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The Effect of Various Training on the Expression of the 5'amp-Activated Protein Kinase A2 and Glucose Transporter - 4 in Type-2 Diabetes Mellitus Rat

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Abstract

BACKGROUND: Exercise is the main pillar in Type 2 Diabetes Mellitus (T2DM) management. The mechanism of glucose uptake mediated by exercise is different from insulin, and this mechanism is not disturbed in T2DM. One of the mechanisms is through the activation of 5'AMP-activated protein kinase (AMPK). AMPK also regulates the glucose transporter 4 (GLUT4) expression. Effect various types of exercise to AMPK α2 and GLUT-4 of the skeletal muscle still limited.

AIM: This study aims to determine the effect of various physical training on the expression of Ampk α2 and Glut 4 in skeletal muscle of T2DM rats.

METHODS: This study used stored skeletal muscles of 25 T2DM Wistar rats. Previously, the rats were divided into groups of K1 (control, not given exercise), K2 (moderate continuous training), K3 (severe continuous training), K4 (slow interval training), and K5 (fast interval training). Running on a treadmill frequency 3 times a week for 8 weeks. The relative expression of *Ampk* α*2* and *Glut 4* were assessed using Real Time-PCR and were compared among the groups using the Livak formula.

RESULTS: Moderate intensity continuous training increased *Ampk* α*2* and *Glut 4* expression by 1.45 and 2.39 times, respectively. Severe intensity continuous training increased the expression of *Ampk* α*2* and *Glut 4* by 1.55 and 2.56 times, respectively. Slow interval training increased the expression of *Ampk* α*2* and *Glut 4* by 4.41 and 3.76 times, respectively. The expression of *Ampk* α*2* and *Glut4* in fast interval training was 4.56 and 4.79 times more than control.

CONCLUSION: Continuous and interval training increase *Ampk* α*2* and *Glut 4* expression. The fast interval training showed the highest expression of *Ampk* α*2* and *Glut 4*.

Introduction

The prevalence of Diabetes Mellitus Type-2 (T2DM) in the world continues to increase every year [1]. Indonesia also experienced an increase in prevalence from 6.9% in 2013 to 8.5% in 2018 [2]. One of the reasons for this increase is the lack of physical activity. Physical inactivity can reduce insulin sensitivity due to mitochondrial dysfunction, especially skeletal muscle because the skeletal muscle was one of the organs with large amount of insulin receptor. Insensitivity of insulin receptor, lead to glucose uptake impairment. As the compensation of energy insufficiency in cell, hepar will produce gluconeogenesis [3], [4].

Exercise is one of the main pillars of managing T2DM [5]. Exercise and muscle contraction stimulate glucose uptake through a different mechanism than insulin-mediated one [6]. In patients with T2DM, insulin-mediated glucose intake is impaired although exercise-mediated glucose uptake is normal or nearly normal [7]. The activation of 5'AMP-activated protein kinase (AMPK) is one of the insulin-independent mechanisms that is mediated by exercise. AMPK is a heterotrimeric protein kinase consisting of α, β, and γ subunits, encoded by different genes (α1, α2, β1, β2, γ1, γ2, and γ3). AMPK is activated by energy stress in response to an increase in ATP consumption or a decrease in ATP production, in the form of a low ratio of ATP to AMP and ADP [8]. When exercising, the amount of ADP and AMP increases rapidly while ATP decreases slightly, activating AMPK and stimulating glucose uptake by translating GLUT4 to the plasma membrane [9], [10].

AMPK also regulates GLUT4 expression [10]. This is supported by the finding that exercise, through AMPK activation, can increase GLUT4 expression in skeletal muscle [11], [12], [13]. Increased expression of skeletal muscle GLUT4 may improve blood sugar control in T2DM [14]. During exercise, only α2β2γ1 and α2β2γ3 complexes are activated and cause

muscle glucose uptake [15]. Exercise activates α2 subunits more than α1 because α2 subunit activation is more dependent on AMP concentration [16]. Exercise, through AMPK α2 mediation, could increase the binding of the transcription factor GLUT4 to the promoter [17].

The most effective exercise strategy in T2DM has not been established [18]. The American Diabetes Association (2019) recommends moderate to severe aerobic exercise [19]. Aerobic exercise comprises continuous and interval training [20], both methods are effective in improving glycemic control [21]. Continuous training is more often recommended as an aerobic exercise option [22]. However, doing high-intensity continuous training for a long duration can be risky and difficult to do in patients with chronic diseases such as T2DM [21]. Interval training can be an alternative since it induces cardiometabolic adaptations that are similar to continuous training and has superior benefits compared to continuous training in T2DM patients [23].

The effect of moderate-to-severe intensity continuous and interval training on AMPK and GLUT4 expression in T2DM is not well known. This study will examine the effect of various training models, moderate-intensity continuous training, severe intensity continuous training, slow interval training, and fast interval training on the expression of *Ampk α2* and *Glut4* in the Wistar rat T2DM model.

Methods

Animals

The ethical clearance for this study was obtained from The Research Ethics Committee, Faculty of Medicine, Universitas Sumatera Utara (No. 562/KEP/USU/2020). This study used stored muscle tissues of male white Wistar rats which have been grouped into 25 T2DM rats [24]. The preparation of experimental animal models was modified from the previous study [25]. The rats were placed in a cage at room temperature, with a light-dark cycle of 12/12 h. The T2DM model was carried out by providing a high-fat diet with a composition of 41% fat, 41% carbohydrates, and 18% protein for 5 weeks and injected 30 mg/kgBW dan 45 mg/kgBW of streptozotocin intraperitonial. This procedure was followed Machrina *et al*. (2018) that was modified from Zhang *et al*. protocol. Rats with fasting glucose levels were >200 mg/dL are grouped as T2DM rats while insulin resistance was determined using HOMA-IR values >6.5. At the end of the study, all the animals were sacrified under sedation. Gastrocnemius from all groups was taken and keep in RNA later [24].

Aerobic training

T2DM rats were divided into five groups, namely, K1: the sedentary group (not treated) as the control group; K2: group with moderate-intensity continuous training: running on a treadmill with a running speed of 20 m/min, for 30 min; K3: group with severe intensity continuous training: running on a treadmill with a running speed of 24–33 m/min, for 30 min; K4: group with slow interval training: running on a treadmill with a running speed of 20 m/min, 10 repetition sessions, with a duration of 2 min/session interspersed with rest for 1 min (20 m/min, 10 × 2 min, rest 1 min); K5: group with fast interval training: running on a treadmill with a running speed of 30 m/min, 15 repetition sessions with a duration of 30 s/session interspersed with a rest for 1 min (30 m/min, 15 × 30 s, rest 1 min). Physical training in the K2, K3, K4, and K5 was carried out for 8 weeks, three times a week [24].

Real time-PCR

RNA was extracted from 30 mg of the muscles using commercial kit (Tiangen Simple Total RNA Kit, Tiangen, China). The Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Newington, NH, USA) was applied to measure the concentration and purity of extracted RNA. RNA samples were reverse-transcribed using ReadyScript cDNA Synthesis Mix (Sigma Aldrich, Darmstadt, Germany). Specific primers were used in this study for *Ampk α2* and *Glut4* and gene expression was normalized using β-actin as a housekeeping gene (Table 1). Primers were purchased from Macrogen, Seoul, South Korea.

Table 1: Primers sequences

Gene	Primer
β actin	F 5'-AGGCCCCTCTGAACCCTAAG-3'
	R 5'- ATGTCACGCACGATTTCCCT-3'
Ampk α2	F 5'-GATCGGACACTACGTGCTGG-3'
	R 5'- GACAGTAGTCCACGGCAGAC-3'
Glut4	F 5'- GGCCGGGACACTATACCCTA-3'
	R 5'- GGAGGAAATCATGCCACCCA-3'

A total of 20 μL real time-PCR reactions were made up as follows in a 36 well plate; 100 ng of cDNA in 6,4 μl of *Rnase free ddH_2O,* 10 μL of SensiFast SYBR No-Rox Mix (Bioline Reagents Ltd. the United Kingdom) and 0.8 μL of both forward and reverse primers at 100 μM concentrations. The amplification process was performed using RotorGene (Qiagen, Germany), which was programmed to perform the following steps; 2 min hold at 95°C, and then 40 cycles at 95°C for 5 s and 60°C for 20 s. The mRNA expression was represented using the Rq which is normalised to the control group or $Rq = 2^{-\Delta\Delta Ct}$ (ΔCt = Ct [target] – Ct [β-*actin*]; ΔΔCt = ΔCt [sample] – ΔCt [control]) [26].

Statistical analysis

Effect aerobic training to fasting blood glucose in treatment groups was analyzed with *paired t*-test. To

compare the cycling threshold from *Ampk* α*2* and *Glut4* gen expression difference among group, we analyzed data with One Way ANOVA. Relative expression of *Ampk* α*2* and *Glut4* in each group was determined by livask formula.

Table 2: The comparation of ΔCt *Ampk* α*2* and ΔCt *Glut4* among group

Groups	ΔCt *Ampk* α*2* X ± SD	ΔCt *Glut4* X ± SD
K1	1.78 ± 3.75	4.72 ± 5.00
K2	1.24 ± 1.37	3.46 ± 1.30
K3	1.15 ± 1.37	3.36 ± 2.85
K4	0.36 ± 3.03	2.80 ± 3.13
K5	0.41 ± 4.85	2.46 ± 3.37

p value ΔCt Ampk α2=0.737, P value ΔCt Glut4=0.0.853. K1: not given training (control); K2: moderate intensity continuous training; K3: severe intensity continuous training; K4: slow interval training; K5 fast type interval training. ΔCt: Ct target - Ct Beta actin.

Results

Aerobic training for 8 weeks significant reduce fasting in all groups (Table 3). Average of cycling threshold (Ct) for AMPK A2 and GLUT-4 in treatment groups were lower than control group (Table 2). Cycling threshold value was inverse to mRNA gene expression, which mean lower Ct, higher mRNA gene expression.There was an increase in gene expression of Ampk α2 and Glut4 after 8 weeks of training. Moderate intensity continuous training increased Ampk α2 expression by 1.45 times and Glut 4 expression 2.39 times. Severe intensity continuous training increased the expression of Ampk α2 by 1.55 times and the expression of Glut 4 by 2.56 times. The highest expression of Ampk α2 and Glut 4 was shown in the fast interval training group. The expression of Ampk α2 and Glut 4 were 4.56 and 4.79 fold higher than the control group, respectively (Figure 1 and Figure 2).

Discussion

The results of this study indicated that there was an increase in *Ampk* α*2* expression in the four groups that were given physical training. The interval training group experienced an increase in *Ampk* α*2* expression higher than the continuous exercise group. The highest increase in the expression was found in the group with fast interval training. In addition, exercise with a higher intensity will further increase the expression of *Ampk* α*2.* The results of this study are in line with Cao *et al.* Cao *et al.*, (2012) reported that swimming exercise in T2DM rats model for 8 weeks increased the expression of *Ampk* α*2* protein 1.5 times compared to rats that were not given exercise [13]. Brandt *et al.*, (2010) also reported that swimming exercise for 4 weeks increased *Ampk* α*2* expression by 43% in insulin resistance-induced rats [27].

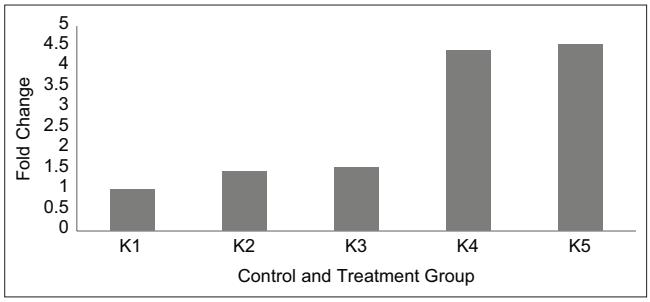

Figure 1: Relative Ampk α*2 Expression in Each Group. Livask formula =* $2^{-\Delta\Delta Ct}$*. K2 expressed Ampk* α*2 1.45 fold than K1; K3 expressed 1.55 fold than K1; K4 expressed 4.41 fold than K1; K5 expressed 4.56 fold than K1*

Table 3: Fasting Blood Glucose before and after treatment among group

Group	Fasting Blood Glucose (mg/dl)		ΔFBG	p value
	Before treatment	After treatment	(After-Before)	
K1	474.8 ± 24.9	214.9 ± 5.7		
K2	339 ± 103.7	191.6 ± 5.4	−147.4	0.014
K3	396.8 ± 25.7	198.2 ± 75.0	−198.6	0.009*
K4	452.6 ± 31.3	227.2 ± 87.8	−225.4	0.001*
K5	451.2 ± 83.2	259 ± 125.0	−192.2	0.006*

(*) mean significant (p<0.005). K1: Control, K2: Moderate intensity continuous training, K3: Severe intensity continuous training, K4: Slow interval tranining, K5: Fast interval training.

During exercise, repeated muscle contractions cause ATP to be consumed faster than it is synthesized. This results in an increase in ADP, which is followed by an increase in AMP due to the action of the adenylate kinase enzyme in the cytosol. This enzyme converts two ADP molecules into one ATP molecule and one AMP molecule. An increase in the AMP/ATP ratio causes AMPK activation and alters the anabolic and catabolic rates to restore intracellular ATP [28]. Apart from muscle tissue, AMPK also plays a critical role in controlling glucose production in the liver. AMPK activation in the liver mediates the hypoglycemic effect of adiponectin [29]. Previous study showed that moderate-intensity continuous training, severe intensity continuous training, slow interval training, and fast interval training increase the expression of *Ampk* α*1* in the liver and decrease fasting glucose levels in T2DM rats [30].

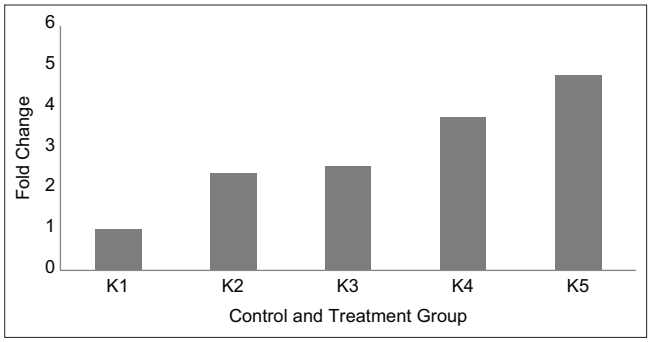

Figure 2: Relative Glut4 Expression in Each Group. Livask formula = $2^{-\Delta\Delta Ct}$*. K2 expressed Ampk* α*2 2.39 fold than K1; K3 expressed 2.56 fold than K1; K4 expressed 3.76 fold than K1; K5 expressed 4.79 fold than K1*

AMPK activation depends on the exercise intensity. AMPK activation requires a high enough intensity, which is above 50% VO_2 max [31]. Greater ATP turnover occurs in greater intensity exercise. ADP

and AMP levels increase along with increasing exercise intensity and trigger AMPK activation [28].

The change in substrate use or mobilization for interval training is steeper than for continuous training. Plasma lactate and glucose levels during interval training are lower than during continuous training. This difference explains the greater energy requirements of interval training compared to continuous training. This results in a significant increase in the AMP/ATP ratio [32]. In addition, the exercise-rest pattern on interval training also has an effect. AMPK activity was 2.9 times higher when the intensity training session was punctuated by rest intervals, compared to when the exercise was done continuously for 30 min [33].

The increase in *Glut4* expression in this study was in line with the results of the increase in *Ampk* $\alpha 2$ expression. T2DM is characterized by insulin resistance, a condition in which cells are unable to respond to insulin, especially in the liver, skeletal muscle, and fat tissue [34]. There are several mechanisms of insulin resistance. One of them is due to a decrease in the number of insulin receptors and their catalytic activity. This change causes a decrease in glucose uptake in the muscles [35]. Previous study showed that moderate-intensity continuous training, severe intensity continuous training, slow interval training, and fast interval training in T2DM rats improve insulin receptor distribution and the highest percentage increase in the distribution is in the fast interval group. The increase in the distribution of insulin receptors was followed by a decrease in insulin resistance [24].

Insulin resistance is also associated with disturbances in GLUT4 expression.[35] The amount of GLUT4 in skeletal muscle is mainly regulated at the transcription level. GLUT4 transcription is mediated by a transcription factor, namely, myocyte enhancer factor 2 (MEF2) [36]. In the resting state, MEF2 is physically associated with the transcription repressor histone deacetylase 5 (HDAC5) [11].

There are two enzymes that can phosphorylate HDAC5, namely Calmodulin-dependent Protein Kinase (CaMK) and AMPK [6]. During the contraction during exercise, each wave of depolarization will remove Ca from the reticulum sarcoplasm and increase the concentration of Ca in the cytosol and activate CaMK [37]. Both CaMK and AMPK which are activated during exercise can phosphorylate HDAC5 so that HDAC5 leaves the nucleus and the MEF2/HDAC5 complex dissociates. The dissociation of the MEF2/HDAC5 complex causes MEF2 binding to PGC-1α and increase GLUT4 expression [6], [38]. An important adaptation of exercise is increased GLUT4 expression in skeletal muscle, increased capacity for glucose transport, and better systemic control [39]. Previous study showed that moderate-intensity continuous exercise, severe intensity continuous exercise, slow interval training, and fast interval training in T2DM rats significantly reduces fasting blood sugar levels compared to the sedentary group [40].

Conclusion

Moderate-intensity continuous training, severe intensity continuous training, slow interval training, and fast interval training increased the *Ampk* $\alpha 2$ and *Glut4* expression. The highest increase in expression was found in the fast interval training group.

References

1. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, *et al*. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. Diabetes Res Clin Pract. 2019;157:107843. https://doi.org/10.1016/j.diabres.2019.107843
   PMid:31518657
2. Ditjen Kesehatan Masyarakat Kementrian Kesehatan. Kementrian Kesehatan Republik Indonesia, Hasil Riskesdas Tahun 2018; 2020. Available from: https://www.kesmas.kemkes.go.id/assets/upload/dir_519d41d8cd98f00/files/Hasil-riskesdas-2018_1274.pdf [Last accessed on 2020 Nov 20].
3. Neill HM. AMPK and exercise: Glucose uptake and insulin sensitivity. Diabetes Metab J. 2013;37(1):1-21. https://doi.org/10.4093/dmj.2013.37.1.1
   PMid:23441028
4. Sharabi K, Tavares CD, Rines AK, Puigserver P. Molecular pathophysiology of hepatic glucose production. Mol Aspects Med. 2015;46:21-33. https://doi.org/10.1016/j.mam.2015.09.003
   PMid:26549348
5. PERKENI. In: Soelistijo SA, Novida H, editors. Konsensus Pengelolaan dan Pencegahan Diabetes Melitus Tipe 2 di Indonesia 2015. Jakarta: PB PERKENI; 2015. p. 10-61.
6. Richter EA, Hargreaves M. Exercise, GLUT4, and skeletal muscle glucose uptake. Physiol Rev. 2013;93(3):993-1017.
   PMid:23899560
7. Stanford KI, Goodyear LJ. Exercise and Type 2 diabetes: Molecular mechanisms regulating glucose uptake in skeletal muscle. Adv Physiol Educ. 2014;38:308-14. https://doi.org/10.1152/advan.00080.2014
   PMid:25434013
8. Yan Y, Zhou XE, Xu HE, Melcher K. Structure and physiological regulation of AMPK. Int J Mol Sci. 2018;19(11):3534. https://doi.org/10.3390/ijms19113534
   PMid:30423971
9. Richter EA, Ruderman NB. AMPK and the biochemistry of exercise: Implications for human health and disease. Biochem J. 2010;418(2):261-75. https://doi.org/10.1042/BJ20082055
   PMid:19196246
10. Pereira RM, Sanchez A. Molecular mechanisms of glucose uptake in skeletal muscle at rest and in response to exercise. Moritz Rio Carlo. 2017;23:1-8.
11. Mcgee SL, van Denderen BJ, Howlett KF, Mollica J, Schertzer JD,

Kemp BE, *et al.* AMP-activated protein kinase regulates GLUT4 transcription by phosphorylating histone deacetylase 5. Clin Exp Pharmacol Physiol. 2008;57:860-7.

12. Hussey SE, McGee SL, Garnham A, Wentworth JM, Jeukendrup AE, Hargreaves M. Research letter in patients with Type 2 diabetes research letter. Diabetes Obes Metab. 2011;13(10):959-62. https://doi.org/10.1111/j.1463-1326.2011.01426.x
PMid:21615668
13. Cao S, Li B, Yi X, Chang B, Zhu B, Lian Z, *et al.* Effects of exercise on AMPK signaling and downstream components to PI3K in rat with Type 2 diabetes. PLoS One. 2012;7(12):e51709. https://doi.org/10.1371/journal.pone.0051709
PMid:23272147
14. Hansen JS, Zhao X, Irmler M, Liu X, Hoene M, Scheler M, *et al.* Type 2 diabetes alters metabolic and transcriptional signatures of glucose and amino acid metabolism during exercise and recovery. Diabetologia. 2015;58(8):1845-54.
15. Lantier L, Fentz J, Mounier R, Leclerc J, Treebak JT, Pehmøller C, *et al.* AMPK controls exercise endurance, mitochondrial oxidative capacity, and skeletal muscle integrity. FASEB J. 2014;28(7):3211-24. https://doi.org/10.1096/fj.14-250449
PMid:24652947
16. Jørgensen SB, Jensen TE, Richter EA. Role of AMPK in skeletal muscle gene adaptation in relation to exercise. Appl Physiol Nutr Metab. 2007;32(5):904-11. https://doi.org/10.1139/H07-079
PMid:18059615
17. Gong H, Xie J, Zhang N, Yao L, Zhang Y. MEF2A binding to the Glut4 promoter occurs via an AMPKα 2-dependent mechanism. Med Sci Sports Exerc. 2011;43(8):1441-50. https://doi.org/10.1249/MSS.0b013e31820f6093
PMid:21233771
18. Francois ME, Little JP. Effectiveness and safety of high-intensity interval training in patients with Type 2 diabetes. Diabetes Spectr. 2015;28(1):39-44. https://doi.org/10.2337/diaspect.28.1.39
PMid:25717277
19. American Diabetes Association. Lifestyle Management: Standards of Medical Care in Diabetes 2019. Diabetes Care. 2019;42:46-60.
20. Spanoudaki S. Interval versus continuous training. J Sports Med Doping Stud. 2011;1(1):4172.
21. Mitranun W, Deerochanawong C, Tanaka H, Suksom D. Continuous vs interval training on glycemic control and macro- and microvascular reactivity in Type 2 diabetic patients. Scand J Med Sci Sport. 2014;24(2):69-76. https://doi.org/10.1111/sms.12112
PMid:24102912
22. Álvarez C, Ramirez-campillo R, Alvarez C, Mancilla R, Ciolac EG. Low-volume high-intensity interval training as a therapy for Type 2 diabetes. Int J Sport Med. 2016;3:1-8. https://doi.org/10.1055/s-0042-104935
PMid:27259099
23. De Nardi AT, Tolves T, Lenzi TL, Signori LU, da Silva AM. High-intensity interval training versus continuous training on physiological and metabolic variables in prediabetes and Type 2 diabetes: A meta-analysis. Diabetes Res Clin Pract. 2018;137:149-59. https://doi.org/10.1016/j.diabres.2017.12.017
PMid:29329778
24. Machrina Y, Damanik HA, Purba A, Lindarto D. Effect various type of exercise to Insr gene expression, skeletal muscle insulin receptor and insulin resistance on diabetes mellitus type-2 model rats. Int J Health Sci. 2018;6(4):50-6.
25. Zhang M, Lv XY, Li J, Xu ZG, Chen L. The characterization of high-fat diet and multiple low-dose streptozotocin induced Type 2 diabetes rat model. Exp Diabetes Res. 2008;2008:704045. https://doi.org/10.1155/2008/70404
PMid:19132099
26. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-delta delta C(T)) method. Methods. 2001;25(4):402-8. https://doi.org/10.1006/meth.2001.1262
PMid:11846609
27. Brandt N, de Bock K, Richter EA, Hespel P. Cafeteria diet-induced insulin resistance is not associated with decreased insulin signaling or AMPK activity and is alleviated by physical training in rats. Am J Physiol Endocrinol Metab. 2010;299(2):E215-24. https://doi.org/10.1152/ajpendo.00098.2010
PMid:20484011
28. Carling D, Mayer FV, Sanders MJ, Gamblin SJ. AMPK-activated protein kinase: Nature's energy sensor. Nat Chem Biol. 2011;7(8):512-8. https://doi.org/10.1038/nchembio.610
PMid:21769098
29. Viollet B, Foretz M, Guigas B, Horman S, Dentin R, Bertrand L, *et al.* Activation of AMP-activated protein kinase in the liver: A new strategy for the management of metabolic hepatic disorders. J Physiol. 2006;574(1):41-53.
30. Machrina Y, Pane YS, Lindarto D. The expression of liver metabolic enzymes ampkα1, ampkα2, and pgc-1α due to exercise in Type-2 diabetes mellitus rat model. Open Access Maced J Med Sci. 2020;8:629-32.
31. Thomson DM. The role of AMPK in the regulation of skeletal muscle size, hypertrophy, and regeneration. Int J Mol Sci. 2018;19(10):3125. https://doi.org/10.3390/ijms19103125
PMid:30314396
32. Torma F, Gombos Z, Jokai M, Takeda M, Mimura T, Radak Z. High intensity interval training and molecular adaptive response of skeletal muscle. Sport Med Health Sci. 2019;1(1):24-32.
33. Combes A, Dekerle J, Webborn N, Watt P, Bougault V, Daussin FN. Exercise-induced metabolic fluctuations influence AMPK, p38-MAPK and CaMKII phosphorylation in human skeletal muscle. Physiol Rep. 2015;3(9):e12462. https://doi.org/10.14814/phy2.12462
PMid:26359238
34. DeFronzo RA, Tripathy D. Skeletal muscle insulin resistance is the primary defect in Type 2 diabetes. Diabetes Care. 2009;32 Suppl 2:S157-63. https://doi.org/10.2337/dc09-S302
PMid:19875544
35. Gutierrez-Rodelo C, Roura-Guiberna A, Olivares-Reyes JA. Molecular mechanisms of insulin resistance: An update. Gac Med Mex. 2017;153:197-209.
PMid:28474708
36. Lehnen AM. Changes in the GLUT4 expression by acute exercise, exercise training and detraining in experimental models. J Diabetes Metab. 2013;1(10):1-8.
37. Ojuka EO, Goyaram V, Smith JA. The role of CaMKII in regulating GLUT4 expression in skeletal muscle. Am J Physiol Endocrinol Metab. 2012;303(3):322-31. https://doi.org/10.1152/ajpendo.00091.2012
PMid:22496345
38. Ling C, Roon T, Nitert MD. Epigenetics and Type-2 Diabetes in Epigenetics Aspect of Chronic Diseases. Berlin, Germany: Springer; 2011. p. 135-45.
39. Koh JH, Hancock CR, Han DH, Holloszy JO, Nair KS, Dasari S. AMPK and PPARβ positive feedback loop regulates endurance exercise training-mediated GLUT4 expression in skeletal muscle. Am J Physiol Endocrinol Metab. 2019;316(5):E931-9. https://doi.org/10.1152/ajpendo.00460.2018
PMid:30888859
40. Machrina Y, Purba A, Lindarto D, Maskoen AM. Exercise intensity alter insulin receptor gene expression in diabetic Type-2 rat model. Open Access Maced J Med Sci. 2019;7(20):3370-5. https://doi.org/10.3889/oamjms.2019.425
PMid:32002053