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The effect of aerobic training with Citrus aurantium L. on Sirtuin 1 and peroxisome proliferator-activated receptor gamma coactivator 1-alpha gene expression levels in the liver tissue of elderly rats

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Abstract

Background and objective: Proper nutrition and exercise are two effective factors in improving liver function in old age. The aim of this study was to investigate the effect of aerobic training (T) with consumption of Citrus aurantium (CA) on Sirtuin 1 (SIR1) and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) gene expression levels in the liver tissue of elderly rats.

Methods: In this experimental study, 25 elderly female rats were with mean weight of 231.12±22.43 g placed in five groups of 5 rats, including 1) control, 2) sham, 3) CA, 4) T and 5) T+CA. Over the course of eight weeks, groups 4 and 5 ran on the treadmill three sessions per week at 65 to 75 percent of the maximum running speed, and groups 3 and 5 received 300 mg/kg/day of CA extract peritoneally. Forty- eight hours after the last training session and CA consumption, SIR1 and PGC-1α gene expression levels in the liver tissue were measured by real-time PCR method.

Results: T had a significant effect on increasing SIR1 ($P = 0.009$) and PGC-1α ($P = 0.001$) gene expression levels; CA had a significant effect on reducing SIR1 gene expression levels and increasing PGC-1α ($P = 0.001$); T + CA had a greater effect on increasing PGC-1α gene expression levels than T and CA ($P = 0.001$).

Conclusion: Although eight weeks of T and CA consumption alone appear to improve PGC-1α gene expression levels in the liver tissue of elderly rats, concurrent T and CA consumption has more favorable effects than each of them alone.

Keywords: Training, Citrus aurantium, Liver, Metabolism, Elderly

Introduction

Aging is a decrease in body readiness as a result of progressive decline in function and the ability to maintain homeostasis. The ability of tissues to repair and maintain their natural structure and function decreases with age. Therefore, older people are more prone to chronic liver disease (1). The liver is an organ that has a very vital function, however liver function decreases with age (2). The liver regulates energy metabolism through hepatic glucose and fat homeostasis, biosynthesis and steroid destruction, and glucose signaling. Therefore, the liver plays an important role in nutritional interventions in aging and age-related diseases. On the other hand, impaired hepatic metabolism brings about insulin resistance, diabetes mellitus, and non-alcoholic fatty liver disease (1). While the liver is highly resistant to aging, the available evidence suggests that the liver experiences all the symptoms of cellular aging. The aging process in the liver leads to impaired mitochondrial function and diet-sensitive pathways, leading to cellular aging and inflammation. These events lead to several changes in the phenotype of the liver and impair the function of liver cells (3). Aging in liver cells increases the accumulation of fat droplets, reduces the oxidation capacity of mitochondria, and increases the reactive species of oxygen (4). One of the most important changes is the change in FOXO, which is a key factor in aging (5). FOXO is regulated by NAD^+/SIRT1, insulin-like growth factor 1, AMPK, and oxidative stress, all of which are affected by aging. SIRT1 is involved in the regulation of several key proteins related to aging, glucose homeostasis, lipid metabolism, autophagy, inflammation, apoptosis, and cellular changes (6, 7). SIRT1 activity is dependent on NAD^+ and has been shown to play a role in the beneficial effects of CR on longevity (8). Age-related decline in NAD^+ impairs SIRT1 function (7). Inactive activity can accelerate the aging process by lowering certain hormones and increasing free radicals. Therefore, physical activity is very important for maintaining the function of the organs. Research has shown that moderate-intensity physical activity on the treadmill reduces liver fibrosis and can repair damaged liver (9). Lindin *et al.* (2016) stated that moderate-intensity aerobic activity reduces the threshold of non-alcoholic hepatic steatosis and liver fibrosis in rats (10). Research has shown that moderate-intensity exercise can repair damaged liver tissue before it is induced by aging (9). In parallel with the increase in the costs of health care in countries, especially in the field of specific groups, the use of non-pharmacological treatments, including therapies and preventive methods of traditional medicine, is expanding rapidly and has become an industry in societies. The most important of these methods is the use of medicinal plants. Citrus aurantium (CA) has great therapeutic potential. These biological effects include anti-cancer, anti-allergic, anti-fat, antibacterial, antioxidant, detoxifying and anti-diabetic effects. The main oils of this plant are limonene, linalool and β-myersen. Photochemically, CA is rich in p-synephrine, alkaloids, and secondary metabolites that affect health. Due to the reduction of physiological processes due to aging and the effect of aging on liver function, physical activity and CA appear to have a beneficial effect on improving metabolic function and delaying the aging process of the liver in the elderly. Therefore, although the cellular mechanisms of exercise and CA activity have not been well identified, the present study

aimed to investigate the effect of aerobic training (T) with CA on SIR1 and PGC-1α gene expression levels in the liver tissue of elderly rats.

Materials and Methods

In this experimental study, 25 elderly female rats with an average age of 14 months and an average weight of 270- 320 grams were purchased and transferred to the Sports Physiology Laboratory of the Islamic Azad University of Marvdasht Branch. All rats were kept under standard conditions (ith humidity of 45 to 55%, dark-light cycle of 12-12 hours and temperature of 23 ± 2 ° C) for one week to adapt to the new environment, with free access to water and food. The rats were then divided into five groups of 5 rats, including 1) control, 2) sham, 3) CA, 4) T, and 5) T+CA. Over the course of eight weeks, groups 4 and 5 ran on the treadmill three sessions per week at 65 to 75 percent of the maximum running speed, and groups 3 and 5 received 300 mg/kg/day of CA extract peritoneally. Also, group 2 received 300 mg/kg/day of solvent CA extract (normal saline) peritoneally. At the end of the study, 48 hours after the last training session and CA injection, rats were anesthetized with ketamine and xylazine, and after extraction, the liver tissue was placed in a nitrogen tank and sent to the laboratory to measure SIRT1 and PGC-1α gene expression levels. SIRT1 and PGC-1α gene expression levels were measured by real-time PCR. The sequence of SIRT1 and PGC-1α primers with control gene (TBP) is reported in Table 1. Researchers received introduction letters from Marvdasht Branch of Islamic Azad University with ethics code IR.IAU.M.REC.1399.032.

Aerobic training protocol

At first, the graded exercise testing protocol was taken with a slope of zero degrees to estimate the maximum running speed. To perform this test, the rats initially run at a speed of 10 meters per minute and then their speed increased by 1 meter per minute for every 1 minute. This process continued until the rats were not able to run anymore (exhaustion stage). After determining the maximum running speed, the rats first heated for 5 minutes at 50 to 60 percent of the maximum speed on the treadmill, then they performed continuous training at 65% of the maximum speed in the first week; 70% of the maximum speed in the second week; and 75% of the maximum speed from the third week onwards. In the end, the rats cooled for 5 minutes at a maximum speed of 50 to 60%.

Table 1. The sequence of SIRT1 and PGC-1α primers with control gene

Genes	Primer Sequences	Sizes (Bp)
TBP	Forward: 5’-GCGGGGTCATGAAATCCAGT -3’	147
	Reverse: 5’-AGTGATGTGGGGACAAAACGA -3’	
SIRT1	Forward: 5’-TCCTGTGGGATACCTGACTT-3’	300
	Reverse: 5’-AAAGGAACCATGACACTGAATGA -3’	
PGC1a	Forward: 5’-CAGAAGCAGAAAGCAATTGAAGA -3’	230
	Reverse: 5’-GTTTCATTCGACCTGCGTAAAG -3’	

CA preparation

To prepare the CA extract, 50 g of the powdered sample of the plant was first weighed by a digital scale and added to a balloon containing 500 ml of distilled water connected to a clevenger apparatus. Extraction was performed for 4 hours and the collected essential oil was dehydrated by waterless sodium sulfate, and the prepared essential oil was stored in a freezer at -20˚ C until use.

Data analysis procedure

The Shapiro-Wilk test was used to evaluate the normal distribution of the findings and one-way ANOVA along with Tukey's *post-hoc* test were used to analyze the findings (P <0.05).

Results

The SIRT1 and PGC-1α gene expression levels are presented in Figures 1 and 2, respectively. The results of one-way analysis of variance showed a significant difference in SIRT1 and PGC-1α gene expression levels in the five research groups (P = 0.001).

The results of Tukey's post- hoc test showed that although there was no significant difference in SIRT1 (P = 0.99) and PGC-1α (P = 0.99) gene expression levels between control and sham groups, SIRT1 gene expression levels in the T group were significantly higher than the control (P = 0.009), CA (P = 0.001) and T + CA (P = 0.001) groups. The levels were also significantly lower in the CA group than the control group (p=0.001) (Figure 1). PGC-1α gene expression levels in the CA, T, and T + CA groups were significantly higher than the control group (P = 0.001); in the CA and T + CA groups, the levels significantly higher than the T group (P = 0.001), and in the T + CA group, the levels were significantly higher than the CA group (Figure 2).

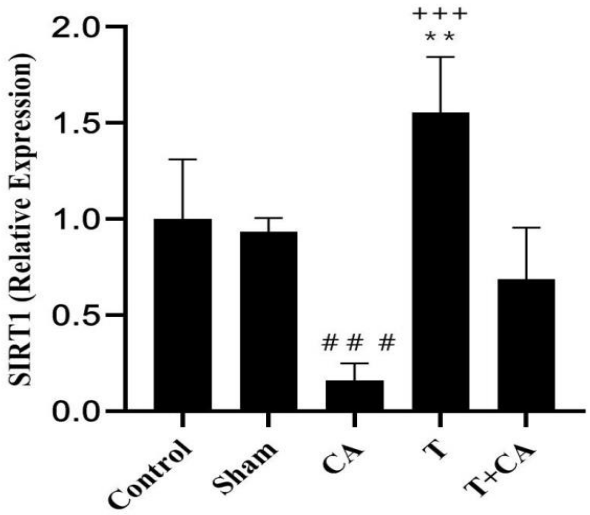

Figure 1. SIRT1 gene expression levels in the five groups of study
P≤0.001 Significant decrease compared to the control group
** P≤0.01 Significant increase compared to the control group
+ + + P≤0.001 Significant increase compared to the CA and T+CA groups
CA: Citrus aurantium and T: training

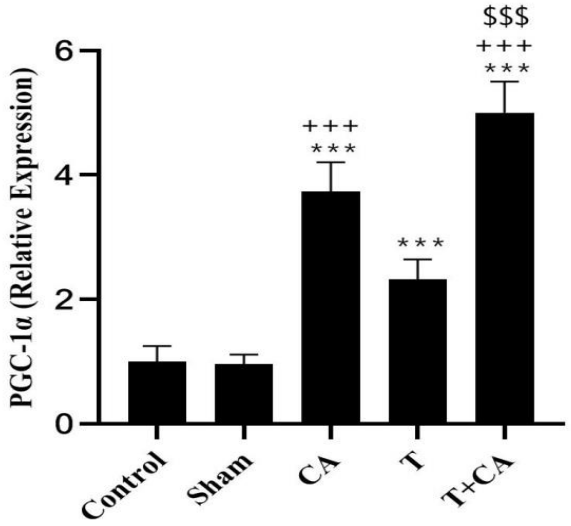

Figure 2. PGC-1α gene expression levels in the five groups of study
*** P≤0.001 Significant increase compared to the control group
+ + + P≤0.001 Significant increase compared to the T group
$$$ P≤0.001 Significant increase compared to the CA group
CA: Citrus aurantium and T: training

Discussion

The results of the present study showed that T significantly increased SIRT1 and PGC-1α gene expression levels in the liver tissue of elderly rats. Aging is associated with a gradual decline in cell biochemical functions such as oxidative phosphorylation and mitochondrial respiration chain. Decreased mitochondrial function leads to increased oxidative stress and leads to aging and modulation at the level of life-enhancing proteins such as SIRT1 (13). In mammals, three members of the sirtoin family (SIRT1, SIRT3, and SIRT6) are involved in aging (14). Excessive expression of SIRT1 in mammals increases life expectancy as well as health in old age (14). Studies show that these epigenetic changes are very dynamic and are influenced by a variety of biological factors, including aging, nutrition, and exercise. In addition, the regulation of SIRT1 by aerobic exercise regulates PGC-1α, p53, and NF-$_k$B tumor suppressors, as well as other transcription factors through diastylase activity (15). Exercise has been shown to reduce oxidative damage to DNA and alter mitochondrial function due to aging by regulating PGC-1 and SIRT1 (16). The results of the present study are consistent with previous studies that have shown that regular exercise increases the expression of SIRT1 and other cytotoxins in many tissues, thereby preventing metabolic diseases or age-related disorders (17). In addition, Van Liu *et al.* (2019) stated that aerobic exercise increases the expression of SIRT1 in the liver and kidneys of diabetic mice and reduces inflammation and metabolic disorders (18). Another result of the present study was an increase in PGC-1α expression in liver tissue following T. Ziegler *et al.* (2019) showed in a study that both aerobic and resistance training increase the expression of PGC-1α in the

visceral adipose tissue of rats (19). Endurance training using calcium- and phosphate-dependent pathways activates adenosine monophosphate and Calmodulin-dependent kinase enzymes, resulting in activation of PGC-1α. Aerobic activity also reduces intracellular energy charge and subsequently activates AMPK and activates intracellular PGC-1α to increase the expression of genes involved in mitochondrial biogenesis and even increase PGC-1α (20). However, a study showed that voluntary exercise did not have a significant effect on the protein content of PGC-1α slow-twitch muscle and liver function (21). Perhaps the difference in the training protocol and the animal model under study has led to differences in results.

In the present study, an increase in SIRT1 expression was associated with increased liver PGC-1α in older rats. Activation of SIRT1 leads to diastylation and activation of PGC-1α, followed by mitochondrial biogenesis. Increased expression of PGC-1α has been shown to facilitate mitochondrial biogenesis and enhance oxidative phosphorylation in the muscle, heart, and fat tissues (22). The expression of PGC-1α is also essential to the antioxidant effects of exercise against cell damage caused by aging in the mitochondria and cellular apoptosis (23). Therefore, SIRT1 is an important mediator in the regeneration of cellular metabolism and suppresses inflammatory signals. SIRT1 is also involved in controlling hepatic fat metabolism, modulating hepatic oxidative stress, and protecting against hepatic oxidative stress due to HFD, glucose intolerance, and hepatic steatosis (24). Therefore, treatment strategies to increase SIRT1 levels could potentially protect liver function.

Another result of this study was a significant reduction in liver SIRT1 expression following CA consumption. Flavonones are the main flavonoids in CA. The most abundant free flavonones identified in VA are hesperin and naringin (25). Contrary to the findings of the present study, Shokri Afra *et al.* (2019) showed that hesperin and resveratrol increased the amount and activity of SIRT1 protein as well as increased phosphorylation of AMPK, although the effect of hesperin was greater than resveratrol (26). Another clinical study has shown that hesperin has antitrogenic effects, regulates fat metabolism, and improves metabolic disorders (27). The effects of CA appear to be mediated by modification in SIRT1. However, the results of the present study showed that CA reduced the expression of SIRT1 in the liver tissue. Perhaps the type of tissue examined or the age of the rats made a difference in the results, which requires further investigation. Studies also show that protein levels and SIRT1 expression are not necessarily the same (28). Therefore, CA may stimulate SIRT1 activity and protein, although it does not affect SIRT1 expression. In the present study, the expression of liver PGC-1α increased significantly after CA consumption and a combination of aerobic training and CA. The results of a study showed that the glycosidase flavonoid in citrus fruits can activate the AMPK-SIRT1-PGC-1α pathway and thus play a role in controlling fat metabolism (29). Naringin has also been shown to increase PGC-1α, CPT1 and adiponectin expression (30). It appears that the naringin present in CA can increase the expression of PGC-1α and PGC-1β by increasing thermogenesis by UCP1 (30).

In the present study, the simultaneous effects of T and CA on PGC-1α were greater than each one alone. Perhaps the two together have synergistic effects and could play a better role in improving the metabolic liver disorder caused by aging. One of the limitations of the

present study is the inability to measure SIRT1 and PGC-1α protein levels by ELISA and Western blot methods, so it is recommended that future studies should examine the effect of T with different intensities and CA consumption with different doses on SIRT1 and PGC-1a protein levels.

Conclusion

Although eight weeks of T and CA consumption alone appear to improve PGC-1α gene expression levels in the liver tissue of elderly rats, simultaneous T and CA consumption has more favorable effects than each one alone.

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