal muscle hypertrophy, and according to the evidence, the mTOR signaling pathway is the primary mechanism in protein synthesis, resistance exercises probably cause muscle hypertrophy by activating this pathway (7). The mTOR is a serine/threonine kinase related to a phosphatidylinositol kinase (PIKKs), selectively inhibited by rapamycin macrolide antibiotics (8, 9). The effects of mTOR include increasing protein translation, ribosome biogenesis (increasing the capacity of a cell for protein synthesis), and inhibiting the autophagy process. In addition, mTOR increases cell division and transcription of some genes. It has been determined that mTOR, in its signaling pathway, causes the phosphorylation of the ribosomal protein 70kDa S6 kinase (p70^{S6K}), which is necessary for protein synthesis (10, 11).

The p70^{S6K} is a member of the AGC protein kinase family and increases mRNA translation by phosphorylating several substrates, including SKAR, eIF4B, eEF-2K, PDCD4, and ribosomal protein S6. The role of p70^{S6K} in increasing mRNA translation is best characterized as a phosphorylating factor of eEF-2kinase (eEF-2k) (11). By phosphorylating eEF2, eEF-2k suppresses it and reduces mRNA translation, but eEF-2k directly phosphorylates and inhibits p70^{S6K} (12). In this way, mTOR and p70^{S6K} increase the translation process and the expression of proteins needed for muscle structure. Since the increase in protein synthesis is the leading cause of skeletal muscle hypertrophy, and according to the evidence, the mTOR signaling pathway is the primary mechanism in protein synthesis; fatigue probably causes muscle hypertrophy by activating this pathway (13). In this study, the value of p70^{S6K} and mTOR signaling pathways were considered in monitoring exercise-induced central fatigue in rats.

Materials and Methods

Samples

Twelve male rats (180 ± 20 grams, eight weeks old) were familiarized with the resistance training protocol and vertical ladder climbing for inducing central fatigue in the Sports Science, Department at the Hebei Sport University for one week. Then, the rats were randomly divided into resistance training (n=6) and control (n=6) groups, and the rats were kept under standard and completely identical conditions for the two groups (except for the training program). The present experimental protocol is approved by the Experimental Animal Ethnic Committee of Hebei Ex & In Vivo Biotechnology Co., Ltd. All procedures were

carried out according to the instructions for the animal care and use of the Laboratory Animals -- Guideline for Ethical Review of Animal Welfare (GB/ 35892-2018).

Implementation and evaluation of the central fatigue protocol

In the first week, the number of weights attached to the rats was 30% of their body weight, which gradually increased and reached about 200% of their body weight in the final week. We carried out this exercise for eight weeks and five weekly sessions of climbing a one-meter ladder with 26 steps, which the research group made. The training load was based on the percentage of the rats' weight. In this way, at the beginning of each week, the rats were weighed, and a percentage of their body weight was considered the week's training load. In the first week, the number of weights tied to the rats' tails was about 30% of their body weight, which gradually increased and reached almost 200% in the last week (Table 1).

Climbing the ladder was done in three rounds of four repetitions, and three minutes between rounds and 10 seconds between repetitions were considered as rest time. Then, to measure the central fatigue, the animal was controlled and monitored for 5 minutes, and a score was given to them. Scores from number 1 to 5 were given to each animal as follows: the average level of consciousness of each animal is number 1, the level of consciousness is lighter than the typical state number 2, the eyelashes fall on each other, and the animal's movements slow down (fatigue state) number 3, animal dozing number 4 and not moving animal number 5 (14-16). The control group was also kept in the training place to experience all the available conditions (environmental noise and researchers during the training).

Measurement of mTOR and p70^{S6K} levels

Forty-eight hours after the last training session, the animals were anesthetized with ketamine and xylazine (30-50 mg/kg body weight). Blood was taken from the corners of the animals' eyes. The blood of the animals was poured into a tube containing some sodium citrate to prevent blood coagulation. Then, the samples were placed inside a flask immediately and kept to measure laboratory variables, including mTOR and p70^{S6K}.

The expression level of the proteins was measured by the sandwich ELISA method and using special kits for rats produced by the Cell signaling company in the United States with serial numbers #7974 for total mTOR, #7976 for phosphorylated mTOR, #2903 for intact p70^{S6K}, and #9205 for phosphorylated p70^{S6K}. Each sample was measured twice, and the average of the two measurement steps was calculated.

Statistical analysis

One-way ANOVA was used to compare the mean levels of total mTOR, phosphorylated mTOR, p70^{S6K}, and phosphorylated p70^{S6K} proteins. Before implementing the statistical design, the assumptions of normal distribution of the data were evaluated with the Shapiro-Wilk test and

Table 1. The weekly program of resistance exercises used in the research.

Weeks	First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth
load (percentage of body weight)	30	70-80	100	120-130	140-150	170-175	180-190	200

homogeneity of variances with the Levan test. All statistical operations were performed by PASW version 18 software, and the significance level of the test was P<0.05.

Results

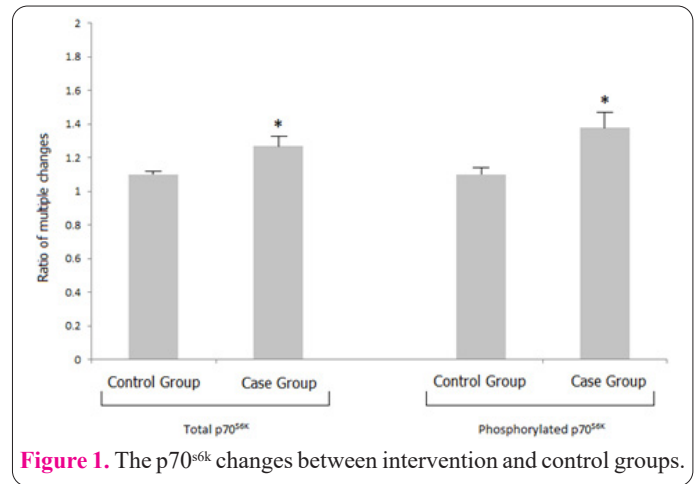
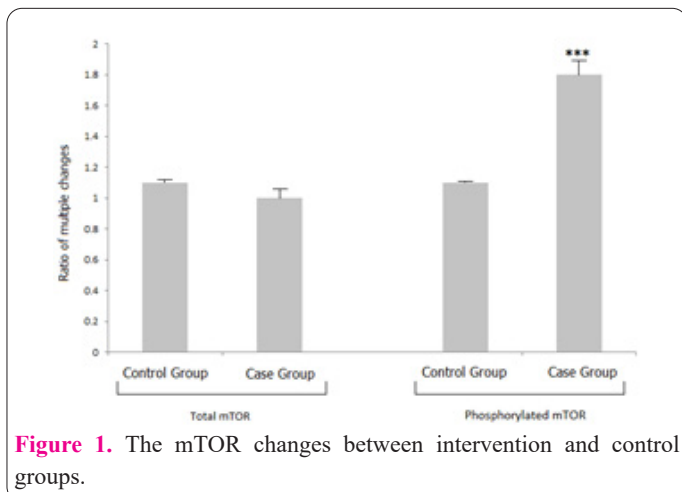
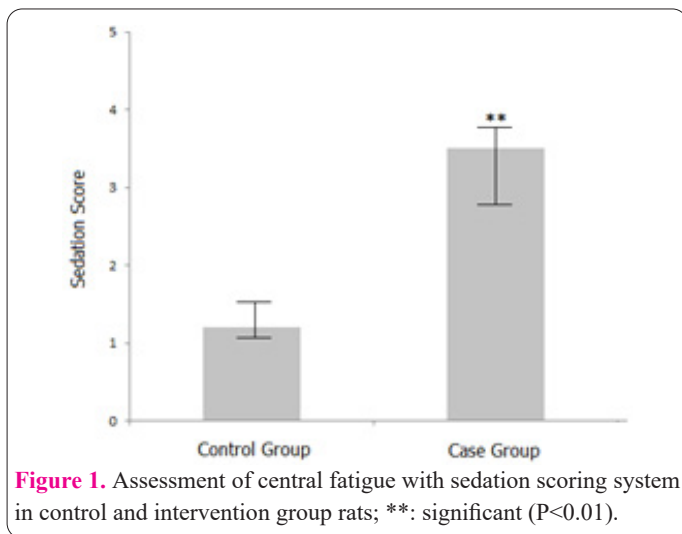
In the present study, central fatigue was determined by the animal sedation scoring system (Figure 1).

This study showed that central fatigue did not significantly affect the total mTOR protein content (F=0.720, P=0.421). However, the level of phosphorylated mTOR in the intervention group had a significant difference compared to the control group (F=684.893, P=0.001, Eta²=0.988) (Figure 2). There was a significant effect for total p70^{S6K} content (F=5.84, P=0.04, Eta²=0.42), also for phosphorylated p70^{S6K}, there was a significant difference between the mentioned groups (F=7.262, P=0.027, Eta²=0.476) (Figure 3).

Discussion

Muscle fatigue is associated with a decrease in the ability of muscles to produce the desired force, which occurs as a result of interrupting the chain of events from the central nervous system to the muscle fiber. Various factors affect muscle fatigue. One of these factors is exercise (17). The purpose of the present study is to investigate the value of p70^{S6K} and mTOR signaling pathways in monitoring exercise-induced central fatigue in rats.

In muscle cells, the change in the total protein content



of mTOR does not indicate a change in the rate of cellular protein synthesis, because as mentioned, mTOR has phosphorylated and non-phosphorylated forms and its phosphorylated form is the active form. But it can indicate mTOR gene expression increase and protein synthesis capacity (18). The findings of this study regarding the lack of increase in the total protein content of mTOR were consistent with Haraguchi *et al.* (19) and inconsistent with Goodman *et al.* (20). The reason for the lack of significant increase in the total content of this protein is not apparent. In this study, its gene expression was not measured to determine whether it increased, but it seems that it must be due to the growth and damage of regulatory protein (REDD1). DNA focused on the mTOR protein synthesis pathway because Hulmi *et al.* (21) showed that increasing REDD1 protein expression decreases mTOR protein expression.

Previous studies have well shown that for the effect of mTOR on the process of fatigue and increasing protein synthesis, the amount of phosphorylation of this protein is practical, and phosphorylated mTOR is the most critical indicator of protein synthesis and muscle hypertrophy (22). Because this protein is activated by phosphorylation, we observed that its amount in the rats' blood increased in this study. The mTOR has several positions to be phosphorylated, but position 2448 is phosphorylated by mechanical stimuli such as resistance exercises, which activate mTOR and phosphorylates its downstream proteins (23). Different pathways can activate mTOR, but it is not completely clear which pathway fatigue causes mTOR phosphorylation. One of the suggested pathways is the pathway of insulin-like growth factor (IGF-1-1) receptor tyrosine kinase-phosphatidylinositol-3-kinase (PI3K) protein kinase B or Akt, which is now questioned because, despite the inhibition of each of these proteins, resistance exercises have caused mTOR activation (24). Recently, the theory of mechanical receptors on the cell surface, such as integrin, dystrophin, and channels activated by stretching, has been proposed, but it needs more studies. The role of phosphatidic acid (PA) in the activation of mTOR in response to resistance training is confirmed by other routes. Unlike other factors that activate mTOR through TSC/Rheb, PA directly activates mTOR by affecting the FRB region (25).

Studies found the RPS6KB1 gene codes the p70^{S6K} protein but based on the searches, no research on the effects of exercise and fatigue on RPS6KB1 gene expression (26). In this study, resistance training has probably caused

an increase in the expression of the RPS6KB1 gene, and subsequently, the expression of the total p70^{S6K} protein has increased significantly. This finding agrees with the study of Goodman *et al.* (20). Their study used the removal of auxiliary muscles to create mechanical pressure. In this research, we showed that fatigue with increasing mechanical pressure causes a 48% increase in the content of this protein.

The most important finding of this research is the 37% increase in phosphorylated p70^{S6K}. In this case, it should be mentioned that p70^{S6K} is phosphorylated and inhibited by AMPK and rapamycin in several places, such as threonine 229, serine 404, and 411. But to be activated, it must be phosphorylated by mTOR at the threonine 389 positions (27). Pearson *et al.* (28) created a mutation at position 389, and alanine was placed at position 389. After that, p70^{S6K} no longer had any activity, which shows the importance of phosphorylation of threonine 389 by mTOR. The increase in p70^{S6K} phosphorylation in this study is consistent with the research of Goodman *et al.* (20). Probably, it can be attributed to fatigue in this research by increasing the phosphorylated mTOR, and its activation has caused the increase in the phosphorylation of p70^{S6K} (26). In fact, p70^{S6K} is one of the most important downstream targets of mTOR. Without it, mTOR cannot increase the translation elongation phase and is one of the determining factors of protein synthesis. In addition, p70^{S6K} increases ribosomal DNA transcription through phosphorylation of upstream binding factor (UBF) and causes ribosome bioproduction (17). Since the ribosome is a protein factory, living organisms need the presence and activity of the ribosome to produce protein. Activating p70^{S6K}, the mTOR pathway increases protein production through eEF-2k and protein production capacity through UBF (27-29).

Conclusion

In summary, it was shown in this study that central fatigue is directly related to the increase in p70^{S6K} production and phosphorylation of p70^{S6K} and mTOR. Therefore, these two proteins can probably be evaluated for monitoring exercise-induced central fatigue, although we need more evaluations.

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Statements and Declarations

The author declares that no conflict of interest is associated with this study.

Authors' contribution

This study was done by the authors named in this article, and the authors accept all liabilities resulting from claims which relate to this article and its contents.

Conflicts of interest

There are no conflicts of interest.

Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon request.

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References

- McMorris T, Barwood M, Corbett J. Central fatigue theory and endurance exercise: Toward an interoceptive model. *Neurosci Biobehav Rev* 2018; 93: 93-107.
- AbuMoh'd MF, Matalqah L, Al-Abdulla Z. Effects of oral branched-chain amino acids (bcaas) intake on muscular and central fatigue during an incremental exercise. *J Hum Kinet* 2020; 72(1): 69-78.
- Park SS, Park HS, Jeong H, Kwak HB, No MH, Heo JW, Yoo SZ, Kim TW. Treadmill exercise ameliorates chemotherapy-induced muscle weakness and central fatigue by enhancing mitochondrial function and inhibiting apoptosis. *Int Neurol J* 2019; 23(Suppl 1): S32.
- Millet GY, Martin V, Temesi J. The role of the nervous system in neuromuscular fatigue induced by ultra-endurance exercise. *Appl Physiol Nutr Metabol* 2018; 43(11): 1151-1157.
- Mira J, Floreani M, Savoldelli A, Amery K, Koral J, Oranchuk DJ, Messonnier LA, Rupp T, Millet GY. Neuromuscular fatigue of cycling exercise in hypoxia. *Med Sci Sports Exerc* 2020; 52(9): 1888-1899.
- Gordon RJFH, Tillin NA, Tyler CJ. The effect of head and neck per-cooling on neuromuscular fatigue following exercise in the heat. *Appl Physiol Nutr Metabol* 2020; 45(11): 1238-1246.
- Torre-Villalvazo I, Alemán-Escondrillas G, Valle-Ríos R, Noriega LG. Protein intake and amino acid supplementation regulate exercise recovery and performance through the modulation of mTOR, AMPK, FGF21, and immunity. *Nutr Res* 2019; 72: 1-17.
- Maiolo F, Giachero S, Fossati SM, Rocchi A, Zullo L. mTOR as a Marker of Exercise and Fatigue in Octopus vulgaris Arm. *Front Physiol* 2019; 10: 1161.
- Aziziarum Z, Mattoo RU. A recombinant chimeric protein of protective antigen and lethal factor from Bacillus anthracis in polymeric nanocapsules showed a strong immune response in mice: a potential high efficacy vaccine against anthrax. *Cell Mol Biol* 2022; 68(3): 1-8.
- Schram AM, Gandhi L, Mita MM, Damstrup L, Campana F, Hidalgo M, Grande E, Hyman DM, Heist RS. A phase Ib dose-escalation and expansion study of the oral MEK inhibitor pimasertib and PI3K/MTOR inhibitor voxalisib in patients with advanced solid tumours. *Br J Cancer* 2018; 119(12): 1471-1476.
- Constantin-Teodosiu D, Constantin D. Molecular mechanisms of muscle fatigue. *Int J Mol Sci* 2021; 22(21): 11587.
- Tsimberidou AM, Shaw JV, Juric D, Verschraegen C, Weise AM, Sarantopoulos J, Lopes G, Nemunaitis J, Mita M, Park H, Ellers-Lenz B, Tian H, Xiong W, Kaleta R, Kurzrock R. Phase 1 study of M2698, a p70S6K/AKT dual inhibitor, in patients with advanced cancer. *J Hematol Oncol* 2021; 14(1): 1-10.
- Senousy MA, Hanafy ME, Shehata N, Rizk SM. Erythropoietin and Bacillus Calmette-Guérin Vaccination Mitigate 3-Nitropropionic Acid-Induced Huntington-like Disease in Rats by Modulating the PI3K/Akt/mTOR/P70S6K Pathway and Enhancing the Autophagy. *ACS Chem Neurosci* 2022; 13(6): 721-732.
- Lee VC, Moscicki JC, DiFazio CA. Propofol sedation produces dose-dependent suppression of lidocaine-induced seizures in rats. *Anesth Analg* 1998; 86(3): 652-657.
- Riessen R, Pech R, Tränkle P, Blumenstock G, Haap M. Comparison of the RAMSAY score and the Richmond Agitation Sedation Score for the measurement of sedation depth. *Crit Care* 2012; 16(1): 1-189.
- Hakimizadeh E, Mahsa H, Kazemi A. The effects of single dose administration of opium on muscular and central fatigue in male

- rats. *J Jiroft Uni Med Sci* 2020; 6(2): 178-186.
17. Meeusen R, Watson P, Hasegawa H, Roelands B, Piacentini MF. Central fatigue. *Sport Med* 2006; 36(10): 881-909.
 18. Perl A. mTOR activation is a biomarker and a central pathway to autoimmune disorders, cancer, obesity, and aging. *Ann N Y Acad Sci* 2015; 1346(1): 33-44.
 19. Haraguchi FK, de Brito Magalhães CL, Neves LX, dos Santos RC, Pedrosa ML, Silva ME. Whey protein modifies gene expression related to protein metabolism affecting muscle weight in resistance-exercised rats. *Nutrition* 2014; 30(7-8): 876-881.
 20. Goodman CA, Frey JW, Mabrey DM, Jacobs BL, Lincoln HC, You JS, Hornberger TA. The role of skeletal muscle mTOR in the regulation of mechanical load-induced growth. *J Physiol* 2011; 589(22): 5485-5501.
 21. Hulmi JJ, Silvennoinen M, Lehti M, Kivelä R, Kainulainen H. Altered REDD1, myostatin, and Akt/mTOR/FoxO/MAPK signaling in streptozotocin-induced diabetic muscle atrophy. *Am J Physiol Endocrinol Metab* 2012; 302(3): E307-E315.
 22. Bar-Peled L, Sabatini DM. Regulation of mTORC1 by amino acids. *Trends Cell Biol* 2014; 24(7): 400-406.
 23. Chantranupong L, Wolfson RL, Orozco JM, Saxton RA, Scaria SM, Bar-Peled L, Spooner E, Isasa M, Gygi SP, Sabatini DM. The Sestrins interact with GATOR2 to negatively regulate the amino-acid-sensing pathway upstream of mTORC1. *Cell Rep* 2014; 9(1): 1-8.
 24. Karar J, Maity A. PI3K/AKT/mTOR pathway in angiogenesis. *Fron Mol Neurosci* 2011; 4: 51.
 25. Wang X, Proud CG. The mTOR pathway in the control of protein synthesis. *Physiology* 2006; 21(5): 362-369.
 26. Wang, W., Wen, Q., Xu, L., Xie, G., Li, J., Luo, J., Chu, S., Shi, L., Huang, D., Li, J., Fan, S. Activation of Akt/mTOR pathway is associated with poor prognosis of nasopharyngeal carcinoma. *PLoS One* 2014; 9(8): e106098.
 27. Zanchi NE, Lancha AH. Mechanical stimuli of skeletal muscle: implications on mTOR/p70s6k and protein synthesis. *Eur J Appl Physiol* 2008; 102(3): 253-263.
 28. Tula, M., Filgona, J., Kyauta, S., Elisha, R. Screening for some virulent factors among bacterial isolates from surfaces of hospital fomites and hands of healthcare workers. *Cell Mol Biomed Rep* 2023; 3(1): 9-16. doi: 10.55705/cmbr.2022.355120.1054.
 29. Pearson RB, Dennis PB, Han JW, Williamson NA, Kozma SC, Wettenhall RE, Thomas G. The principal target of rapamycin-induced p70s6k inactivation is a novel phosphorylation site within a conserved hydrophobic domain. *EMBO Rep J* 1995; 14(21): 5279-5287.