ORIGINAL ARTICLE

The Effect of 12 Weeks Aerobic Training on Expression of AKT1 and mTORc1 genes in the Left Ventricle of Type 2 Diabetic Rats

Mona Mirsepasi[1], Abdolali Banaeifar[2*], Mohammad-Ali Azarbayjani[3], Sajad Arshadi[4]

1. PhD Candidate, Department of Exercise Physiology, Islamic Azad University, South Tehran Branch, Tehran, Iran.
2. Associate Professor, Department of Exercise Physiology, Faculty of Physical Education, Islamic Azad University, South Branch, Tehran, Iran.
3. Professor, Department of Exercise Physiology, Islamic Azad University, Central Tehran Branch, Iran.
4. Assistant Professor, Department of Exercise Physiology, Faculty of Physical Education, Islamic Azad University, South Branch, Tehran, Iran.

***Correspondence:**
Abdolali Banaeifar, Associate Professor, Department of Exercise Physiology, Faculty of Physical Education, Islamic Azad University, South Branch, Tehran, Iran.
Tel: (98) 912 225 1779
Email: alibaneifar@yahoo.com

Received: 22 July 2018

Accepted: 12 August 2018

Published in November 2018

Abstract

Objective: The purpose of this study was to investigate the effect of 12 weeks aerobic training on the expression of AKT1 and mTORc1 genes in the left ventricle of T2DM rats.

Materials and Methods: The statistical sample consisted of 21 male wistar rats from the Pasteur Institute in Tehran. (10 weeks old weighing 220 ± 20 grams). They were divided into three groups: diabetic aerobic training, diabetic control and healthy control groups. The diabetic aerobic training group participated in a period of aerobic training for 12 weeks in 5 sessions per week with gradual increase in speed (18-26 m / min) and 10 to 55 minutes in the form of running on treadmill. AKT1 mRNA and mTORc1 mRNA were evaluated by RT-Real time PCR technique by the Roturer 6000 system.

Results: According ANOVA findings diabetics rat significantly alters the expression of Akt1 (*P*-value: 0.002) and mTORc1 (*P*-value: 0.001). However, aerobic exercise significantly increase the expression of Akt1 (*P*-value: 0.002) and mTORc1 (*P*-value: 0.001) in comparison with the diabetic untrained control groups.

Conclusion: Although this study was conducted on animals and it was necessary to caution people in generalizing these findings, it seems that an aerobic training period with an increase in the expression of Akt1 and mTORc1 genes leads to improved cardiac function and it prevents heart disease from diabetes.

Keywords: Diabetes, Aerobic exercise, Heart disease, Akt1, mTORc1

Introduction

Type 2 diabetes mellitus (T2DM) is one of the most common metabolic disorders (1). Long-term exposure to high blood glucose levels is known as one of the major causes of diabetes mellitus (2). Different factors such as, inactivity, obesity, stress and genetic factors are also other causes of T2DM. On the other hand, cardiovascular disease is the leading cause of mortality in patients with T2DM (3). These patients are at

risk of high blood pressure, atherosclerosis, coronary artery disease, and heart attack (3).

In this regard, some studies have shown that there is a relationship between diabetes and left ventricular function (3). But physical activity can be considered as a cardio-protective factor for T2DM. Many studies have shown that during exercise, structural and functional changes in the left ventricle are greater than other parts of the heart (5-4).

Ebert et al, reported that aerobic exercise increases the left ventricular diameter and improves left ventricular diastolic function (6). Also, Rawlins et al, concluded that participation in an intense regular exercises increased the thickness of the left ventricular wall and size of the cavities, which is a physiological change due to exercise (7). Structural and functional adaptations in the heart muscle depend on the type of exercise activity. Research has shown that adaptability resulting from strength and endurance exercises in the heart and cavities is different (8). Aerobic exercises lead to eccentric hypertrophy, which is due to volumetric overload (9). Fathi et al, reported that endurance training increases the diastolic diameter of the left ventricle in heart of the mice (10). The AKT / mTOR pathway is the main route that causes hypertrophy in exercise. (11) Training causes cardiac growth, which is regulated by the GH / IGF axial signaling path via PI3k / AKT or AKT / Mtor. The activity of the AKT protein interacts with different intracellular substrates to regulate growth, metabolism and phosphorylation. (11) The high expression of the IGF-1 receptor induces AKT activity, resulting in physiological hypertrophy by increasing calcium flow through the L-type calcium channel and SERCA (sarco-endoplasmic reticulum ca^{2+}_ATP_{ase}). (12) When AKT-1 is suppressed, the physiological growth and hemodynamic adaptations slow down. Glycogen synthase kinase-3 is an important negative regulator of protein synthesis that inhibited by AKT (11).

Providing information on effect of exercise training on the heart of diabetic patients is important because diabetic cardiomyopathy is associated with impairment in cardiac function and structure. The study on the effect of exercises on structural and functional adaptations of the heart in animal samples has always been considered with heart scarification and cardiac evaluating (13).

Meanwhile, the most important signaling pathway for left ventricular hypertrophy is the Akt / mTOR pathway, so that measuring the expression of the Akt and mTOR genes together with structural changes in the heart can provide useful information on the growth and improvement of cardiac conditions as a result of exercise training. Liao et al, designed their study to determine whether mTOR signaling responds to exercise with different intensities. Male rats were divided into three groups: control, moderate training, and high intensity training. Exercise was designed for 8 weeks with a 2-day rest for each week. Both exercises led to hypertrophy of the heart, while its rate increased with increased exercise intensity. Signaling activation of Akt, mTOR and P70 (S6K) was observed in the moderate exercise group but not in the severe exercise group. The results showed that prolonged severe exercise leads to hypertrophy of the heart, but the Akt / mTOR pathway is involved in moderate exercise, not severe exercise (14). However, the effect of training on cardiac hypertrophy signaling pathway genes in T2DM rats has been less studied, and it is important that cardiovascular disease and mortality are resulting from the complications of diabetes mellitus.

The purpose of this study was to investigate the effect of 12 weeks aerobic training on the expression of AKT1 and mTORc1 genes in the left ventricle of T2DM rats.

Materials and Methods

It was an experimental study on 21 male wistar rats from the Pasteur Institute in Tehran. They were10 weeks old and weighing 220 ± 20 grams. Rats were divided randomly

to 3 groups: diabetic aerobic training, diabetic control and healthy control groups. After a night fasting, for induction of type 2 diabetes, the nicotinamide solution at a dose of 110 mg / kg was injected intraperitoneally. After 15 minutes, freshly prepared STZ solution was injected intraperitoneally at 60 mg / kg in citrate buffer with pH = 4.5. The healthy control group received only the same volume of citrate buffer (15). After 72 hours, fasting blood glucose and glucose levels of 126 mg / dl were considered as diagnose of T2DM (16). Glucose oxidase was used to measure glucose. Rats were kept in the animal house of physical education faculty under controlled light conditions (12 hours of light and 12 hours of darkness, starting the lighting 6pm and starting darkness at 6am) temperature (22 ± 3° C), and humidity (about 45%). Throughout the study, rats were also moved and manipulated by one person. The first group was diabetes training group which include 7 diabetic wistar rats and after 13 weeks were performed aerobic training on the treadmill with a gradual increase in speed (18-26 m / min) and time (10 to 55 minutes) in running treadmill for 12 weeks and 5 sessions each weeks (17). The second group did not practice the diabetic group. This group includes7male 10 weeks old wistar rats who received diabetes nicotine amide injection and STZ and did not attend any exercise program. The third group included 7healthy male wistar 10 weeks as a healthy control group.

The training program was done as table 1. To measure AKT1 mRNA and mTORc1 mRNA the Real time PCR (RT-PCR) was done.

This study was accorded to ethical principles in animal research adopted by international institute of health and adhering to the principles of the Declaration of Helsink. It was approved by Islamic university of Islamic Azad university Tehran south branch (ir.iau.thehran.rec.51/410/16).

The statistical analysis of the variables was done by SPSS-20. The variables were first described by mean and standard deviation (SD). For comparing AKT1 and mTORc1 among three groups, One-way analysis of variance (ANOVA) was used at a significant level of *P*-value ≤0.05.

Results

The mean and SD of the studied variables are presented in Table 2. The results of independent analysis of variance analysis for comparison between three groups are presented in Tables 3. With respect to the weight ratio of heart to body weight, diabetes was not significantly affected, but aerobic exercise significantly increased this ratio (*P*-value: 0.001). However, the ratio of left ventricular weight to heart weight did not change significantly between the three groups (*P*-value: 0.08). The most important finding was that diabetes lead to a significant change in the expression of Akt1 and mTORc1 genes, and aerobic training resulted in a significant increase in the expression of these two genes compared to two unhealthy and untrained conditions and the untrained diabetic patient.

Discussion

Based on the findings of this study, 12 weeks of aerobic training led to an increase in the expression of Akt1 and mTORc1 genes in T2DM rats, also creating desirable changes in the heart structure of these rats. These results are consistent with the findings of Lee et al. (18).

Table 1. The training program of rats

Weeks	Time of running (min)	Speed of running (m / min)
First	10	18
Second and third	20	20
Fourth and fifth	30	22
Sixth and seventh	40	22
Eighth and ninth	50	24
Tenth, eleventh and twelfth	55	26

Table 2. Mean and standard deviations of variables

Variables	Group	Before intervention	After intervention	*P*-value
Weight (gr)	Healthy control group	2.28±221.28	6.72±290.28	0.001*
	Diabetic control group	3.25±219.28	5.82±254.57	
	Aerobic diabetic training group	2.99±225.57	2.29±241.42	
Heart weight(mg)	Healthy control group	-	25.60±1016.42	0.001*
	Diabetic control group	-	9.19±920.57	
	Aerobic diabetic training group	-	20.49±1130.42	
Left ventricular weight(mg)	Healthy control group	-	13.52±420.14	0.001*
	Diabetic control group	-	5.30±382.14	
	Aerobic diabetic training group	-	8.30±461.42	
Heart weight ratio to body weight(mg/gr)	Healthy control group	-	0.15±3.50	0.001*
	Diabetic control group	-	0.08±3.61	
	Aerobic diabetic training group	-	0.10±4.68	
The ratio of the left ventricle to the heart's weight (mg/mg)	Healthy control group	-	0.003±0.413	0.08
	Diabetic control group	-	0.006±0.415	
	Aerobic diabetic training group	-	0.006±0.408	
AKT1 (bp)	Healthy control group	-	0±1	0.002*
	Diabetic control group	-	0.35±0.56	
	Aerobic diabetic training group	-	1.06±2.004	
mTORc1 (bp)	Healthy control group	-	0±1	0.001*
	Diabetic control group	-	0.65±0.75	
	Aerobic diabetic training group	-	1.51±3.15	

* Significantly at the level of $P \leq 0.05$

Table 3. Tukey'spost hoc results

Variables	Compare the pairs	*P*-value
Weight	Healthy control group- Diabetic control group	0.001*
	Healthy control group- aerobic diabetic training group	0.001*
	Diabetic control group - aerobic diabetic training group	0.001*
Heart weight	Healthy control group- Diabetic control group	0.001*
	Healthy control group- aerobic diabetic training group	0.001*
	Diabetic control group - aerobic diabetic training group	0.001*
Left ventricular weight	Healthy control group- Diabetic control group	0.001*
	Healthy control group- aerobic diabetic training group	0.001*
	Diabetic control group - aerobic diabetic training group	0.001*
Heart weight to body weight ratio	Healthy control group- Diabetic control group	0.20
	Healthy control group- aerobic diabetic training group	0.001*
	Diabetic control group - aerobic diabetic training group	0.001*
AKT1	Healthy control group- Diabetic control group	0.007*
	Healthy control group- aerobic diabetic training group	0.024*
	Diabetic control group - aerobic diabetic training group	0.002*
mTORc1	Healthy control group- Diabetic control group	0.022*
	Healthy control group- aerobic diabetic training group	0.001*
	Diabetic control group - aerobic diabetic training group	0.001*

*** Significantly at the level of P≤0.05**

Launay and colleagues also reported similar findings (19). But in contradiction with these findings, Sturgeon et al. did not see any significant changes in cardiac hypertrophy induced by Akt-mTOR-related signaling pathways in female mice after two months of moderate training on treadmill (20). This difference in results may be due to differences

in exercise protocols, especially exercise intensity .It seems that insufficient training intensity was due to the lack of significant results in Sturgeon et al study (20).

Generally, diabetes is associated with a high incidence of cardiovascular disease, which is the leading cause of death (20-21).

Characteristics of chronic heart failure has been shown to reduce left ventricular function and loss of cardio-myocytes through apoptosis or necrosis (22). It has been shown that a diabetic heart has a 85-fold increase in cardio-myocytes apoptosis (21). Exercise trainings is an important non-pharmacological approach that can be used to improve the quality of life and reduce the pathological symptoms in patients with chronic heart failure (23). Studies have shown that training reverses abnormal functional and molecular Characteristics that is depended on cardiac pathology (24-27). The underlying mechanism which shows that training has role in preventing apoptosis in diabetes is still not well known. The IGF-1 / IGF-1-R axis and the downstream signaling pathways PI3K and Akt have been shown to contribute to the mediation of critical response and apoptosis in cardiac tissue (28,29).

Additionally, the IGF-1 / PI3K / Akt pathway is a vital mediator for physiological cardiovascular growth-And exercise intervention have shown that abnormal functional and molecular abnormalities associated with cardiac pathology reversed by increasing the activity of IGF-1 or PI3K (24-27).

Cheng et al, reported a decrease in IGF-1 / PI3K / Akt pathways in diabetic hearts, but significantly increased after a period of exercise trainings (30).

Among the family of protein kinases associated with PI3K, mTOR is a unique protein that plays a role in signaling PI3K / Akt. Although mTOR is coded by a single gene in mammals, it is attached to a special regulating protein in the form of two sets, which include mTORC1 and mTORC2, both of which have distinct effects and mechanisms.Insulin and IGF-I stimulate receptor tyrosine kinase and its known receptors.thus activating the signaling pathways of PI3K / Akt and Ras.The effective phosphorylated kinases Akt and ERK1 / 2 (kinase regulated extracellular signal 1/2) directly phosphorylate and inactivate the HSC TSC1 / 2 (stem cell sclerosis 1/2) (31,32). TSC1 / 2 acts as a GAP (GTPase activating protein) for Rheb (a ram-enriched homologue in the brain) and reduces GTPase, Rheb-GAP. Hence, Rheb-GAP stimulates mTORC1 kinase activity, and eventually insulin / IGF-I signaling activates mTORC1 activity via the PI3K / Akt signaling axis and thereby inhibits TSC1 / 2 (33). Akt also can activate mTORC1 kinase activity independently of TSC1 / 2 by direct phosphorylation and separating PRAS40 (mTORC1 inhibitor) from RAPTOR (34).

Among the mTORC1 downstream molecular processes, protein synthesis is the best feature, and in this context two mTORC1 targets are well defined (35). When mTORC1 is activated, S6K1 (kinase 1 of the ribosomal S6 protein) phosphorylates, which propagate various cellular processes that include the biogenesis of mRNA, the translation of ribosomal proteins, cell growth and cell metabolism (36). mTORC1 and S6K1 have an important negative feedback activity in inhibiting IRS-1 (substrate 1 insulin receptor) (37). Another target is mTORC1, 4E-BP1 (the elF4E-binding protein or the eukaryotic transcription factor-4E binding factor), which accelerates the dissolution of elF4E, which regenerates the conditions that synthesize the protein (38) Hence, 4E-BP1 phosphorylation by mTORC1 is necessary to initiate the translation of mRNA and protein synthesis. Additionally, mTORC1 regulates cell growth and proliferation by controlling autophagy, which is an important process in maintaining the metabolic cell homeostasis. (39)

So, after 12 weeks of aerobic training in T2DM rats, these changes led to improvement of functional and structural features of the heart, which is probably the result of improving cardiac death-related mortality.

However, in order to achieve more reliable results further studies in this area should be done by measuring other indicators, such as IGF-I, PI3K, etc.

Conclusions

Although this study was conducted on animals, It seems that an aerobic training increases the gene expression of Akt1 and mTORc1, which can improve cardiac function and probably prevents clinical symptom of heart disease resulted from diabetes.

Acknowledgments

Thanks and gratitude to all those who have contributed to this research

Funding

This study was the result of a PhD thesis in sport physiology which was approved by the physical education department of Islamic Azad university of Tehran- South Branch.

References

1. De Luca A, Stefani L, Pedrizzetti G, Pedri S, Galanti G. The effect of exercise training on left ventricular function in young elite athletes. Cardiovascular ultrasound. 2011;9(1):1-9.
2. Sheikhzadeh F, Khajehnasiri N, Khojasteh SMB, Soufi FG, Dastranj A, Taati M. The effect of regular moderate exercise, on cardiac hypertrophy and blood glucose level in diabetic adult male rats. International Research Journal of Applied and Basic Sciences. 2013;6(4):499-503.
3. Barauna VG, Rosa KT, Irigoyen MC, De Oliveira EM. Effects of resistance training on ventricular function and hypertrophy in a rat model. Clinical medicine & research. 2007;5(2):114-20.
4. Daryanoosh F, Tanideh N, Bazgir B, Alizadeh H. Effect of aerobic trainings on heart's functioned and structure in diabetic Sprague-dawely albino species male rats. Res Applied Exercise Physiology. 2010;6(12):59-72. (In Persian)
5. Pluim BM, Zwinderman AH, van der Laarse A, van der Wall EE. The athlete's heart a meta-analysis of cardiac structure and function. Circulation. 2000;101(3):336-44.
6. Obert P, Mandigout S, Vinet A, N'guyen L, Stecken F, Courteix D. Effect of aerobic training and detraining on left ventricular dimensions and diastolic function in prepubertal boys and girls. International journal of sports medicine. 2001;22(2):90-6.
7. Rawlins J, Bhan A, Sharma S. Left ventricular hypertrophy in athletes. European Heart Journal-Cardiovascular Imaging. 2009;10(3):350-6.
8. Badalzadeh R, Shaghaghi M, Mohammadi M, Dehghan G, Mohammadi Z. The Effect of Cinnamon Extract and Long-Term Aerobic Training on Heart Function, Biochemical Alterations and Lipid Profile Following Exhaustive Exercise in Male Rats. Advanced pharmaceutical bulletin. 2014;4(2):515-20. (In Persian)
9. Kazemi F, Zahedi AS. The Correlation of Plasma Levels of Apelin-13 with Insulin Resistance Index and Plasma Leptin of Diabetic Male Rats after 8-Week Aerobic Exercise. Arak Medical University Journal. 2015;18(99):51-60. (In Persian)
10. Fathi M, Gharakanlou R, Rezaei R. The effect of endurance training on left ventricle serum response factor gene expression in Wistar male rats. Journal of Shahrekord University of Medical Sciences. 2015;17(1):78-86. (In Persian)
11. Kate L. Weeks and Julie R. McMullen. The Athlete's Heart vs. the Failing Heart: Can Signaling Explain the Two Distinct Outcomes? Physiology. 2011;26:97-105.
12. Hunter JJ, chien KR. Signaling Pathways for cardiac hypertrophy and failure. New England journal of Medicine. 1999;341(17):1276-83.
13. Soufi FG, Saber MM, Ghiassie R, Alipour M. Role of 12-week resistance training in preserving the heart against ischemia-reperfusion-induced injury. Cardiol J. 2011;18(2):140-5. (In Persian)
14. Liao J, Li Y, Zeng F, Wu Y. Regulation of mTOR Pathway in Exercise-induced Cardiac Hypertrophy.Int J Sports Med. 2015;36(5):343-50.
15. Diabetes Prevention Program Research Group: Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med. 2002; 346:393-403.
16. Garrow JS. Obesity: definition, Aetiology and Assessment. Encyclopedia of human nutrition. Academic press. 1999:1430-34.
17. Eizadi M, Ravasi AA, Soori R, Baesi K, Choubineh S. Effect of three months aerobic training on TCF7L2 expression in pancreatic tissue in type 2 diabetes rats induced by streptozotocin-nicotinamide. Feyz. 2017;21(1):1-8.
18. Lee Y, Kang EB, Kwon I, Cosio-Lima L, Cavnar P, Javan GT. CardiacKinetophagy Coincides with Activation of Anabolic Signaling. Med Sci Sports Exerc. 2016;48(2):219-26.

19. Launay T, Momken I, Carreira S, Mougenot N, Zhou XL, De Koning L, et al. Acceleration-based training: A new mode of training in senescent rats improving performance and left ventricular and muscle functions. ExpGerontol. 2017;95:71-6.
20. Sturgeon K, Muthukumaran G, Ding D, Bajulaiye A, Ferrari V, Libonati JR. Moderate-intensity treadmill exercise training decreases murine cardiomyocyte cross-sectional area. Physiol Rep. 2015;3(5):12406.
21. Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. Nature. 2001;414:782-7.
22. Frustaci A, Kajstura J, Chimenti C, Jakoniuk I, Leri A, Maseri A, et al. Myocardial cell death in human diabetes. Circ Res. 2000;87:1123-32.
23. Petrovic D. Cytopathological basis of heart failure–cardiomyocyte apoptosis, interstitial fibrosis and inflammatory cell response. Folia Biol (Praha). 2004;50:58-62.
24. Flynn KE, Pina IL, Whellan DJ, Lin L, Blumenthal JA, Ellis SJ, et al. Effects of exercise training on health status in patients with chronic heart failure: HF-ACTION randomized controlled trial. JAMA. 2009;301:1451-9.
25. Konhilas JP, Watson PA, Maass A, Boucek DM, Horn T, Stauffer BL, et al. Exercise can prevent and reverse the severity of hypertrophic cardiomyopathy. Circ Res. 2006;98:540-8.
26. McMullen JR, Amirahmadi F, Woodcock EA, Schinke-Braun M, Bouwman RD, Hewitt KA, et al. Protective effects of exercise and phosphoinositide 3-kinase(p110alpha) signaling in dilated and hypertrophic cardiomyopathy. ProcNatlAcadSci USA. 2007;104:612-7.
27. Scheuer J, Malhotra A, Hirsch C, Capasso J, Schaible TF. Physiologic cardiac hypertrophy corrects contractile protein abnormalities associated with pathologic hypertrophy in rats. J Clin Invest. 1982;70:1300-5.
28. Bernardo BC, Weeks KL, Pretorius L, McMullen JR. Molecular distinction between physiological and pathological cardiac hypertrophy: experimental findings and therapeutic strategies. Pharmacol Ther. 2010;128:191-227.
29. Kuo WW, Chung LC, Liu CT, Wu SP, Kuo CH, Tsai FJ, et al. Effects of insulin replacement on cardiac apoptotic and survival pathways in streptozotocin-induced diabetic rats. Cell Biochem Funct. 2009;27:479-87.
30. Sun HY, Zhao RR, Zhi JM. Insulin-like growth factor I inhibits cardiomyocyte apoptosis and the underlying signal transduction pathways. Methods Find Exp Clin Pharmacol. 2000;22:601-7.
31. Cheng SM, Ho TJ, Yang AL, Chen IJ, Kao CL, Wu FN, et al. Exercise training enhances cardiac IGFI-R/PI3K/Akt and Bcl-2 family associated pro-survival pathways in streptozotocin-induced diabetic rats. International Journal of Cardiology. 2013:(167):478-5.
32. Inoki K, Li Y, Zhu T, Wu J, Guan KL. TSC2 is phosphorylated and inhibited by Akt and suppresses mTORsignalling. Nat Cell Biol. 2002;4(9):648-57.
33. Potter CJ, Pedraza LG, Xu T. Akt regulates growth by directly phosphorylating Tsc2. Nat Cell Biol. 2002;4(9):658-65.
34. Garami A, Zwartkruis FJ, Nobukuni T, Joaquin M, Roccio M, Stocker H, et al. Insulin activation of Rheb, a mediator of mTOR/S6K/4E-BP signaling, is inhibited by TSC1 and 2. Mol Cell. 2003;11(6):1457-66.
35. Sancak Y, Thoreen CC, Peterson TR, Lindquist RA, Kang SA, Spooner E, et al. PRAS40 is an insulin-regulated inhibitor of the mTORC1 protein kinase. Mol Cell. 2007;25(6):903-15.
36. Ma XM, Blenis J. Molecular mechanisms of mTOR-mediated translational control. Nat Rev Mol Cell Biol. 2009;10(5):307-18.
37. HolzMK, Ballif BA, Gygi SP, Blenis J. mTOR and S6K1 mediate assembly of the translation preinitiation complex through dynamic protein interchange and ordered phosphorylation events. Cell. 2005;123(4):569-80.
38. Um SH, Frigerio F, Watanabe M, Picard F, Joaquin M, Sticker M, et al. Absence of S6K1 protects against age- and diet-induced obesity while enhancing insulin sensitivity. Nature. 2004;431(7005):200-5.
39. Shigeyama Y, Kobayashi T, Kido Y, Hashimoto N, Asahara S, Matsuda T, et al. Biphasic response of pancreatic beta-cell mass to ablation of tuberous sclerosis complex 2 in mice. Mol Cell Biol. 2008;28(9):2971-9.
40. Kim J, Kundu M, Viollet B, Guan KL. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. Nat Cell Biol. 2011;13(2):132-41.