The Regulation of the Concentrations of Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1-Alpha and Sirtuin 1 Protein in the Soleus Muscle by Aerobic Exercise Training in Obese Wistar Rats

Keyvan Hejazi [1, *], Seyyed Reza Attarzadeh Hosseini [2], Mehrdad Fathi [2] and Mohammad Mosaferi Ziaaldini [2]

[1]Department of Sport Physiology, Hakim Sabzevari University, Sabzevar, Iran
[2]Department of Sport Physiology, Ferdowsi University of Mashhad, Mashhad, Iran

Corresponding author: Department of Sport Physiology, Hakim Sabzevari University, Sabzevar, Iran. Email: k.hejazi@hsu.ac.ir

Abstract

Background: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1a) and Sirtuin 1 (SIRT1) are significant indicators of obesity and other metabolic disorders.

Objectives: The present study aimed to investigate the regulation of the concentrations of PGC-1a and SIRT1 protein in the soleus muscle by aerobic exercise training in obese Wistar rats.

Methods: This study was conducted on 24 obese male rats, which were randomly categorized into three groups of control, medium-intensity training (28 m/min), and high-intensity training (34 m/min) after obesity induction through a high-fat diet. A series of aerobic trainings in five sessions of 60-minute aerobic training per week was performed for eight weeks. Data analysis was performed using one-way ANOVA to examine the mean differences between the groups. In addition, Tukey's post-hoc test was used for the paired comparisons of the groups.

Results: Significant differences were observed in the concentrations of the PGC-1a ($P < 0.001$) and SIRT1 proteins between the study groups ($P < 0.001$). Tukey's post-hoc test revealed a significant difference between the moderate-intensity aerobic exercise and control groups ($P < 0.01$) regarding their mean concentration of the PGC-1a protein. However, the high- and moderate-intensity groups showed no difference in this regard ($P < 0.028$). Moreover, there was a significant difference in the concentration of the SIRT1 protein between the moderate-intensity aerobic exercise and control groups ($P < 0.02$), and the high-intensity training and control groups ($P < 0.005$).

Conclusions: According to the results, aerobic exercise training could activate SIRT1 and PGC-1a and might enhance mitochondrial biogenesis in the subcutaneous fat. Therefore, aerobic training is recommended as a therapeutic approach to obesity and several other metabolic diseases.

Keywords: Aerobic Training, PGC-1a, SIRT1, Obese Rat

1. Background

Obesity is considered to be a significant health concern across the world, and one of its main consequences is epigenetic changes, which has not been investigated adequately (1). The transformation of the white adipose tissue into the brown adipose tissue is a solution for increased energy consumption and obesity. Contrary to the white dispose tissue, the brown dispose tissue is where heat is produced (2). The white dispose tissue produces heat through expressing unpaired protein-1 (UCP-1) and increasing mitochondrial density (3). Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1a) regulates heat production by inducing UCP-1 expression and the mitochondrial respiratory chain key enzymes (4). Numerous transcription factors are involved in metabolic and physiological adaptations, leading to mitochondrial biogenesis; such examples are transcription factors PGC-1a (5) and Sirtuin 1 (SIRT1) (6). PGC-1a factor plays a key role in increasing energy consumption and mitochondrial biogenesis as a co-activator (7) and is expressed in the brown dispose tissue of the heart, kidneys, skeletal muscles, brain, and other oxidative tissues. The expression of PGC-1a in the

heart and skeletal muscles increases under the impact of physical training ([8]). In addition, PGC-1a regulates numerous genes in the metabolic pathway, including glycogen genesis, glycolysis, and fatty acid oxidation ([9]). The SIRT1 protein residing in the nucleus is among the first genes known to impact the cellular responses to stress ([10]) and fatty acid excursion from the fat cells ([11]) This is also one of the essential nuclear factors contributing to PGC-1a activation (deacetylation). In fact, the increased mitochondrial activity suggests the deacetylase activation of the SIRT1 protein ([12]). SIRT1-increasing stimuli contribute to PGC-1a activation, the increased mitochondrial enzymes attributed to the fat oxidation process, and energy metabolism alternation in the hepatocytes, white adipocytes, and hepatocytes ([13]).

According to the literature, the adaptations achieved through physical training could contribute to the changes in the function and structure of contractile proteins ([14]), mitochondrial function ([15]), metabolic regulation ([16]), intracellular signaling ([17]), and transcriptional responses ([18]). In this regard, regular high-intensity training has been reported to activate the signaling pathway that contributes to the mitochondrial biogenesis in the skeletal muscles ([19]). Suwa et al. ([20]) explored the impact of severe endurance training and both high-intensity and low-intensity training on the expression of the SIRT1 and PGC-1a proteins in the skeletal muscles of rats, reporting that severe endurance training with a treadmill (20 m/min with 18.5% elevation for 45 minutes) could improve the expression of the PGC-1a protein for 18 hours after the training, as well as the expression of the SIRT1 protein in the horseshoe muscles for two hours after the training.

In another study, Gurd et al. ([21]) investigated the impact of three training sessions per week containing 10 sets of training at 90% of the peak oxygen consumption level with two minutes of rest between every two sets for six weeks, discovering that the SIRT1 activity increased along with the mitochondria biogenesis in the skeletal muscles after the training program. In addition, the mentioned study indicated that the SIRT1 levels had no significant changes after six weeks of regular training at the intensity of 90% of the peak oxygen consumption ([21]).

2. Objectives

According to the literature, aerobic training is among the important influential factors in the phenotypic conversion of the white dispose tissue into the brown dispose tissues, while it also plays a pivotal role in weight loss. On the other hand, the association of aerobic exercises with PGC-1a and SIRT1 could influence obesity. Considering the diversity of the findings in this regard, there seems to be

no coherent knowledge of the general impact of aerobic training and trainings with various intensities on PGC-1a and SIRT1. The present study aimed to determine which aerobic training intensities could possibly alter the SIRT1 and PGC-1a levels in obese male Wistar rats. A comparative experiment was also performed to assess the impact of high-intensity and moderate-intensity aerobic training over eight weeks the SIRT1 and PGC-1a proteins levels of the rats.

3. Methods

3.1. Experimental Animals

In the present experimental study, 24 Wistar rats, healthy, male, 14 weeks old, weighing from 250 to 300 grams, and with a body mass index more than 30 g/cm², acquired from Razi Institute of Iran and fed/grown on/with a set diet from Behparvar Company and thus adapted to laboratory research, were used as models. After the week 6, the rats began to receive a calorie-rich diet to gain weight/become obese, leading to them weighing from 250 to 300 grams at the week 14. Their living conditions consisted of polycarbonate cages, and a controlled environment (the average temperature = $23 \pm 1°C$; humidity = $50 \pm 3\%$; and kept in a cycle of light and darkness for 12 hours each). Also, for 14 days, the rats were provided with unlimited water and food, specially made for Wistar rats. After the 14-day period, the rats were separated in three groups, randomly: moderate-intensity aerobic exercise (n = 8); high-intensity aerobic exercise (n = 8); and control (n = 8).

3.2. Induction of Obesity in the Rat

The obese group continued to receive a fat- and calorie-rich diet, containing 4.8 kcal in g energy and 39% fat, in comparison to the standard food, containing 3.9 kcal in g, and 3.5% fat. The rats' unlimited access to water and food continued for another 14 weeks. The normal range of body mass index (BMI) of rats, which is the definitive measure of their obesity, is 0.45 to 0.68 g/mc² ([22]). When the rats finished the obesity phase at 14 weeks of age and before training, their BMI had exceeded the normal range at 0.84 g/cm².

3.3. Familiarization Stage and Exercise Protocol

The rats underwent 60-minute-long high- and medium-intensity aerobic training sessions, lasting eight weeks, five times per each (week). The rat rodent treadmills used for the training were provided by Mobin Company (Iran), with an adjustable 15-15 degrees elevation range and various consecutive trainings with different

speeds, shocks, elevations, and accelerations. First, the rats were accustomed to the program and learned the aerobic training protocol. At the start of the program, they began to walk on the treadmill with no elevation and at the speed of 10 meters/minute. The training became steadily longer and faster in the course of the second and the third weeks. The rats in the medium-intensity group could run on the treadmill with a speed of 28 meters/minute (70% - 75% VO2max), and those in the high-intensity group with a speed of 34 meters/minute (80% - 85% VO_{2max}) (23). For the rats to cool off, the treadmill would stop (inversely to zero) after each session was finished.

3.4. Muscle Tissue Biopsy

The rats were anesthetized using a combination of xylazine (3 - 5 mg/kg of body weight) and ketamine (30 - 50 mg/kg of body weight) 48 hours after the last training session and 12 hours of fasting. After confirming the anesthesia by examining leg retraction, an incision (5 - 6 cm) was made through the abdominal area of the rats, Moreover, the horseshoe muscle was removed quickly, transferred to a microtube (1.5 mL), immersed in liquid nitrogen immediately, and preserved at the temperature of -80°C until examination (24). The tissue samples were initially pulverized using a mortar and pestle and mixed with a radioimmunoprecipitation assay buffer solution in homogenizer tubes for 15 minutes. Following that, the samples were retrieved from the tube using a sampler, poured in microtubes (1.5 mL), and centrifuged at 20,000 rpm and the temperature of 4°C for four minutes. At the next stage, the microtubes were removed from the centrifuge device, drawn from the tubes using a supernatant sampler with the contents poured into another tube, and preserved in a freezer at the temperature of-80°C. The levels of PGC-1a and SIRT1 (intra-assay: CV < 10%) were measured using the special Rat ELISA Kit.

3.5. Statistical Analysis

SPSS version 16 was used for data analysis (significance level = P $\leq$ 0.05). First, the normal distribution of the data was attested by the Shapiro-Wilk test, and the variance homogeneity confirmed by Levene's test. Next, the differences between the three groups were investigated using One-way ANOVA and the paired comparisons between the groups was performed using Tukey's post-hoc test.

4. Results

According to the information in Table 1, eight weeks of moderate-intensity and high-intensity aerobic exercises caused significant differences in the protein concentrations of PGC-1a (F [2,29] = 11.81; P < 0.001) and SIRT1 (F [2,28] = 5.34; P < 0.001] between the three groups. Tukey's post-hoc test revealed that there was a significant difference between the moderate-intensity aerobic exercise and control groups (P < 0.01) regarding their mean concentration of the PGC-1a protein. However, the high- and moderate-intensity groups showed no such difference (P < 0.028). Moreover, the concentration of the SIRT1 protein between the high-intensity training and control groups (P < 0.005), and moderate-intensity aerobic exercise and control groups (P < 0.02), was significantly different, and the high-intensity training and control groups (P < 0.005). However, the high-intensity and moderate-intensity training groups had a significant difference in this regard (P < 0.37).

Table 1. Variation of PGC-1a and SIRT1 Levels in Study Groups[a]

Variable	C	MI	HI
PGC-1a, ng/mL	0.27 ± 0.01	0.31 ± 0.01[b]	0.30 ± 0.008[b]
SIRT1, ng/mL	0.73 ± 0.08	0.82 ± 0.09[b]	0.86 ± 0.11[b]

Abbreviations: C, control; HI, high-intensity aerobic exercise; MII moderate-intensity aerobic exercise.
[a]Data expressed as mean ± standard deviation.
[b]The level of significant at 0.05.

5. Discussion

The present study aimed to regulation of the concentrations of PGC-1a and SIRT1 protein in the soleus muscle by aerobic exercise training in obese Wistar rats. According to the obtained results, the moderate- and high-intensity aerobic exercise program significantly increased the PGC-1a protein concentration in the obese male rats compared to the control group. The results of the present study are consistent with some studies (25, 26) and inconsistent with the results of Alvehus et al. (27) and Ikeda et al. (12). By altering the NADH/NAD ratio, aerobic physical activity could stimulate the SIRT1 activity in rat muscles (12). Furthermore, upstream mechanisms seem to be stimulated by the physical activity, thereby leading to the stimulation of SIRT1 and PGC-1a activity, increased AMPK activity, and activation of the cell surface receptors by epinephrine (26, 28). In this process, SIRT1 interacts physically and functionally with PGC-1a (29). In addition, hormonal stimulation plays a pivotal role in enhancing PGC-1a expression in the visceral and subcutaneous white adipose tissues, so that chronic physical activity would increase the level of thyroid hormone secretion, which in turn may increase PGC-1a expression and stimulate UCP-1 expression (30).

Aerobic activity has been shown to decrease adenosine triphosphate (ATP) levels and increase intracellular calcium levels, thereby triggering the activation of the AMPK and CaMK pathways (31). The activation of these pathways results in the activation of MEF2 transcription and increased synthesis of PGC-1a (32). By regulating the expression of both contractile and enzymatic proteins, the working capacity increases, thereby providing the required energy (33). Among the other potential benefits of aerobic exercises of varying intensities is the stimulation of the upstream of the mechanisms that influence mitochondrial production and diminish the adverse effects of obesity through increasing exothermicity and energy expenditure.

According to the results of the present study, the moderate and high-intensity aerobic exercise programs significantly increased the SIRT1 protein compared to the control group. The results of Huang et al. (34) and Vizvari et al. (35) are similar to the present study. Hence, findings of Marton et al. (36) is inconsistent with present study. Sirtuins regulate fat metabolism and lipogenesis. PPARγ is a nuclear receptor that regulates lipogenesis. SIRT1, along with N-CoR, suppresses PPARγ transcriptional activity, thereby inhibiting lipogenesis (37). Under starvation conditions, the activation of PGC-1a by SIRT1 increases fatty acid oxidation and ketogenesis (38). PGC-1a stimulates fatty acid oxidation enzymes, such as MCAD, CPT1, and PDK-4, acting as a key regulator of metabolic transition to fatty acid oxidation under nutrient depletion conditions. SIRT1 also binds to PPARα and enhances its transcriptional activity along with its coactivator (i.e., PGC-1a), thereby improving fatty acid oxidation (39). LXRS and FXRS are hepatic X receptors and the nuclear receptors that regulate lipid metabolism. LXRS regulates lipid and cholesterol metabolism and increases the transfer of cholesterol from the peripheral tissues to the liver, while FXR decreases serum lipid and glucose levels by regulating glucose, fat, and acidic metabolism. SIRT1 deacetylates and activates this nuclear receptor and improves the metabolic status. The deacetylation of LXRS and FXR by SIRT1 also increases ubiquitinase and its degradation (40). However, the activation of these nuclear receptors by this rapid modernization remains unclear, which also deacetylates SIRT1, SREBP-18, and SREBP-2; these are the transcription factors that increase the expression of cholesterogenic and lipogenic genes for fat storage and are also active in nutrition and satiety, and deacetylation renders them as targets for ubiquitinase, thereby decreasing their activity (41). Through deacetylation and the subsequent activation of LCAD (fatty acid oxidation pathway), SIRT3 increases fatty acid oxidation during starvation (42). Therefore, the substances that activate syringes (especially SIRT1) have the potential to be used in the treatment of

metabolic disorders such as obesity. Furthermore, sirtuin is able to regulate energy metabolism. Physical exercise activates AMPK, which increases oxidative phosphorylation to produce ATP and reduce its consumption by inhibiting anabolic pathways, such as protein synthesis pathways (43).

5.1. Conclusions

According to the results, the PGC-1a and SIRT1 proteins significantly increased in the obese male Wistar rats of the moderate-intensity and high-intensity aerobic training groups compared to the control group. However, therapeutic interventions and exercise activities (e.g., aerobic exercises) are recommended for the activation of PGC-1a and SIRT1 as a treatment for obesity and several other metabolic diseases.

Footnotes

References

1. van Dijk SJ, Tellam RL, Morrison JL, Muhlhausler BS, Molloy PL. Recent developments on the role of epigenetics in obesity and metabolic disease. *Clin Epigenetics*. 2015;**7**:66. doi: 10.1186/s13148-015-0101-5. [PubMed: 27408648]. [PubMed Central: PMC4940755].

2. Payab M, Abedi M, Foroughi Heravani N, Hadavandkhani M, Arabi M, Tayanloo-Beik A, et al. Brown adipose tissue transplantation as a novel alternative to obesity treatment: a systematic review. *Int J Obes (Lond)*. 2020. doi: 10.1038/s41366-020-0616-5. [PubMed: 32499525].

3. Lee DH, Chang SH, Yang DK, Song NJ, Yun UJ, Park KW. Sesamol Increases Ucp1 Expression in White Adipose Tissues and Stimulates Energy Expenditure in High-Fat Diet-Fed Obese Mice. *Nutrients*. 2020;**12**(5). doi: 10.3390/nu12051459. [PubMed: 32443555]. [PubMed Central: PMC7284577].

4. Cereijo R, Giralt M, Villarroya F. Thermogenic brown and beige/brite adipogenesis in humans. *Ann Med*. 2015;**47**(2):169–77. doi: 10.3109/07853890.2014.952328. [PubMed: 25230914].

5. Hood DA. Invited Review: contractile activity-induced mitochondrial biogenesis in skeletal muscle. *J Appl Physiol (1985)*. 2001;**90**(3):1137–57. doi: 10.1152/jappl.2001.90.3.1137. [PubMed: 11181630].

6. Zhao Q, Tian Z, Zhou G, Niu Q, Chen J, Li P, et al. SIRT1-dependent mitochondrial biogenesis supports therapeutic effects of resveratrol against neurodevelopment damage by fluoride. *Theranostics*. 2020;**10**(11):4822–38. doi: 10.7150/thno.42387. [PubMed: 32308752].

7. Lira VA, Benton CR, Yan Z, Bonen A. PGC-1α regulation by exercise training and its influences on muscle function and insulin sensitivity. *Am J Physiol Endocrinolo Metab*. 2010;**299**(2):E145–61. doi: 10.1152/ajpendo.00755.2009. [PubMed: 20371735].

8. Hawley JA. Molecular responses to strength and endurance training: Are they incompatible? *App Physiol Nutr Metab*. 2009;**34**(3):355–61. doi: 10.1139/h09-023. [PubMed: 19448698].

9. Fernandez-Marcos PJ, Auwerx J. Regulation of PGC-1α, a nodal regulator of mitochondrial biogenesis. *Am J Clini Nutr*. 2011;**93**(4):884S–90S. doi: 10.3945/ajcn.110.001917. [PubMed: 21289221].

10. Sin TK, Yung BY, Siu PM. Modulation of SIRT1-Foxo1 Signaling axis by Resveratrol: Implications in Skeletal Muscle Aging and Insulin Resistance. *Cell Physiol Biochem*. 2015;**35**(2):541–52. doi: 10.1159/000369718. [PubMed: 25612477].

11. Gariani K, Menzies KJ, Ryu D, Wegner CJ, Wang X, Ropelle ER, et al. Eliciting the mitochondrial unfolded protein response by nicotinamide adenine dinucleotide repletion reverses fatty liver disease in mice. *Hepatology*. 2016;**63**(4):1190–204. doi: 10.1002/hep.28245. [PubMed: 26404765].

12. Ikeda S, Kawamoto H, Kasaoka K, Hitomi Y, Kizaki T, Sankai Y, et al. Muscle type-specific response of PGC-1? and oxidative enzymes during voluntary wheel running in mouse skeletal muscle. *Acta Physiol*. 2006;**188**(3-4):217–23. doi:10.1111/j.1748-1716.2006.01623.x. [PubMed: 17054661].

13. Um JH, Park SJ, Kang H, Yang S, Foretz M, McBurney MW, et al. AMP-Activated Protein Kinase-Deficient Mice Are Resistant to the Metabolic Effects of Resveratrol. *Diabetes*. 2009;**59**(3):554–63. doi: 10.2337/db09-0482. [PubMed: 19934007].

14. Adams GR, Hather BM, Baldwin KM, Dudley GA. Skeletal muscle myosin heavy chain composition and resistance training. *J Appl Physiol*.1993;**74**(2):911–5. doi:10.1152/jappl.1993.74.2.911. [PubMed: 8458814].

15. Spina RJ, Chi MM, Hopkins MG, Nemeth PM, Lowry OH, Holloszy JO. Mitochondrial enzymes increase in muscle in response to 7-10 days of cycle exercise. *J Appl Physiol*. 1996;**80**(6):2250–4. doi: 10.1152/jappl.1996.80.6.2250. [PubMed: 8806937].

16. Green HJ, Helyar R, Ball-Burnett M, Kowalchuk N, Symon S, Farrance B. Metabolic adaptations to training precede changes in muscle mitochondrial capacity. *J Appl Physiol*. 1992;**72**(2):484–91. doi: 10.1152/jappl.1992.72.2.484. [PubMed: 1559923].

17. Benziane B, Burton TJ, Scanlan B, Galuska D, Canny BJ, Chibalin AV, et al. Divergent cell signaling after short-term intensified endurance training in human skeletal muscle. *Am J Physiol Endocrinolo Metab*. 2008;**295**(6):E1427–38. doi: 10.1152/ajpendo.90428.2008. [PubMed: 18827172].

18. Pilegaard H, Saltin B, Neufer P. Exercise induces transient transcriptional activation of the PGC-1α gene in human skeletal muscle. *J Physiol*. 2003;**546**(3):851–8. doi: 10.1113/jphysiol.2002.034850. [PubMed: 12563009].

19. Gibala MJ, McGee SL, Garnham AP, Howlett KF, Snow RJ, Hargreaves M. Brief intense interval exercise activates AMPK and p38 MAPK signaling and increases the expression of PGC-1α in human skeletal muscle. *J Appl Physiol*. 2009;**106**(3):929–34. doi: 10.1152/japplphysiol.90880.2008. [PubMed: 19112161].

20. Suwa M, Nakano H, Radak Z, Kumagai S. Endurance exercise increases the SIRT1 and peroxisome proliferator-activated receptor γ coactivator-1α protein expressions in rat skeletal muscle. *Metabolism*. 2008;**57**(7):986–98. doi: 10.1016/j.metabol.2008.02.017. [PubMed: 18555842].

21. Gurd BJ, Perry CG, Heigenhauser GJ, Spriet LL, Bonen A. High-intensity interval training increases SIRT1 activity in human skeletal muscle. *Appl Physiol Nutr Metab*. 2010;**35**(3):350–7. doi: 10.1139/H10-030. [PubMed: 20555380].

22. Novelli EL, Diniz YS, Galhardi CM, Ebaid GM, Rodrigues HG, Mani F, et al. Anthropometrical parameters and markers of obesity in rats. *Lab Anim*. 2007;**41**(1):111–9. doi:10.1258/002367707779399518. [PubMed: 17234057].

23. Garekani ET, Mohebbi H, Kraemer RR, Fathi R. Exercise training intensity/volume affects plasma and tissue adiponectin concentrations in the male rat. *Peptides*. 2011;**32**(5):1008–12. doi: 10.1016/j.peptides.2011.01.027. [PubMed: 21291933].

24. Lee S, Farrar RP. Resistance training induces muscle-specific changes in muscle mass and function in rat. *J Exercise Physiol Online*. 2003;**6**(2).

25. Mathai AS, Bonen A, Benton CR, Robinson DL, Graham TE. Rapid exercise-induced changes in PGC-1alpha mRNA and protein in human skeletal muscle. *J Appl Physiol (1985)*. 2008;**105**(4):1098–105. doi: 10.1152/japplphysiol.00847.2007. [PubMed: 18653753].

26. Li L, Pan R, Li R, Niemann B, Aurich AC, Chen Y, et al. Mitochondrial biogenesis and peroxisome proliferator-activated receptor-gamma coactivator-1alpha (PGC-1alpha) deacetylation by physical activity: intact adipocytokine signaling is required. *Diabetes*. 2011;**60**(1):157–67. doi: 10.2337/db10-0331. [PubMed: 20929977]. [PubMed Central: PMC3012167].

27. Alvehus M, Boman N, Soderlund K, Svensson MB, Buren J. Metabolic adaptations in skeletal muscle, adipose tissue, and whole-body oxidative capacity in response to resistance training. *Eur J Appl Physiol*. 2014;**114**(7):1463–71. doi: 10.1007/s00421-014-2879-9. [PubMed: 24711079].

28. Bostrom P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, et al. A PGC1-alpha-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature*. 2012;**481**(7382):463–8. doi: 10.1038/nature10777. [PubMed: 22237023]. [PubMed Central: PMC3522098].

29. Nemoto S, Fergusson MM, Finkel T. SIRT1 functionally interacts with the metabolic regulator and transcriptional coactivator PGC-1{alpha}. *J Biol Chem*. 2005;**280**(16):16456–60. doi: 10.1074/jbc.M501485200. [PubMed: 15716268].

30. Fortunato RS, Ignacio DL, Padron AS, Pecanha R, Marassi MP, Rosenthal D, et al. The effect of acute exercise session on thyroid hormone economy in rats. *J Endocrinol*. 2008;**198**(2):347–53. doi: 10.1677/JOE-08-0174. [PubMed: 18539729].

31. Khalafi M, Mohebbi H, Symonds ME, Karimi P, Akbari A, Tabari E, et al. The Impact of Moderate-Intensity Continuous or High-Intensity Interval Training on Adipogenesis and Browning of Subcutaneous Adipose Tissue in Obese Male Rats. *Nutrients*. 2020;**12**(4). doi: 10.3390/nu12040925. [PubMed: 32230849]. [PubMed Central: PMC7231004].

32. Oka SI, Sabry AD, Cawley KM, Warren JS. Multiple Levels of PGC-1alpha Dysregulation in Heart Failure. *Front Cardiovasc Med*. 2020;**7**:2. doi: 10.3389/fcvm.2020.00002. [PubMed: 32083094]. [PubMed Central: PMC7002390].

33. Czubryt MP, Olson EN. Balancing contractility and energy production: the role of myocyte enhancer factor 2 (MEF2) in cardiac hypertrophy. *Recent Prog Horm Res*. 2004;**59**:105–24. doi: 10.1210/rp.59.1.105. [PubMed: 14749499].

34. Huang CC, Wang T, Tung YT, Lin WT. Effect of Exercise Training on Skeletal Muscle SIRT1 and PGC-1alpha Expression Levels in Rats of Different Age. *Int J Med Sci*. 2016;**13**(4):260–70. doi: 10.7150/ijms.14586. [PubMed: 27076782]. [PubMed Central: PMC4829538].

35. Vizvari E, Farzanegi P, Abbas Zade Sourati H. Effect of Vigorous Aerobic Exercise on Serum Levels of SIRT1, FGF21 and Fetuin A in Women with Type II Diabetes. *Med Lab J*. 2018;**12**(2):1–6. doi:10.29252/mlj.12.2.1.

36. Marton O, Koltai E, Takeda M, Koch LG, Britton SL, Davies KJ, et al. Mitochondrial biogenesis-associated factors underlie the magnitude of response to aerobic endurance training in rats. *Pflugers Arch*. 2015;**467**(4):779–88. doi: 10.1007/s00424-014-1554-7. [PubMed: 24943897]. [PubMed Central: PMC4272336].

37. Picard F, Kurtev M, Chung N, Topark-Ngarm A, Senawong T, Machado De Oliveira R, et al. Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR-gamma. *Nature*. 2004;**429**(6993):771–6. doi: 10.1038/nature02583. [PubMed: 15175761]. [PubMed Central: PMC2820247].

38. Gerhart-Hines Z, Rodgers JT, Bare O, Lerin C, Kim SH, Mostoslavsky R, et al. Metabolic control of muscle mitochondrial function and fatty acid oxidation through SIRT1/PGC-1alpha. *EMBO J*. 2007;**26**(7):1913–23. doi: 10.1038/sj.emboj.7601633. [PubMed: 17347648]. [PubMed Central:

PMC1847661].

39. Jiang H, Horiuchi Y, Hironao KY, Kitakaze T, Yamashita Y, Ashida H. Prevention effect of quercetin and its glycosides on obesity and hyperglycemia through activating AMPKalpha in high-fat diet-fed ICR mice. *J Clin Biochem Nutr.* 2020;**67**(1):74–83. doi: 10.3164/jcbn.20-47. [PubMed: 32801472]. [PubMed Central: PMC7417802].

40. Kemper JK, Xiao Z, Ponugoti B, Miao J, Fang S, Kanamaluru D, et al. FXR acetylation is normally dynamically regulated by p300 and SIRT1 but constitutively elevated in metabolic disease states. *Cell Metab.* 2009;**10**(5):392–404. doi: 10.1016/j.cmet.2009.09.009. [PubMed: 19883617]. [PubMed Central: PMC2785075].

41. Peng CH, Cheng JJ, Yu MH, Chung DJ, Huang CN, Wang CJ. Solanum nigrum polyphenols reduce body weight and body fat by affecting adipocyte and lipid metabolism. *Food Funct.* 2020;**11**(1):483–92. doi: 10.1039/c9fo02240f. [PubMed: 31833514].

42. Hirschey MD, Shimazu T, Goetzman E, Jing E, Schwer B, Lombard DB, et al. SIRT3 regulates mitochondrial fatty-acid oxidation by reversible enzyme deacetylation. *Nature.* 2010;**464**(7285):121–5. doi: 10.1038/nature08778. [PubMed: 20203611]. [PubMed Central: PMC2841477].

43. Lan F, Cacicedo JM, Ruderman N, Ido Y. SIRT1 modulation of the acetylation status, cytosolic localization, and activity of LKB1. Possible role in AMP-activated protein kinase activation. *J Biol Chem.* 2008;**283**(41):27628–35. doi: 10.1074/jbc.M805711200. [PubMed: 18687677]. [PubMed Central: PMC2562073].