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Original Article

The combined effect of eight-week resveratrol supplementation and interval training on some regulatory and executive factors of hepatocyte apoptosis in male rats with diabetes

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Abstract

Background and aims: The aim of this study was to investigate the combined effect of interval training and resveratrol consumption on the regulatory and executive factors of hepatocyte apoptosis in male rats with diabetes.

Methods: Frothy-two male Wistar rats were randomly divided into healthy control, diabetic, saline diabetic, resveratrol supplement, intermittent exercise, and resveratrol + intermittent exercise groups. The interval training program consisted of 8 weeks of training with an intensity of 50%-70% of the maximum oxygen consumption. Resveratrol was injected in two training groups with resveratrol supplement and resveratrol + supplement at a dose of 20 mg.

Results: The concentrations of caspase-3 and BAX in diabetic saline and diabetic groups were significantly higher than in control groups ($P=0.001$). On the other hand, Bcl-2 (B-cell lymphoma 2) levels in diabetic saline and diabetic were significantly lower than in the control groups ($P=0.001$). The concentration of the BAX/Bcl-2 ratio in diabetic and diabetic saline was significantly higher compared to control groups ($P=0.001$) The effect of interval training, administration of resveratrol alone, or especially with interval training caused a significant decrease in the concentration of BAX and caspase-3 ($P=0.001$), a significant increase in the mean Bcl-2 concentration ($P=0.001$), and a significant decrease in the mean BAX/Bcl-2 ratio ($P=0.001$) in male rats with diabetes compared with the diabetic and diabetic groups.

Conclusion: The combined effect of eight-week resveratrol supplementation and interval training decreased apoptosis markers, while it increased the concentration of Bcl-2 in male rats with type 2 diabetes.

Keywords: Diabetes, Interval training, Resveratrol, Apoptosis

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Introduction

The latest estimate by the World Health Organization shows that the number of diabetics in the world will increase to 300 million in 2025. More than 10% of the adult population are diabetic in Iran, and half of them are still unaware of their diabetes (1). This disease is characterized by three pathophysiological abnormalities, including impaired insulin secretion, environmental resistance to insulin, and excessive production of glucose by the liver (2). Previous evidence exists regarding investigating the relationship between the complications of diabetes and the increase in oxidative stress (OS) reactions in the body. OS can cause tissue damage through the peroxidation of lipids, proteins, and the activation of pathways that lead to apoptosis (3). It further activates some transcription factors and cell signaling pathways, including nuclear factor kappa B (NF-κB) and mitogen-activated protein kinases (MAPK) which are involved in and can activate cell death processes such as autophagy and apoptosis (4). The lipid breakdown product (4-HNE) has also been reported to induce apoptosis (5).

Apoptosis is the programmed cell death and generally causes the activation of a special group of aspartate-dependent proteases called caspases, especially caspase-3, from two pathways. The mentioned pathways are the extrinsic pathway, which is activated by the stimulation of membrane death receptors, and the intrinsic or mitochondrial pathway, which is regulated by Bcl-2 (B-cell lymphoma 2) family members. There are death receptors in the plasma membrane of most cells. Death receptors are members of the tumor necrosis factor (TNF) receptor superfamily. When these receptors are stimulated by the respective ligands, caspases are activated in such a way that procaspase-8 becomes active caspase-8, which activates executive caspases (caspase-3, 6, and 7), leading to apoptosis. However, in the internal pathway, mitochondrial membrane permeability to cytochrome C increases with the relative change of pro-apoptotic (BAX)

and anti-apoptotic (Bcl-2) mediators. The apoptosome is formed with the release of cytochrome C, activating caspase (6). As a control point between the cell surface and internal signals for the formation of apoptosis and activation of the caspase cascade, the proteins of the Bcl-2 family play an important role and are divided into anti-apoptotic proteins or inhibitors and pre-apoptotic proteins or promoters It has been found that Bcl-2 itself does not perform the antioxidant activity, but it may have an indirect effect on increasing the antioxidant activity inside the cell. Therefore, the increase in Bcl-2 protein allows cells to better deal with free radicals, and this is obtained as a result of increasing the activity of antioxidant defense enzymes (7). In general, diabetes can lead to apoptosis through increasing OS. In this regard, France´s et al reported hyperglycemia and diabetes induction in rats through increasing OS causes apoptosis in the liver (8). In this process, the role of caspase-3 and Bcl-2 is highly important. Caspase-3 promotes apoptosis, but Bcl-2 protects the body in the process of apoptosis.

Accordingly, compounds that can inhibit free radicals in cells are of great importance. Botanical studies demonstrate that resveratrol, having important anti-inflammatory and antioxidant elements such as flavonoids and saponins, plays an effective role in inhibiting inflammatory reactions; from this point of view, it may be effective in inhibiting apoptosis or cell destruction (9). In this regard, Sia et al found the positive effect of resveratrol administration on reducing the apoptosis of cardiomyocyte cells (10). Jiang et al also indicated that resveratrol inhibits interleukin 1 beta-mediated nucleus pulposus cell apoptosis by regulating the PI3K/Akt pathway (11). Zhu et al also reported that resveratrol reduces OS and apoptosis in the lung tissue of newborn mice (12).

Exercise is also one of the main methods of treating type 2 diabetes by reducing insulin resistance and stimulating insulin secretion. Moreover, Tanoorsaz et al represented that aerobic exercise reduces cardiac apoptosis in diabetic patients (13). Matinfar et al concluded that exercise reduces brain OS in diabetic rats (14). Additionally, Tofighi et al showed that the combined use of resveratrol and aerobic exercise protects the heart against the production of OS after ischemia through the activation of antioxidant defenses (15). Some studies revealed that interval exercise significantly improves insulin sensitivity, increases nitric oxide availability, and improves lipid metabolism (16) compared to continuous exercise. Carvalho et al also reported that interval exercise inhibits the apoptotic signaling pathway in healthy rat myocardium (17).

However, no clear results have been provided regarding the effects of interval exercise training and resveratrol supplementation on liver damage and apoptosis caused by diabetes. Therefore, the present study sought to investigate the long-term effect of interval exercise on the regulatory markers of the internal pathways of apoptosis, including BAX and Bcl-2. The ratio of BAX to Bcl-2 and caspase-3 as the most effective factor of the caspase family was determined in the hepatocyte tissue of diabetic male rats treated with streptozotocin (STZ).

Materials and Methods

Subjects

In this research, the samples of the current research were laboratory mice. Considering that the subjects were under control in terms of many variables in the laboratory, the current research is of an experimental type.

The statistical sample of the research project included male Wistar rats of about 2 years old with an average weight of 250-300 g. These animals were selected from the laboratory animal breeding and maintenance center of Islamic Azad University, Sari branch. They were familiarized with the exercise protocol and divided into 6 groups (7 heads in each group), including healthy control, diabetic, saline diabetic (placebo), resveratrol supplemented groups, interval exercise, and resveratrol supplement + interval exercise. After the animals were transferred to the laboratory, they were placed in polycarbonate cages (4 mice per cage) with a temperature of 22 ± 2°C, a humidity of 55 ± 5%, and a 12:12 light-dark cycle with proper ventilation. In all stages of the research, the animals had free access to food and water in a 500 mL special bottle suitable for laboratory animals.

In this project, diabetes was induced by injecting a single dose of STZ 50 mL/kg intraperitoneally, and blood sugar above 250 mg/dL 48 hours after the injection was considered induced diabetes (18).

Exercise training

The animals were familiarized with the treadmill and how to run on it for 10 minutes every day for a week. Forty-eight hours after the last familiarization session, the maximum speed of the rats was tested, and the maximum oxygen consumption was predicted using the maximum speed during the delay time. To determine the maximum rate of oxygen consumption, the standard increase test of Bedford et al (19) was used, which was standardized for Wistar rats by Leandro et al (20). The test consisted of 10 stages of three minutes. In the first stage, the speed of 0.3 km/hour was added to the speed of the turntable: the same speed was also applied for the next stages. Considering Leandro et al, five regenerative test methods were introduced to determine the maximum oxygen consumption, which has different slopes. In this research, zero slopes were used to measure the maximum speed of oxygen consumption and the speed achieved in the last stage in such a way that the animal could not run. The maximum running speed of the animal was used for running (20).

The interval training program consisted of 8 weeks, 5 sessions per week, and each session consisted of 10 sets of 1-minute activity with an intensity of 50-70% of the maximum oxygen consumption and 2 minutes of rest (active) between sets, which started with 14 meters per

minute in the first week. Each week, the speed increased by 2 meters per minute until it reached 28 meters per minute in the eighth week (21,22). The active rest consisted of low-intensity exercise of about 4 meters per minute. Exercises were performed on a 5-lane rodent treadmill. At the beginning of the movement line, there was an electric shocker that was used when the mice did not want to move. Of course, this shocker was applied as little as possible. The exercises were performed under the supervision of a sports physiology specialist (Table 1).

Resveratrol supplement

A resveratrol supplement (Swanson Resveratrol 500 mg) was prepared based on previous studies. For each administration of resveratrol, 100 microliters of 7% ethanol or 10% DMSO with water were prepared for each rat, and resveratrol was suspended in it and administered to reduce the percentage. The solution was prepared for all subjects at once. In the resveratrol supplement and resveratrol + interval training groups, they were injected intraperitoneally (3 hours before training) with a dose of 20 mg per kilogram of body weight. This process was performed for 8 weeks (23). To make sure that all rats received the same amount of resveratrol, it was injected intraperitoneally.

Laboratory

After conducting the research, all animals were subjected to completely similar conditions. After the study, all animals were placed in exactly the same conditions and after 12 to 14 hours of fasting and 48 hours after the last training session and supplementation, with an intraperitoneal injection of ketamine (60 mg base per kg) and Xylosin (5 mg base per kg body weight) were anesthetized at a ratio of 5:2. Then their liver tissue was separated from the umbilical region and sent to the laboratory and kept at -80°C. Bcl-2 and BAX levels were determined using special commercial kits manufactured by Zelbio, Germany, respectively, with a sensitivity of less than 0.078 ng/mL and 15.6 pg/mL by the ELISA method (23). Furthermore, the activity measurement kit manufactured by Zelbio, Germany was used to quantify the activity of caspase-3 (24). To extract the desired variables, first, the liver tissue was powdered by placing it in liquid nitrogen, and then 1 mL of buffer containing anti-protease was added to the samples with the help of a refrigerated centrifuge for 10 minutes at 4000 revolutions per minute. The samples was separated, and then its value was checked by the Bradford measurement method and then the values using commercial kits. The ratio of BAX to Bcl-2 was also obtained by dividing it into two numbers.

Statistical method

The results were presented as the mean ± standard deviation. Considering the normality of data distribution (through the Shapiro-Wilk test), the independence of the groups, and the equality of variances (using Levene's test), the data were analyzed using one-way analysis of variance and, if necessary, Tukey's post hoc test. The considered significance level was 0.05, and all statistical analyses were performed by SPSS software, 20 version.

Results

The mean and standard deviation of the regulatory and executive factors of hepatocyte apoptosis in different groups are provided in Table 2.

The average concentration of BAX and caspase-3 in diabetic saline and diabetic patient groups was significantly higher compared to the control group ($P=0.001$). The effect of interval exercise, the administration of resveratrol alone, or especially with interval exercise could significantly decrease the average concentration of BAX and caspase-3 ($P=0.001$) in male rats with diabetes compared to the diabetic and saline diabetic groups (Figures 1 and 2).

A comparison of the Bcl-2 concentration among different groups also represented a significant difference ($P=0.001$). The concentration of Bcl-2 in diabetic and saline diabetic patient groups was significantly lower compared to the control group ($P=0.001$). The effect of interval exercise, the administration of resveratrol alone, or especially with interval exercise caused a significant increase in the average concentration of Bcl-2 ($P=0.001$) in male rats with diabetes in comparison to the diabetic and saline diabetic groups (Figure 3).

A significant difference was observed in the average concentration of the BAX/Bcl-2 ratio among the groups ($P=0.001$). The average concentration of the BAX/Bcl-2 ratio in the diabetic and saline diabetic patient groups was significantly higher compared to other groups ($P=0.001$). The effect of interval exercise, the administration of resveratrol alone, or especially with interval exercise caused a significant decrease in the average ratio of BAX/Bcl-2 ($P=0.001$) in male rats with diabetes compared to the diabetic and saline diabetic groups (Figure 4).

Discussion

This research evaluated the effect of interval exercise and resveratrol supplementation alone and the interactive effect of exercise and resveratrol supplementation on

Table 1. Exercise Training Protocol

Times	1 Week	2 Weeks	3 Weeks	4 Weeks	5 Weeks	6 Weeks	7 Weeks	8 Weeks
Speed (m/min)	14	16	18	20	22	24	26	28
Time (min)	30	30	30	30	30	30	30	30

Note. 10 one-minute sets and 2 minutes of active rest between sets.

Table 2. The mean and standard deviation of regulatory and executive factors of hepatocyte apoptosis

Variable	Control	Diabetic	Saline diabetic	Supplement	Training	Supplement+Training	*P* value
Caspase-3	1.2±0.27	2.35±0.36	2.29±0.32	1.69±0.35	1.69±0.27	1.44±0.35	0.001
BAX	9.52±1.28	20.31±3.18	21.87±9.72	14.1±2.64	14.32±2.22	12.45±2.12	0.001
Bcl-2	4.82±1.12	2.32±0.38	2.42±0.47	3.61±0.54	3.78±0.48	4.09±0.65	0.001
BAX/Bcl-2	1.38±0.4	5±0.7	6±0.8	2.66±0.6	2.39±0.5	2±0.7	0.001

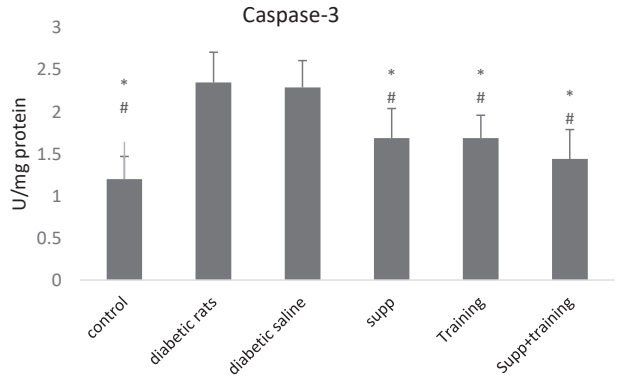

Figure 1. The concentration of hepatocyte caspase-3 in the studied groups. *Note*. * Significance compared to diabetic rats; #: Significance in comparison to diabetic saline

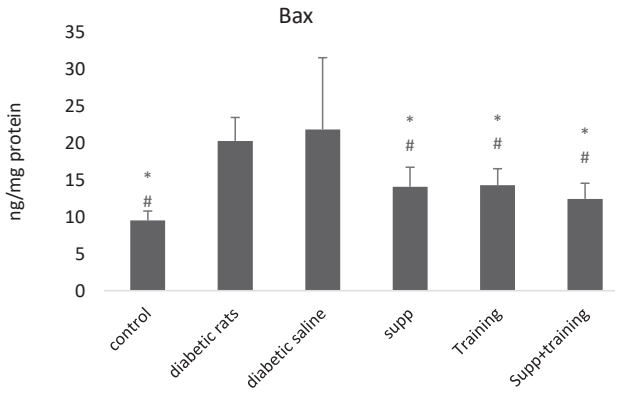

Figure 2. The concentration of hepatocyte BAX in the studied groups. *Note*. *Significance in comparison to diabetic rats; #Significance compared to diabetic saline

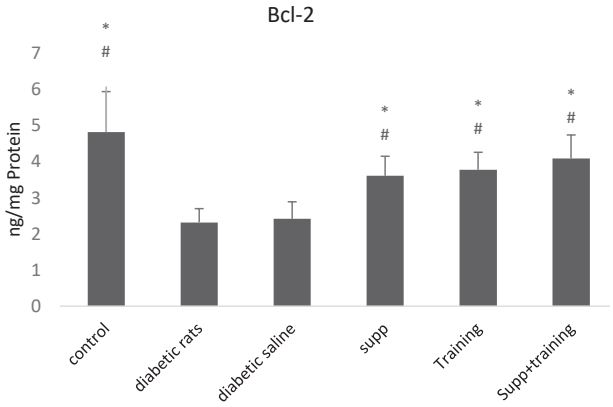

Figure 3. The Concentration of Hepatocyte Bcl-2 in the Studied Groups. *Note*. *Significance compared to diabetic rats; # Significance in comparison to diabetic saline

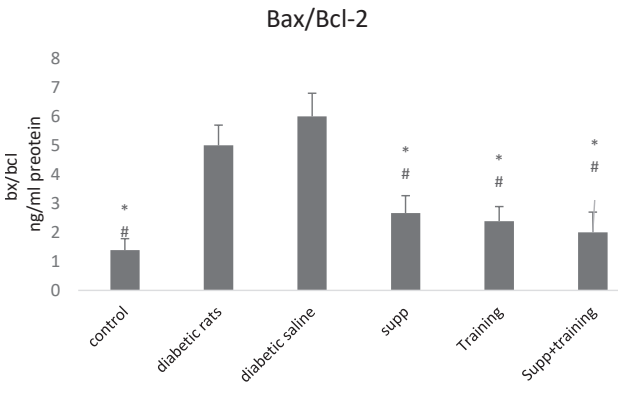

Figure 4. Hepatocyte BAX/Bcl-2 Ratio in the Studied Groups. *Note*. *Significance in comparison to diabetic rats; #: Significance compared to diabetic saline

changes in the regulatory and executive biomarkers of hepatocyte tissue apoptosis in diabetic male rats. The results of the research showed that the induction of diabetes was associated with a significant increase in the concentration of BAX, caspase-3, and the concentration of the BAX/Bcl-2 ratio while a significant decrease in Bcl-2 in the diabetic control group. In line with these findings, He et al (25) and Mohamed et al (26) also reported the induction of apoptosis due to diabetes. An increase in OS and a decrease in the level of antioxidants are probably among the mechanisms of the effect of diabetes on the hepatocyte tissue, which can be associated with apoptosis or death of hepatocyte cells. Although the exact mechanism of this process is not well known, it is likely that the increase in the level of free radicals that occurs in diabetic conditions leads to the damage of intracellular organelles, including the mitochondrial membrane, and thus the activation of the apoptosis pathway (27). Considering that mitochondria are considered energy production factories, as well as the controlled amounts of intracellular free radicals, mitochondrial membrane damage leads to the release of apoptotic signals, including cytochrome C and the uncontrolled production of reactive oxygen species (ROS) along the chain. Electrons are transferred, which is ultimately associated with more severe cell damage and apoptosis or cell death (28-30). Apoptotic activity is regulated by some proteins that play an important role in its control. For example, an increase in the ratio of BAX to Bcl-2 indicates an increase in apoptosis or cell death, while a decrease in the ratio of BAX to Bcl-2 by the inhibition of cytochrome C and inactivation of caspase-3 causes a decrease in apoptosis or cell death. Therefore,

the increase in BAX and the decrease in Bcl-2, along with the increase in the ratio of BAX to Bcl-2 in the hepatocyte tissue of diabetic mice observed in our study can indicate an increase in the rate of apoptosis or death of hepatocyte cells in these animals. The results of this research are consistent with those of Farzanegi et al (31,32).

Likewise, in the current research, eight weeks of interval training and resveratrol supplementation alone caused a significant decrease in the concentration of BAX, caspase-3, and the concentration of BAX/Bcl-2 ratio while a significant increase in the concentration of Bcl-2, indicating that exercise and resveratrol supplementation alone can have anti-apoptotic effects. Chen et al also found that the chronic exercise of running on a treadmill (30 or 60 minutes per session and 3 sessions per week) led to a decrease in the expression of BAX, an increase in the expression of Bcl-2, and a decrease in the concentration of BAX/Bcl-2 ratio. In rats suffering from chronic kidney disease, it was accompanied by a decrease in the activity of initiator caspase-9 and executive caspase-3, which could prevent apoptosis and DNA fragmentation from both internal and external pathways (33). In addition, Kwak et al stated that exercise activity reduces the increased levels of BAX/Bcl-2 in the cardiac tissue of old rats by decreasing BAX protein expression and increasing cardiac Bcl-2 levels (34), confirming the supportive role of sports activity by reducing the process of apoptosis in body tissues, which corroborates to the results of this study. In another study, Mehri et al indicated that exercise and resveratrol alone caused a significant decrease in the average concentration of BAX and caspase-3 whereas a significant increase in the concentration of Bcl-2 in the cardiomyocyte tissue (35). Salehi et al also reported a significant increase in Bcl-2 levels while a significant decrease in cardiac BAX levels with the effect of various periodic and continuous exercises on some indices of cardiac tissue apoptosis in diabetic rats (36), which is consistent with our results. Moradi et al also concluded that interval training decreased BAX while increasing Bcl-2 in the skeletal muscle of diabetic rats fed a fatty diet (37).

The capacity to prevent the formation of ROS can be one of the possible mechanisms in the field of the protective ability of sports training. Free radicals are produced in the electron transport chain, but they can lead to cell death when their levels exceed the body's antioxidant capacity. OS caused by ROS is strongly associated with diabetes and its complications and can initiate cell death through various pathways (38). Resveratrol supplement is a plant compound belonging to the group of polyphenols and exerts part of its anti-apoptotic effects through antioxidant properties and apoptosis regulatory factors in the liver tissue of diabetic rats (39).

Although the administration of resveratrol and exercise alone could affect factors influencing apoptosis, their interactive effect led to better results. In this research, the interaction of interval training and resveratrol caused a significant decrease in the concentration of BAX, caspase-3, and the concentration of BAX/Bcl-2 ratio whereas a significant increase in the concentration of Bcl-2. Hajighasem et al also demonstrated that the administration of resveratrol, especially in combination with exercise, is associated with a significant increase in Bcl-2 protein and a significant decrease in BAX protein. Moreover, Farzanegi et al investigated the effect of resveratrol combined treatment and interval and continuous exercises on the level of the apoptotic biomarkers of the heart tissue in male rats with non-alcoholic fatty liver disease. Decreased BAX levels, BAX/Bcl-2 ratio concentration, and increased Bcl concentration were observed as a result of exercise and administration of resveratrol alone and as a combination of resveratrol supplement with exercise (40), all of which are consistent with the results of this research for the use of resveratrol as an anti-apoptotic substance. It seems that exercise and resveratrol together increase the mitochondrial level of an important antioxidant enzyme such as superoxide dismutase 2 (15). Interval training and resveratrol can reduce OS caused by apoptosis in special tissues, the effect of which is more prominent in cardiac muscles. At the same time, as the OS increases, BAX protein is transferred, and placement in the mitochondrial outer membrane represents an increase (41). This discussion can partly depend on the activation of cytosolic c-Jun N-terminal kinase (JNK); thus, JNK inhibits Bcl-2 protein by being phosphorylated by cellular stress stimuli. Inside the mitochondria, JNK increases the permeability of the mitochondrial membrane, causing the release of pro-apoptotic factors such as the apoptotic-inducing factor and cytochrome C, thereby triggering the caspase cascade (41,42). Another way to inhibit apoptosis and increase cell lifespan is the relationship between exercise and resveratrol, as well as silent information regulator 1 proteins, which increase during exercise. These proteins cause the activation of downstream proteins, including peroxisome proliferator-activated receptor-gamma coactivator 1-alpha, which subsequently improves mitochondrial function and prevents apoptosis (41). Additionally, interval training and resveratrol can increase the expression of mitochondrial adenosine triphosphate-sensitive potassium channels. This process occurs, along with an increase in the other mitochondrial proteins that help protect the heart (43). On the other hand, inflammatory factors are also important. TNF-α can promote apoptosis by increasing caspase (44). However, the anti-inflammatory effects of exercise and resveratrol on reducing TNF-α have been reported as well (43). Therefore, interval exercise and resveratrol together can reduce apoptosis by reducing TNF-α.

Conclusion

The combined effect of eight-week resveratrol supplementation and interval training caused a significant decrease in the concentration of BAX, caspase-3, and the concentration of BAX/Bcl-2 ratio, while a significant

increase in the concentration of Bcl-2 in type 2 diabetes rats.

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Conflict of Interests

The authors declare that there is no conflict of interests.

Ethical Approval

All the experiments were conducted in accordance with the customary policy of Iranians for the protection of vertebrate animals and experimental scientific purposes, and it was approved by the Ethics Committee of Payam Noor University, Sari (with ethics ID IR.PNU.REC.1400.059).

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