

The Effect of Feeding with Resveratrol on Myogenin and mTOR Levels in Mice

Resveratrol ile Beslenmenin Farelerde Myogenin ve mTOR Düzeyleri Üzerine Etkisi

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ABSTRACT

Aim: It is reported that resveratrol may induce muscle hypertrophy and myogenesis. Accordingly, resveratrol is promising for the treatment of muscle diseases and ergogenic aid for athletes. In this study it is aimed to observe the effects of resveratrol on myogenin and mTOR levels and muscle mass.

Material and Methods: The study is performed on 14 Swiss albino young adult mice at Başkent University Experimental Animal Research Center. The mice are divided randomly into study (n:7) and control (n:7) groups. During consecutive 7 days, 20 mg/kg trans-resveratrol was given to the study group intraperitoneally. After the seventh day administration, the right gastrocnemius muscles are dissected, weighted and stored at -80 °C for mTOR and myogenin analysis. Then, myogenin and mTOR levels are determined by enzyme-linked immunosorbent assay (ELISA) method in homogenised muscle mass.

Results: According to mean myogenin and mTOR levels, there was no significant difference between groups ($p>0.05$). Total body weight and the right gastrocnemius muscle weight of the study group (22.11 ± 3.22 g, 0.09 ± 0.03 g, respectively) at the 7th day was found significantly lower than control group (28.63 ± 3.80 g, 0.12 ± 0.02 g, respectively) ($p<0.05$).

Conclusion: It was observed that resveratrol administration had no effect on myogenin and mTOR levels which is a fore-step for muscle hypertrophy. On the other hand, interestingly total body weight and muscle mass decrease was observed in the study group. The effects of resveratrol on muscle tissue is a relatively less studied topic. However, the authors are suggesting more studies on the resveratrol usage or administration for the effective dose and time interval investigation.

Keywords: Resveratrol, gastrocnemius, weight, mTOR, myogenin

ÖZET

Amaç: Resveratrolün kas hipertrofisi ve miyogenezi indükleyebileceği bildirilmektedir. Bu nedenle, resveratrol kas hastalıklarının tedavisi ve sporcular için ergojenik yardım olarak umut vermektedir. Mevcut çalışmada, resveratrolün miyogenin ve mTOR düzeyleri ve kas kütlesi üzerindeki etkilerinin gözlenmesi amaçlanmıştır.

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Gereç ve Yöntem: Çalışma, Başkent Üniversitesi Deneysel Hayvanları Araştırma Merkezi'nde 14 Swiss albino genç erişkin fare üzerinde yürütülmüştür. Fareler rastgele olarak çalışma (n:7) ve kontrol grubuna (n:7) ayrılmıştır. Çalışma grubuna ardışık 7 gün boyunca 20 mg/kg transresveratrol intraperitoneal yol ile verilmiştir. Yedinci gün uygulamasından sonra farelerin gastroknemius kas diseksiyonları yapılarak, ağırlıkları kaydedilmiş ve miyogenin, mTOR analizleri için -80 °C'de saklanmıştır. Miyogenin ve mTOR düzeyleri homojenize kas dokusundan enzim bağlı immunosorbent analizi (ELISA) yöntemi kullanılarak saptanmıştır.

Bulgular: Gruplar arasında miyogenin ve mTOR düzey ortalamaları açısından anlamlı farklılık saptanmamıştır ($p>0.05$). Çalışma grubunun vücut ağırlığı ve sağ gastroknemius kasının 7. gün ağırlığı (sırasıyla, 22.11±3.22 g, 0.09±0.03 g), kontrol grubuna göre anlamlı derecede düşük bulunmuştur (sırasıyla, 28.63±3.80 g, 0.12±0.02 g) ($p<0.05$).

Sonuç: Bu çalışmada resveratrol uygulamasının kas hipertrofisi için önemli bir basamak olan miyogenin ve mTOR düzeyleri üzerinde bir etkisinin olmadığı gözlenmiştir. Buna ek olarak çalışma grubunda ilginç olarak vücut ve kas dokusu ağırlığı daha düşük saptanmıştır. Resveratrolün kas dokusu üzerindeki etkileri nispeten daha az çalışılmış bir konudur bu nedenle etkilerinin belirlenebilmesi için etkili doz ve zaman aralığı hakkında daha fazla çalışmaya ihtiyaç vardır.

Anahtar kelimeler: Resveratrol, gastroknemius, ağırlık, mTOR, miyogenin

INTRODUCTION

Resveratrol (RSV), is a stilbenoid that is present in many plants, especially in grape, has two isomers: cis and trans forms, but trans form seems to have major biological effects (1). RSV has demonstrated many beneficial effects, including antiinflammatory, antioxidant, neuroprotective, and anticancer effects (2). Besides these effects it is also indicated that RSV can regulate the skeletal muscle fiber type transformation (3), increase exercise performance (4), and exhibit protective effects against oxidative damage and muscle atrophy in muscle disuse models (including hindlimb unloading and denervation) (5). In addition, RSV promotes muscle differentiation and hypertrophy by controlling muscle-specific proteins like myogenic regulatory factors (MRFs) (6).

Mammalian target of rapamycin (mTOR) is one of the key regulator of cell growth, proliferation, and metabolism (7). It is shown that insulin like growth factor-1 (IGF-1)/phosphatidylinositol 3-kinase (PI3K)/Akt (Protein B kinase)/mTOR signaling pathway has a role in the regulation of skeletal muscle hypertrophy (8). Myogenesis depends on the differentiation potency of the satellite cells providing the formation of new muscle cells to differentiate to new fibrillary structures (9). The myogenesis process is under the control of a wide variety of myogenic transcriptional

factors and kinases (10,11). These basic helix-loop-helix transcription factors which are specific for muscle, also known as myogenic regulatory factors (MRFs), [e.g. Myogenic Factor 5 (Myf5), Myoblast Determination Protein (MyoD), myogenin, and muscle specific regulatory factor 4 (MRF4)] (9). Myogenin expression, beginning with myotubule formation, is necessary for the development of functional skeletal muscle (12) and is the critical step in myogenesis (13).

Resveratrol, can directly affect skeletal muscle metabolism and development (14), inducing myogenesis by stimulating Akt (protein kinase B)/mTOR pathway (15,16), one of the major pathways in muscle hypertrophy (17), and myogenic factors. However its effect on myogenic factors has not been studied sufficiently. The aim of this study, is to investigate the possible effect of resveratrol on myogenic transcription factor, a key factor in the late stages of myogenic transcriptional factors, as well as on mTOR levels which is an important kinase for myogenesis and on muscle mass.

MATERIAL AND METHOD

This study was approved by the decision of Başkent University Medical and Health Sciences Research

Council and Experimental Animal Ethics Board, dated 11/05/2015 under number of 15/23. Mus musculus-Swiss albino type, young-adult (7-weeks old) with normal body weight male mice were used. The study was conducted at Başkent University Experimental Animal Research Center. Experimental animals were brought to the laboratory one week before the study for adaptation. Mice were housed and maintained at room temperature 22 ± 1 °C, relative humidity 50-60%, light period as, 12 hours light/12 hours dark. Water and pellet access of mice was provided as *ad libitum*, throughout the study. According to the results of power analysis, this study was performed with a total of 14 Swiss Albino male mice, including the study group and control group, consisted of 7 mice. The mice were randomly selected for each of the study groups. The study group received 20 mg/kg/day trans-RSV intraperitoneal injection for 7 consecutive days at the same time. The effects of RSV intervention on body weight, gastrocnemius muscle weight, mTOR and myogenin levels were examined in homogenised muscle mass. At the beginning and the seventh day of the study, weights of the mice were measured and recorded using a precision scale.

Muscle Weights Determination

On the 17th day, 1 hour after the intraperitoneal (IP) administration of trans-resveratrol, the right gastrocnemius muscle of the mice were isolated under general anesthesia. General anesthesia was given by intraperitoneal administration of ketamine at a dose of 90 mg/kg and xylazine at a dose of 10 mg/kg. The weights of the dissected muscles were measured using a precision weighing scale and stored at -80 °C in sterile containers for ELISA analysis and the sacrifice was performed with 250 mg/kg IP ketamine application.

mTOR and Myogenin ELISA Analyzes

Protein levels of the homogenised muscle samples were determined by the Bradford Assay. Myogenin (MyBioSource) and mTOR (MyBioSource) were measured by Elisa according to the manufacturer's

instructions. Briefly, samples were homogenized in a glass homogenisator. Standards were prepared using albumin. Both standards and samples were incubated with Bradford reagent in a dark room. After incubation all wells were read at 596 nm. The slope was prepared using standards and protein levels of samples were calculated. Myogenin (MyBioSource, USA, Cat. No: MBS065750) and mTOR (MyBioSource, USA, Cat.No: Cat No. MBS260864) were measured by Elisa according to the manufacturer's instructions. Briefly, samples and standards supplied by the manufacturer were added to precoated 96 well ELISA plates and incubated at 90 min in 37 °C. At the end of the incubation period ELISA plates were washed and avidin biotin peroxidase complex were added. ELISA plates were incubated again and antigen antibody complexes were colorized by horseradish peroxidase (HRP) and 3,3',5,5"-tetramethylbenzidine (TMB). Density of wells was evaluated by spectrophotometry.

Statistical Analysis

Data were analysed using Statistical Package for the Social Sciences (SPSS) version 22 (Turkey). The normal distribution of parameters were evaluated by Kolmogorov-Smirnov and Shapiro Wilks tests and the parameters were found to be distributed normally. Student t-test was used to compare quantitative data between two groups. Paired sample t-test was used in the first and final weight comparisons of the parameters in the group, and Pearson correlation analysis was used when the relations between the parameters were examined. Means (\bar{x}) and standard deviations (SD) were calculated where applicable. Significant difference was assessed at $p < 0.05$ level.

RESULTS

Weight Change of Groups

The mean weight of the study and control group at the first day was 26.63 ± 3.02 g and 27.06 ± 2.27 g, respectively. There was no statistically significant difference between the groups in terms of initial weight averages ($p > 0.05$). When the groups are compared in

terms of weight average on the seventh day; the study group had significantly lower mean weight on the day 7 (22.11±3.22 g) than the control group (28.63±3.80 g) (p<0.05). The decrease in the weight averages of 1st and 7th days was also statistically significant (p<0.05) in the study group. In the control group, the weight averages of 1st and 7th days were increased, but the differences were not significant (p>0.05) (Table 1).

Right Gastrocnemius Muscle Weights

The mean right gastrocnemius muscle weights of the study and control groups after sacrifice were 0.09±0.03 g and 0.12±0.02 g, respectively. The mean right gastrocnemius muscle weight of the study group was found to be significantly lower than the control group (p<0.05).

mTOR and Myogenin Levels

There were no significant differences in the mean values of mTOR and myogenin levels between groups after the administration of resveratrol (p=0.227 and p=0.548, respectively) (Table 2).

There were no relationship between mTOR and myogenin levels and 7th day total body weight and muscle weight for both groups (p>0.05) (Table 3).

Table 1. Comparison of groups 1st and 7th day weights

Weight (g)	Study Group	Control Group	p
	$\bar{X}\pm SD$	$\bar{X}\pm SD$	
1st day	26.63±3.02	27.06±2.27	0.769
7 th day	22.11±3.22	28.63±3.80	0.005 [†]
Difference	-4.51±2.89	1.57±1.73	0.001 [†]
†p	0.006[‡]	0.054	

* Student t test † paired sample t test ‡ p<0.05

DISCUSSION

Resveratrol can exert an interesting benefit in skeletal muscle (18) and it is proposed that; it can protect muscle mass in catabolic processes thought affecting mTOR and various signaling pathways (15,19). Therefore RSV is suggested to be an important strategy for the development of muscle mass (15). In this study, we aimed to investigate RSV effect on muscle mass and myogenesis (myoglobin), and anabolic (mTOR) markers.

In a study, treatment with RSV caused an increase in myotubul size, elongation in heights and it highlighted that RSV can control myogenesis and hypertrophy (16). It has also been reported in several studies that resveratrol reduces protein degradation (17,20). In another study RSV treatment not only attenuated TNF-α induced atrophic response but also promoted hypertrophic processes in myotubules by regulating the activity of Akt/mTOR pathway (15). In contrast to literature; in the present study, muscle weights of the study group were significantly lower than control groups' (respectively, 0.09±0.03 g and 0.12±0.02 g, p<0.05) (Table 1).

mTOR participates in many biological functions such as gene transcription, protein translation, and ribosome

Table 2. Evaluation of groups according to mTOR and myogenin levels

	Study Group	Control Group	*p
	$\bar{X}\pm SD$	$\bar{X}\pm SD$	
mTOR (ng/g)	4.40±0.35	4.63±0.32	0.227
Myogenin (ng/g)	6.17±0.23	6.12±0.04	0.548

Student t test p<0.05

Table 3. Relationship of mTOR, myogenin levels and 7th day body weight and muscle weight for study and control groups

	Study Group				Control Group			
	mTOR (ng/g)		Myogenin (ng/g)		mTOR (ng/g)		Myogenin (ng/g)	
	r	p	r	p	r	p	r	p
7th Day								
Body weight (g)	-0.075	0.873	-0.038	0.935	0.074	0.875	-0.123	0.793
Muscle weight (g)	-0.364	0.422	-0.107	0.819	0.123	0.794	-0.183	0.694

Pearson correlation coefficient test p<0.05

synthesis by integrating extracellular signals such as nutrients, growth factors, and energy substances in the physiological and pathophysiological processes of cells (21). One of the most widely recognized major players in controlling muscle mass. A decreased activation of the Akt-mTOR pathway contributes to protein synthesis reduction, which can occur under disuse conditions or after a low protein diet (18). In a study RSV and exercise combination effects on muscle mass, mTOR and myogenin levels were studied. It was found that at the 7th day, mTOR levels of resveratrol and resveratrol+exercise groups were increased compared to other groups and the authors concluded that on the behalf of mTOR expression, RSV may have stimulated anabolic pathway (22). Although there are lots of studies indicating that RSV can regulate Akt/mTOR process, so give a contribution to muscle gain (15,16). In our study RSV didn't make any difference on the mTOR and myogenin levels. And despite mTOR level was not statistically different from the control group and we randomly found muscle mass decrease in the study group ($p=0.227$) (Table 2). However according to the literature if there is a muscle loss it is often related with Akt/mTOR pathway down-regulation (23), in contrary with that we didn't find any relation between muscle mass and mTOR- myogenin levels (respectively, $p=0.422$, $p= 0.819$) (Table 3).

Resveratrol has been reported to have active role in muscle cell differentiation (16) and upregulates muscle pre-differentiation markers and transcriptional factors (myogenin, Scrp3), and strongly enhances myosin heavy chain content (24). According to a study RSV stimulates early expression of MRFs (such as Myf-5, MyoD, and myogenin) and promotes muscle indicator proteins (myosin heavy chain) and skeletal structural protein processes and exerts positive effect on hypertrophy through stimulating IGF-1 pathway. As a result, RSV can control myogenesis and hypertrophy (16). In a study investigating RSV and RSV+exercise combination effect on muscle mass, mTOR and myogenin levels; it was found that at the 7th day, RSV group myogenin expression increased compared to control group. With that results authors

concluded that, RSV is likely to favor differentiation by increasing myogenin, possibly contributing to enhanced myogenesis in addition to increased protein synthesis and muscle anabolism (22). Conflicting with literature, in our study RSV did not cause any difference in myogenin levels compared with control group ($p=0.548$) (Table 2). It was unexpected results that we found weight and muscle loss, while we were trying to examine myogenic factors but not examining the pellet consumption or other conditions which could give such a reason. There is also data about RSV and weight loss effect (25-27) but it wasn't the main purpose of this study so we don't have any date to argue it.

CONCLUSION

Resveratrol is a frequently studied compound in the literature, but its effects on skeletal muscle are relatively less studied. Myogenin and mTOR are stated to be key molecules for prevention of muscle atrophies and are very important kinase and transcriptional factors for muscle hypertrophy. In our study unexpectedly we experience weight loss and gastrocnemius muscle mass decrease in resveratrol group. Despite the muscle mass decrease in the study group, there was no difference in mTOR and myogenin levels among the groups. The effects of resveratrol on muscle tissue is a relatively less studied issue. However, the authors are suggesting more studies about the resveratrol usage or administration for the effective dose and time interval investigation.

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

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