International Journal of Applied Exercise Physiology
2322-3537 www.ijaep.com
Vol.8 No.1
Received: September 2018 , Accepted: December 2018 , Available online: March 2019

DOI: 10. 30472 /ijaep.v8i1.363

The Effect of High Intensity Interval Training and Endurance Training on the cAMP Gene Expression and Glycerol in the Heart Tissue of Obese Rats

Narges Ahmadi [1], Sirous Farsi [2*], Mohammad Ali Azarbayjani [3]
[1]*Sport Physiology Department, Larestan Branch, Islamic Azad University, Larestan, Iran*
[2]*Sport Physiology Department, Marvdasht Branch, Islamic Azad University, Marvdasht, Iran*
[*] *Corresponding author:* Sirous.farsi@gmail.com ,
[3]*professor of exercise physiology ،Islamic Azad University,Central Tehran Branch,Tehran,Iran.*

Abstract

Introduction: Cyclic adenosine monophosphate (cAMP), transmits intracellular signaling information, resulting in release of free fatty acids. The purpose of this study was to investigate the effect of twelve weeks of high intensity interval training (HIIT) and endurance training on the cAMP gene expression and glycerol in the heart tissue of obese rats.

Methods: In this experimental study, 20 rats were divided into four groups, including: (1) high-fat endurance training, (2) high-fat HIIT, (3) normal diet control, and (4) high-fat control.

Group 1 performed endurance training, and group 2 performed HIIT for 12 weeks. Data were analyzed by one-way ANOVA with repeated measures and one-way ANOVA with Tukey's post hoc test.

Results: Endurance training and HIIT had a significant effect on the decrease in glycerol levels; also the both had the same effects on reducing the concentration of glycerol in the heart tissue of obese rats. However, endurance training and HIIT had no significant effect on cAMP gene expression in the heart tissue of obese rats.

Conclusion: It seems that endurance and interval training with the intensity and duration of the present study protocol reduced the concentration of glycerol in the heart tissue of obese rats. However, they have no significant effect on cAMP gene expression in the heart tissue of obese rats.

Keywords: Endurance training, high intensity interval training, cAMP, Glycerol

Introduction

Although obesity has been described worldwide as an epidemic for years, efforts to reduce this epidemic are not effective and there is a need for new strategies for the prevention and treatment of obesity [1]. The prevalence of obesity worldwide is rising and it is estimated that half of the adult population will be obese in the United States in 2030 [2, 3].

Studies have shown that obesity is a multifaceted disease in which afflicted patients are at risk of environmental and genetic factors, such as type 2 diabetes, high blood pressure, dyslipidemia, coronary heart disease, stroke, and cancers [3, 4]. Hence, the diagnosis of metabolic disorders in the heart has been addressed in response to the challenge of high-fat diet [5].

Given the progress of the biological sciences and the ability to recognize the complex pathways of cellular metabolism, information is being sought on the role of these micro-molecules in preventing, treating or reducing the complications of some diseases. Recent studies have shown that the synthesis, accumulation and secretion of fraction-dependent components such as very low density lipoprotein are a function of hormonal and metabolic control. Therefore, fat metabolism is not only dependent on factors such as catecholamines, prostaglandins, calcium antagonists, but also is inhibited by cyclic adenosine monophosphate cAMP pathway agents such as glucagon, cAMP derivatives and cAMP-dependent protein kinase [6]. cAMP, known as a carrier, transmits intracellular signaling information, resulting in the release of lipid profiles and free fatty acids, both of which provide the background for tissue fat loss [7]. Also, cAMP is the second major messenger of beta-adrenergic signal pathway receptors and phosphorylation of voltage valves and ryanodine receptor, which increases Ca^{2+} for contraction of the heart muscle [8]. Researchers have argued that increased inflammation following obesity reduces the function of beta-adrenergic receptors in adipose cells, resulting in decreased catecholamine function by decreasing intracellular cAMP [7]. Furthermore, exogenous fatty acids are known to inhibit cAMP production in adipocytes [9].

On the other hand, lifestyle change seems to be the most important factor in the treatment of obesity. The most commonly used therapeutic ways to reduce weight in the

obese population include behavioral interventions (physical activity and dietary changes), pharmacology and surgery [3, 10,11]. Among these interventions, sport exercises as a non-pharmacological and low-cost strategy cause changes in the body that increase physical health and reduce the risk factors in the lives of inactive people [12, 13]. However, Studies on the effect of intensity and type of exercise on metabolism and its molecular pathways are contradictory. For example, participation in activities with intensity of 63%, 86% of maximal heart rate had a significant effect on the increase of cAMP, glycerol and adrenaline, but super-maximal activity only managed to affect blood glucose levels, cortisol and glycogen following these exercise intensities in ergometer bike [14]. Four weeks of endurance training with intensity of 50 to 60% of running speed had a significant effect on the increase of serum glycerol levels and the expression of insulin pathway proteins [15]. Twelve weeks of aerobic exercise did not significantly affect increasing cAMP expression in adipose tissue of rats [16].

Although therapeutic advantages of physical exercises are extensively recognized, many people, especially the obese population, do not participate in a regular sporting event. These people consider the "lack of time" as the main obstacle to a regular exercise program [17]. High intensity interval training (HIIT) has been suggested as an effective option to address the lack of time for exercise [18], but such exercises are controversial in the studies on obesity measurement indices [19-20].

Based on our information, no research has examined the effects of HIIT on expression of cAMP in the heart tissue of rats. Therefore, the aim of present study is to investigate the effect of 12 weeks of endurance training and HIIT on cAMP gene expression and glycerol levels in the heart tissue of obese rats.

Methodology

In this experimental study, 20 male Wistar rats with mean weight of 156.71 ± 27.42 were purchased from the Pasteur Institute of Amol, and held for one week for compatibility in appropriate laboratory conditions; the light and dark cycle of 12:12 hours; temperature of 22 ± 3 ° C, and humidity of 40-50%, in polycarbonate transparent cages of 30, 15 and 15 cm in length, width and height, manufactured by Razi Rad Co.

The present study was carried out in two stages including fattening stage and training stage. The weight of the rats was measured at the beginning, at the end of the fattening phase and the end of the 12-week training period. Rats were randomly divided into four groups of

five, including: 1- high-fat endurance training (Under the diet, 40% fat, 13% protein and 47% carbohydrate), 2- high-fat HIIT, 3- high-fat control, and 4- normal diet control (A special diet of rats prepared by Behparvar Company containing 58% carbohydrate, 13% fat, and 28% protein).For each 100 grams weight of each rat, 5 grams of food were put in a cage once a week, based on weighing, and the water they needed was freely available in a 500ml bottle of laboratory animals.

Rats in groups 1, 2 and 3 with high fat diets with 40% fat (20% soybean oil and 20% animal fat), 13% protein and 47% carbohydrates were studied before and during the protocol.

The Lee's index was used to measure obesity. In this criterion, rats over 310 grams are known to be obese [16].

It should be noted that this study was carried out in accordance with the guidelines for the use and care of laboratory animals [21].

Training protocol

The warm-up phase included running for 3 minutes at 10 m / min, followed by running for 2 minutes at an intensity of 15 m / min, and after performing the main training in each group, rats were cooled-down for 1 minute at an intensity of 15 m / min and then for 2 minutes at an intensity of 10 m / min.

For determining the maximum oxygen consumption, the mean of maximum speed obtained by using the Bedford et al.'s (1997) incremental test [16]. For the rats in high-fat endurance training groups, 65% of the maximum speed evaluated and was considered as the given intensity in the endurance training groups in the first week. The endurance training protocol was progressive and started at a speed of 20 m / min for 15 minutes in the first week and gradually progressed to 25 m / min for 13 minutes in the 12^{th} week.

The HIIT protocol was performed at the intensity of 85 to 90% of the maximum running speed, including 7 repetition of 1-minute attempts at 31 m / min speed and active rest between the intervals with 6 attempts at 15 m / min speed in the first week, which with an average increase of 2 m / min per week, gradually reached 10 repetition of 1-minute attempts at 55 m / min speed and active rest with 9 repetition of 1-minute attempts (between the intervals) at 25 m / min speed in week 12. The trainings were conducted for 12 weeks and five sessions per week.

Measurement and examination of tissues

48 hours after the last training session, rats were intraperitoneally anesthetized with a combination of ketamine (70 mg / kg) and zylozine (3-5 mg / kg) and then sacrificed. The heart tissue was taken and weighed with a digital scale of 0.001 grams accurately. The tissue was placed in a tube of Falcon 15 and for

each 0.5 gram of tissue 200 µl of a single-phase lubricating solution was poured into it.

To maintain tissue proteins, aprotinin was added to it and was homogenized using a homogenizer for 5 minutes at 8000 rpm. Then, the achieved solution was centrifuged at 3000 rpm for 15 minutes.

The supernatant was transferred to the microtubule by sampler and analyzed for the variables considered and the remaining sediment was discarded. Extracting RNA and examining the expression of the gene was carried out by Real Time PCR. To extract RNA, 50 mg of frozen heart tissue of rats was homogenized and the RNA solution was extracted using the Plus-RNA kit manufacturer (Sina gen, Iran), and using the DNAse I enzyme any contamination with DNA and RNA degrading enzymes was purified.

Of sample, 2 µg of mRNA was used to synthesize the first cDNA strand. For synthesis of cDNA, a cDNA synthesis kit (Fermentas, Germany) was used. Relative cAMP gene expression in the heart was measured by cAMP specific primers using time real time PCR method.

Information on the design of the cAMP gene primer was obtained in the form of CGTGCTGTGGATGACTTCAA. The glycerol tissue levels were measured by a glycerol kit and analyzed by an auto-analyzer and spectrophotometer.

Statistical procedures

For statistical analysis of the findings, Kolmogorov-Smirnov test, one-way ANOVA with repeated measures and Bonferroni's post hoc test, as well as one-way ANOVA with Tukey's post hoc test were used in SPSS version 20 ($p \leq 0.05$).

Findings

In Table 1, the rats' weight in the four groups of research in different periods is presented.

To examine changes in the weight of rats at different times and groups (Figure 1), the results of one-way ANOVA with repeated measures showed a significant difference in the weight of rats at the time before the fattening stage, after the fattening stage, and the end of the protocol ($p = 0.001$ and $F = 296.66$), but the time and group interaction in weight levels is not significant ($p = 0.31$ and $F = 1.26$).

Table 1: Weight of rats prior to high fat diet, after high fat diet and at end of protocol in the four groups of research

Group	Weight (Before Fattening)	Weight (After Fattening)	Weight (End of Protocol)

Normal Diet Control	149.26±24.05	384.17±11.63	391.32±16.37
High-Fat Control	180.30±08.54	351.73±51.44	397.61±87.0355
Endurance Training	154.41±20.96	376.56±93.76	334.56±81.99
HIIT	143.35±31.28	398.26±21.31	346.21±46.81

Also, the results of Bonferroni's post hoc test showed that the weight of rats in the fattening stage increased significantly compared to the pre-fattening stage (p = 0.001 and M = 220.98); however, there was no significant difference in weight levels after the completion of the protocol compared to the post-fattening stage of rats (p = 0.09 and F = 10.11) (figure1).

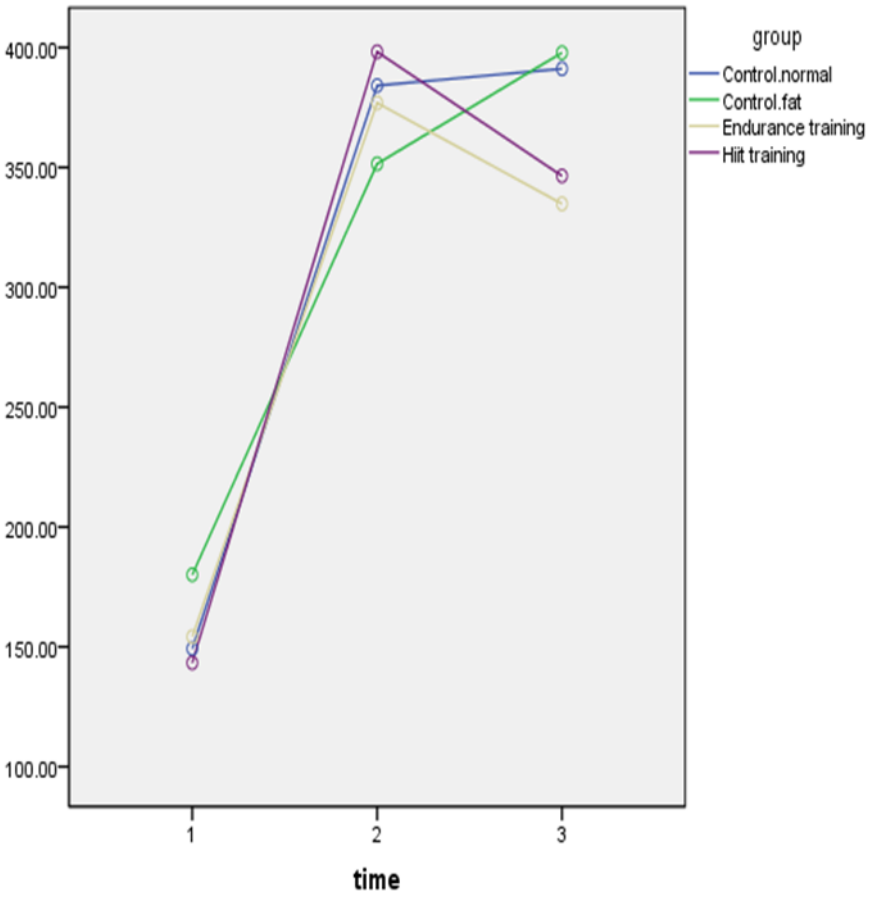

Figure1. Rats' weight levels at different time periods and groups of research

The results of one-way ANOVA showed that there was no significant difference in cAMP gene expression in the heart tissue of the four groups of rats (P = 0.13, F = 2.161). However, there was a significant difference in the level of Glycerol in the heart tissue of rats in four groups of research (P = 0.001, F = 371.70) (Table2).

Table 2: Results of one-way ANOVA test to compare cAMP and glycerol in the study groups

Variable	Group	Mean	Standard Deviation	F	P
cAMP	Normal Diet Control	0.05678	0.0053	2.161	**o.138**
	High-Fat Control	0.00030	0.0012		
	Endurance Training	0.00050	0.0023		
	HIIT	0.00024	0.0012		
Glycerol	Normal Diet Control	0.4000	0.0351	371.70	**0.001***
	High-Fat Control	1.5564	0.1062		
	Endurance Training	0.5020	0.05669		
	HIIT	07277	0.02777		

The results of Tukey's post hoc test in Table 3 showed that high fat diet significantly increased the tissue concentration of glycerol in the heart tissue of rats (p = 0.001, M = -1.15). Also, endurance training (p = 0.001, M = 1.05) and HIIT (p = 0.001, M = 1.10) had a significant effect on glycerol reduction in the heart tissue of rats with high-fat diet. Endurance training and HIIT had the same effects on reducing the amount of glycerol in the heart tissue of rats with high-fat diet (p = 0.62, M = 0.04) (Table3).

Table 3: Results of Tukey's post hoc test to determine the difference in glycerol content in rats in four groups of research

	High-Fat Control	Endurance Training	HIIT
Normal Diet Control	M=-1.15 P=0.001	M=-0.10 P=0.09	**M=-0.05** **P=0.58**
High-Fat Control		M=1.05 P=0.001	**M=1.1** **P=0.001**
Endurance Training			**M=-0.04** **P=0.62**

Discussion

The results of this study showed that endurance training and HIIT had no significant effect on cAMP expression in the heart tissue of obese rats. However, endurance training and HIIT had a significant effect on reducing the concentration of glycerol in the heart tissue of obese rats. Endurance training and HIIT also had the same effects in reducing the concentration of glycerol in the heart tissue of rats.

It has been shown that cAMP as a messenger has a variety of biological effects: for example, maintaining vascular homeostasis. Stimulation of contractions in the heart occurs by various types of cAMP-dependent protein kinases [22]. It also transmits various receptors by transmitting signals generated by stimulation and activates various cellular functions. The cAMP activates the protein kinase a molecules that are adjacent to the T tubules and also seems to create slow channels of Ca2 + in the heart cells [22],

Inactive and active forms of both phosphorylase and triglycerid lipase enzymes responsible for mobilizing carbohydrates and lipids are ultimately controlled by cAMP. As a result, it appears that cAMP variations may be important for mobilizing fat and carbohydrates during sports exercise [23].

Researchers believe that sports exercises stimulate beta-adrenergic receptors due to increased sympathetic nerve activity and epinephrine and norepinephrine secretion. This, in turn, activates cAMP and thus activates fat degrading enzymes and ultimately increases lipolysis, and the transmission of intracellular signaling data via cAMP leads to the release of triglycerides (TGs) from fatty scids (FA) [24].

Researchers believe that lifestyle change with reduced physical activity has a direct relationship with the increased risk of obesity and higher incidence of metabolic disorders [25, 26]. Today, exercise is widely recognized as a nonpharmologic intervention against obesity and diseases associated with obesity [27]. In line with the findings of the present study, the researchers stated that a training session with 40% maximum oxygen consumption had no significant effect on cAMP receptor expression in human skeletal muscle [28]. In addition, twelve weeks of aerobic exercise did not significantly affect cAMP expression in adipose tissue in rats [16]. Three months of high intensity training had no effect on the level of cAMP synthesis in adipose tissue of rats [29].

On the other hand, it has reported that one bout training with an intensity of 80% of maximum oxygen consumption increased cAMP in human skeletal muscle [30]. In another study, six weeks of moderate-intensity strength training had a significant effect on the expression of different AMPK isoforms in men with type 2 diabetes [24].

Participating in activities with intensity of 63%, 86% of maximal heart rate had a significant effect on the increase of cAMP, glycerol and adrenaline, but the supermaximal activity only affected blood glucose levels, cortisol, and glycogen in men following these exercise intensities on ergometer bike [14]; the reason for the inconsistency of this study with the present study is the difference in the statistical population, and the difference in the intensity and duration of the training. Also, the results of this study showed that endurance training and HIIT had a significant and equal effect on reducing the concentration of glycerol in the heart tissue of obese rats. Along with this finding, a previous study has revealed the effect of cAMP on the release of glycerol and fatty acids, which found that there was a relationship between glycerol and cAMP [31].

It was also found that regular physical training has a significant effect on the increase in glycerol circulation levels, and the researchers have acknowledged that physical activities significantly increase glycerol levels as an indicator of total lipolysis in the body [32]. Given the fact that glycerol is a final product of lipolysis and blocks intake of food in different conditions, it is likely that increased lipolysis of fat cells by exercise affects the energy intake [33].

Also, in animal research, it has been shown that during exercise, an increase in heart hormones increased the concentration of adenosine. In this way, ATP decomposition and increased AMP concentration lead to adenosine accumulation to increase energy supply and demand and eventually increase cAMP [34]. Consistent with this study, four weeks of endurance training with intensity of 50 to 60% of running speed had a significant effect on serum glycerol levels and the expression of insulin pathway proteins [15].

In one study, researchers argued that increase in adenosine acts as a progressive loop and increases the expression of cAMP to activate thermogenic genes [35]. They concluded that the accumulation of adenosine increases the metabolism of the heart, which increases the cAMP and lipolysis [36].

Conclusion

Regarding to the results of this study, it seems that endurance training and HIIT, with intensity and duration of the present study protocol, have no significant effect on expression of cAMP in the heart tissue of obese rats. However, with regard to the significant and equal effect of the endurance training and HIIT on reducing the concentration of glycerol in the heart tissue of obese rats, the HIIT could be considered as a possible new strategy for the prevention and treatment of obesity, although more studies are needed.

Our study has several limitations. Due to the role of ATP regulation and AMP concentration, lack of measurement of their concentration is one of the limitations of the

present study. Therefore, it is recommended in future studies to study the concentration of these factors along with the variables of this research. In addition, since the previous studies indicate that effect of physical activities on expression of cAMP is dependent on some hormonal and metabolic factors, it is suggested that these mechanisms be investigated in future studies. Also Regarding the role of diet in weight loss, it seems that the lack of daily meal control measures to accurately control the calorie intakes and calories consumed are the limitations of the present study. Therefore, it is recommended that in future studies the amount of food used in rats be accurately measured and its relationship with regulatory factors of metabolism should be considered.

Acknowledgement: The researcher expresses her thanks and gratitude to all individuals who assisted in conducting the present research study.

References

1. Apovian, C.M., et al., Pharmacological management of obesity: an endocrine Society clinical practice guideline. J Clin Endocrinol Metab, 2015. 100(2): p. 342-62.
2. Bae, J.Y., Aerobic Exercise Increases Meteorin-Like Protein in Muscle and Adipose Tissue of Chronic High-Fat Diet-Induced Obese Mice. Biomed Res Int, 2018. 2018: p. 6283932.
3. Nozari N, Pourshams A. A review on the position of endoscopy techniques in obesity treatment. Govaresh, 2016; 21(1): 7-19.
4. Fernandes, T., et al., Exercise Training Restores the Cardiac Microrna-16 Levels Preventing Microvascular Rarefaction in Obese Zucker Rats. Obes Facts, 2018. 11(1): p. 15-24.
5. Snyder, E.E., et al., The human obesity gene map: the 2003 update. Obes Res, 2004. 12(3): p. 369-439.
6. Rasooli M, Zahraee M. Inhibition of Triacyleglycerol Secretion dependent on VLDL Fraction by α and β Adreno receptors Agonists in Rat Hepatocyte . J Mazandaran Univ Med Sci. 2000; 9 (25) :10-19
7. Mowers, J., et al., Inflammation produces catecholamine resistance in obesity via activation of PDE3B by the protein kinases IKKepsilon and TBK1. Elife, 2013. 2: p. e01119.
8. Boularan, C. and C. Gales, Cardiac cAMP: production, hydrolysis, modulation and detection. Front Pharmacol, 2015. 6: p. 203.
9. Fain, J.N. and R.E. Shepherd, Free fatty acids as feedback regulators of adenylate cyclase and cyclic 3':5'-AMP accumulation in rat fat cells. J Biol Chem, 1975. 250(16): p. 6586-92.
10. Castelnuovo, G., et al., Cognitive behavioral therapy to aid weight loss in obese patients: current perspectives. Psychol Res Behav Manag, 2017. 10: p. 165-173.
11. Kushner, R.F., Weight loss strategies for treatment of obesity. Prog Cardiovasc Dis, 2014. 56(4): p. 465-72.
12. Hoseini S A. Haghjoo M, zar A,The Effect of 8 weeks Zumba Training on Women's Body Composition with Overweight. jmj. 2016; 14 (2) :21-30
13. Warburton, D.E., C.W. Nicol, and S.S. Bredin, Health benefits of physical activity: the evidence. Cmaj, 2006. 174(6): p. 801-9.
14. Naveri, H., Blood hormone and metabolite levels during graded cycle ergometer exercise. Scand J Clin Lab Invest, 1985. 45(7): p. 599-603.
15. de Bem, G.F., et al., Antidiabetic effect of Euterpe oleracea Mart. (acai) extract and exercise training on high-fat diet and streptozotocin-induced diabetic rats: A positive interaction. PLoS One, 2018. 13(6): p. e0199207.

16. Bedford, T.G., et al., Maximum oxygen consumption of rats and its changes with various experimental procedures. J Appl Physiol Respir Environ Exerc Physiol, 1979. 47(6): p. 1278-83.
17. Fox, A.M., et al., Barriers to physical activity in East harlem, new york. J Obes, 2012. 2012: p. 719140.
18. De Feo, P., Is high-intensity exercise better than moderate-intensity exercise for weight loss? Nutr Metab Cardiovasc Dis, 2013. 23(11): p. 1037-42.
19. Keating, S.E., et al., Continuous exercise but not high intensity interval training improves fat distribution in overweight adults. J Obes, 2014. 2014: p. 834865.
20. Mirghani S. Yousefi M. The effect of interval recovery periods during HIIT on liver enzymes and lipid profile in overweight women. Science & Sports, 2015. 30(3): 147-154.
21. National Research Council Committee for the Update of the Guide for the, C. and A. Use of Laboratory, The National Academies Collection: Reports funded by National Institutes of Health, in Guide for the Care and Use of Laboratory Animals, th, Editor. 2011, National Academies Press (US)
22. Billington C K, Ojo O O, Penn R B, Ito S. cAMP regulation of airway smooth muscle function. Pulm Pharmacol Ther, 2012; 26(1): 112-20.
23. Lezoualc'h, F., et al., Cyclic AMP Sensor EPAC Proteins and Their Role in Cardiovascular Function and Disease. Circ Res, 2016. 118(5): p. 881-97.
24. Kolditz, C.I. and D. Langin, Adipose tissue lipolysis. Curr Opin Clin Nutr Metab Care, 2010. 13(4): p. 377-81.
25. Ha, T.H., et al., The Effect of Metabolic Syndrome on Myocardial Contractile Reserve during Exercise in Non-Diabetic Hypertensive Subjects. J Cardiovasc Ultrasound, 2011. 19(4): p. 176-82.
26. Xiao, J., et al., Therapeutic approaches to non-alcoholic fatty liver disease: past achievements and future challenges. Hepatobiliary Pancreat Dis Int, 2013. 12(2): p. 125-35.
27. Sheikholeslami-vatani D, Ebrahimi A. The Effect of Moderate-Intensity Continuous Training Vs. High-Intensity Interval Training on Visceral and Subcutaneous Fats in Obese Women. JRUMS. 2018; 16 (11) :999-1012
28. Egan, B., et al., Exercise intensity-dependent regulation of peroxisome proliferator-activated receptor coactivator-1 mRNA abundance is associated with differential activation of upstream signalling kinases in human skeletal muscle. J Physiol, 2010. 588(Pt 10): p. 1779-90.
29. Carey, G.B., et al., Extracellular cyclic AMP and adenosine appearance in adipose tissue of Sus scrofa: effects of exercise. Exp Biol Med (Maywood), 2004. 229(10): p. 1026-32.
30. Wojtaszewski, J.F., et al., 5'AMP activated protein kinase expression in human skeletal muscle: effects of strength training and type 2 diabetes. J Physiol, 2005. 564(Pt 2): p. 563-73.
31. Rutkowski, J.M., J.H. Stern, and P.E. Scherer, The cell biology of fat expansion. J Cell Biol, 2015. 208(5): p. 501-12.
32. Romijn, J.A., et al., Strenuous endurance training increases lipolysis and triglyceride-fatty acid cycling at rest. J Appl Physiol (1985), 1993. 75(1): p. 108-13.
33. Pistor, K.E., et al., Lipolysis, lipogenesis, and adiposity are reduced while fatty acid oxidation is increased in visceral and subcutaneous adipocytes of endurance-trained rats. Adipocyte, 2015. 4(1): p. 22-31.
34. Farney T. M. Relationship Between Metabolic By-Products and Nervous System Failure/Fatigue. 2016.
35. Gnad, T., et al., Adenosine activates brown adipose tissue and recruits beige adipocytes via A2A receptors. Nature, 2014. 516(7531): p. 395-9.
36. Johansson, S.M., et al., Eliminating the antilipolytic adenosine A1 receptor does not lead to compensatory changes in the antilipolytic actions of PGE2 and nicotinic acid. Acta Physiol (Oxf), 2007. 190(1): p. 87-96.