The Effect of Eight-week Caffeine Supplementation and High-Intensity Interval Training on the Serum Urea and Creatinine Levels and Morphological Changes of Glomerular Unit in Diabetic Rats

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Abstract

Background: Glomerulonephritis is the one of disorders associated with diabetes. The present study was conducted to investigate the effect of 8-week high-intensity interval training (HIIT) and caffeine supplementation on the levels of serum creatinine (Cr) and blood urea nitrogen (BUN) and morphological changes of kidney tissue in diabetic rats.

Methods: 50 diabetic rats as streptozotocin (STZ)-induced diabetic rats were randomly assigned to 5 equal groups including control, diabetic, diabetic + caffeine, diabetic + training, and diabetic + training + caffeine. The groups were given supplements (intraperitoneal injection of 70 mg/kg of caffeine powder for five days in each week) and underwent training (5 sessions including 6 to 12 times of 2-minutes training at the rate of 85-90% of maximal speed each week) for 8 weeks. The serum Cr and BUN levels and the morphological changes of kidney tissue after 48 hours of the last training session were measured. Statistical analysis was performed by one- and two-way ANOVA (P<0.001).

Results: It was revealed that HIIT reduced the levels of Cr (P=0.001) and BUN (P=0.013), as well as the urinary tract area (P=0.015) in diabetic rats. Although caffeine supplementation significantly reduced glomerular area (P=0.001) but increased BUN level (P=0.001) and Bowman's capsule (P=0.001) in them. There was a significant interaction between treatments regarding BUN level and urinary tract area changes (P=0.001, P=0.014, respectively).

Conclusion: HIIT and caffeine supplementation had significant effects on the serum Cr and BUN levels and morphological changes in rats' kidney tissues, but given doses and time of consumption of caffeine, as well as duration and intensity of training were more effective than the other indicators.

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Introduction

Diabetes has been known as one of the main threats to human health which can disrupt organs and different functions of the body (1). Diabetes is the main cause of chronic kidney failure and is associated with some notable disorders such as kidney failure and glomerulonephritis (2). Biochemical

markers play important roles in precise diagnosis, risk evaluation, and treatment adoption for improving the clinical outcomes (3). Nitrogenous compounds, such as ammonia, are from protein metabolites which are not used in the body and are excreted in the form of urea, and creatine transforms to creatinine in physiological conditions and will be excreted as a useless product through kidney (4). Generally, the increased serum levels of these substances demonstrate their decreased refining and kidneys inability in excreting the substances from blood which can be caused by kidney failure, accordingly, glomerular filtration rate (GFR) is known as the best and efficient the index of renal function (5). Disease diagnosis is confirmed by the presence of urea in urine and erythrocytes, and creatinine refinement level is mostly the best indicator for measuring glomerular filtration in patients (6).

Inactivity is the main cause of emerging chronic diseases, and exercises are the most standard treatments for patients with type 2 diabetes (7). Regular exercises may help preventing or postponing type 2 diabetes. Intensity and duration are two factors of effective exercises which help improving conditions of patients with diabetes as well as types of exercises which have key roles in achieving this goal (8). Depending on different refueling paths of physical activities associated with diabetes, aerobic exercise has been mostly mentioned (9). However, studies have indicated that due to the lack of free times for people, HIIT is superior to moderate-intensity continuous training (MICT), and their results are usually much more desirable (9). This training pattern consists of interval trainings (explosive and short-term high-intensity movements) associated with short-term rests (10). Francois and Little reported that HIIT is an appropriate alternative for decreasing the blood sugar level in people who are at risk of type 2 diabetes

(8). In another study, Robinson et al. found that short-term HIIT could be a good alternative for aerobic exercises for improving the risk factors for diabetes (11).

In addition to exercise, nutrition and eating habits play key roles in improving patients with diabetes. One of the fundamental elements in controlling type 2 diabetes is diet modification which helps control the metabolism (12). Accordingly, caffeine supplementation associated with exercise is of great importance due to its metabolism effects and energy, and it has been studied for its nature in changing the metabolism and its effects on glucose homeostasis (13, 14). Alkaloid caffeine is an organic compound belonging to the family of Methylxanthines (15). Previous studies have indicated that coffee consumption reduces the risk of several chronic diseases (16). Its consumption also results in the decreased level of insulin sensitivity and there is a reverse relationship between coffee consumption and the risk of type 2 diabetes (17). Based on the previous epidemiological studies, there is a relationship between coffee consumption and the slow progression of chronic kidney disease (CKD) (18). However, some reports show that there is no relationship between the level of coffee consumption and death caused by the CKD (17). Several studies have affirmed the diuretic effect of caffeine and have reported higher urine volume in healthy individuals after caffeine consumption (19). Conversely, a meta-analysis study of data collected from four studies consisted of more than 10,000 participants has indicated that there is no relationship between coffee consumption and the risk of CKD (20). Different studies on this issue showed that caffeine has both useful and adverse effects on different kidney diseases. Apparently, the key factors determining the caffeine effects are the amount of and habits of caffeine consumption (21). Since

there are contradictory and insufficient reports about the caffeine effects on diabetes glomerulonephritis, this study was conducted to evaluate the effect of caffeine consumption and HIIT on the serum BUN and Cr levels and morphological changes in kidney tissue of diabetic rats as some factors for glomerular filtration.

Materials and Methods

The research was approved by the Ethics Committee of Imam Khomeini International University (Ethical code: 1397-17682). This interventional-clinical animal study was conducted in a two-factor post-test design on 5 equal groups including healthy control group (C), diabetes group (D), diabetes + caffeine group (D+CA), diabetes + training group (D+T), and diabetes + caffeine + training (D+T+CA) group of male Wistar rats, according to the rules for working on laboratory animals. For this purpose, 50 male Wistar rats with the age of about 3 months and weigh of 225-300 g were purchased from Laboratory Animals Breeding Center of Tabriz University of Medical Sciences and studied there. In order to adapt to the environment, prevent stress, and change physiological conditions, the subjects, 3 to 5 rats in a polycarbonate cage, were tested at temperature of $20 \pm 2^\circ$C, relative humidity of $50 \pm 5^\circ$C, minimum noise, and 12 hour darkness cycle (lightness: 7:00 am to 7:00 pm) in animals specialized laboratories. During this course, animals had access to water and animal standard food (pellets prepared by Isfahan Khourak Sazan Company) for two months, which had been measured precisely.

Diabetes induction method

Following two weeks of adaption to the laboratory environment, in order to induce type 2 diabetes based on the methods reported by Saccidarians et al. (2013), researchers and Isfahan Khourak Sazan Company prepared an amount of high-fat food for 2 weeks, and then, intraperitoneal injection (IP) of streptozotocin (Sigma Aldrich Co. USA) in a singles dose of 35 mg / kg dissolved in citrate buffer pH = 0.1 molar, was applied after 6 h fasting. (22). The healthy control and diabetic groups (without supplement and training) got the same amount of physiological serum in order to make completely equal conditions the same as the supplemented groups. One week after diabetes induction, blood samples were collected from the caudate vein and blood glucose levels were analyzed using enzyme-linked glucose oxidase assay, and animals with blood glucose concentrations above 250 mg/dL were considered as type 2 diabetic rats. The weight of animals was measured at the beginning, middle, and end of the study by digital scales to have their weight under control.

Treatment with caffeine supplementation

Studies have proven that caffeine consumption and bioavailability curves levels which are concentration-time based (AUC), are the same in human beings and rats. Thus, 99% of the dose is absorbed during 45 min within approximately 1 hour after ingestion of over 10 mg of body weight, these values depend on dose effects (23); hence, in order to enhance the plasma caffeine level during the activity, caffeine was injected 60 min before training program. The caffeine treatment procedure began with the injection of caffeine powder prepared by Merck Company (Germany) under license number of 2518359435571020 for 5 days of the

week before training program according to the body weight of the animals was done in the form of hydrated caffeine and intraperitoneal injection (IP) (14 mg of caffeine per 200 g of body weight of rats).(24).

Training Method

The fatigue testing was performed to calculate the maximum velocity of animals before the protocol implantation. For this purpose, an incremental exhaustive exercise test was implemented to assess maximal speed, commenced with 10 m/min, with an increase of 3 m/min every 2 min until failure. Rats' failure in running on treadmill despite electrical shock showed exhaustion moment. The participants of two groups (diabetic + training and diabetic + trainings + supplement groups) intended in the present study for 5 days of a week

(Sunday, Saturday, Tuesday, Wednesday, and Thursday), which was held from 4 to 6 p.m. on treadmill for 8 weeks. HIIT included three stages of warm-up, core exercise, and cooling. Exercises in warm up and cooling stages lasted for 5 min with a speed of 10 m/sec (30-40% VO2 max). Core exercises were equal to 85-90% of the maximum velocity at 6 to 12 times (An exercise time was added to the animals' activities once a week) Table 1. Furthermore, 1-min active-rest intervals consisting of continuous running on treadmill were applied within activity times. Also, healthy control group, which had not been in any activity programs, was placed on immobilized treadmill for 5 days in a week for 10 to 15 min to be adapted to the environment and to be in the same conditions with other groups. To stimulate rats to run, low-voltage electrical stimuli were embedded at the end of the treadmill (25).

Table 1. 8-week HIIT including warm-up, core exercise, and cooling stages

Week	No. of Running Intervals (2 min)	Running Speed (m.min)	Treadmill Grade (%)	Speed of Return to Active Initial State (m.min)
1	6	Max speed 85-90%	0	10
2	7	Max speed 85-90%	0	10
3	8	Max speed 85-90%	0	10
4	9	Max speed 85-90%	0	10
5	10	Max speed 85-90%	0	10
6	11	Max speed 85-90%	0	10
7	12	Max speed 85-90%	0	10
8	12	Max speed 85-90%	0	10

Blood glucose, Insulin, Serum Cr and BUN measurement method

In order to eliminate the trainings' intense effects, 48 hours after the last training session and after 12 to 14 hours fasting, animals were anesthetized by intraperitoneal injection with ketamine (90 mg/kg) and xylazine (10 mg/kg) and underwent

surgery by experts. Then, an adequate volume of blood was taken from the left ventricle of heart by a syringe. In order to provide serum, the blood samples were centrifuged at 3000 rpm for 15 min and their serum was separated and stored in a refrigerator at -80°C. Fasting blood glucose was measured by enzymatic calorimetric method with glucose oxidase

technology using glucose kit prepared by Sarai Cellular Research Company (Iran). Fasting serum insulin level was measured based on the Sandwich ELISA method (Rat Insulin Elisa Kit, Elabscience, USA). The serum Cr density level in a healthy person is constant and its fluctuations do not depend on the amount of consumed liquids, exercises, and excreted urine. Thus, increased serum Cr level is always the sign of decreased glomerular filtration and kidney dysfunctions (26). Serum Cr level was examined using calorimetric method without proteins removal based on the JAFFE method. The basis of the experiment was the formation of a colorful complex by combining creatinine and alkaline picrate that is consistent with the Cr level in the sample. The solutions were stored in special vials at temperature of 15-25°C. The Cr sustainability in serum was maintained at -20°C.

In BUN examination, enzymatic method was used based on Urease-GLDH (27). The solutions were stored at 2 to 8°C. The Cr and BUN standard or TruCal U calibrator were used for calibration and TruLab N and TruLab P of Pars Azmoon company for controlling separately. The Cr sustainability in serum was maintained at -20°C. The experiment process was performed at a wavelength of 500 nm for Cr and 340 nm for BUN, cuvette diameter of 1 cm, temperature of 20 to 25°C, and the measurement was performed as a photometer with a blockett set to zero.

Morphological changes of kidney tissue

Analysis of renal microscopic sample images is an imperative tool for several disease diagnoses. For this purpose, We used the image j software (NIH) and automated detection and measurement of renal corpuscles objects Plugin for estimating the Bowman's capsule area, Glumerolar area and urinary space area (28) as follows:

After dissection and opening the abdominal cavity and removing the viscera of rats, the right kidney of animals after weighing and determining the volume, was separated and placed in Bouin's fixation solution. In order to microscopically study the tissue structure of the kidney, the stabilized tissue samples were dehydrated and paraffinized using a histocint device (Leica TP 1020) after anatomical study by the usual method of preparing tissue sections.

After performing the tissue passage steps and preparing the paraffin block, 5 μm thick sections were prepared by rotating Leica RM 2245 microtome. The sections were then stained using hematoxylin-eosin (H&E), and examined using a light microscope (Olympus, Tokyo, Japan) at different magnifications and suitable images were taken by a camera mounted on a Dino-Lite digital microscope and a computer system connected to the camera equipped with Image J software at a scale of one micron were prepared and stored (29).

Image J is a public domain Java image processing program which can be freely downloaded from the Internet. Many of the functions in Image J are often all that is needed to quantify renal histopathology (30).

Statistical Analysis

Kolmogorov-Smirnov and Levene's tests were used to investigate the normal data distribution and homogeneity of variances, respectively. Due to the normality of data distribution and according to the purpose of the research, first, one-way ANOVA was used to investigate the effect of independent variable on dependent variables and then Bonferroni post hoc test was used to examine the differences

between groups of variables. All statistical analyses were performed using SPSS version 24 and statistical significance level was considered at P<0.05.

Results

The initial weight (before intervention) and the second weight (after intervention) of different study groups are presented in Table 2. The Kolmogorov-Smirnov test showed that data were normally distributed. The Mean ± SD of the variables are presented in Table 3.

Glucose

Regarding glucose levels, it was revealed that HIIT and caffeine consumption significantly reduced blood glucose level in diabetic rats (P=0.002 and P=0.0001, respectively). The relationship between the variables and glucose level showed that caffeine with the effect size of 63% had the greatest effect on glucose level (P<0.001). Additionally, there was no significant interaction between caffeine and HIIT on the glucose changes (P=0.14).

Insulin

Fasting insulin levels in the groups of D+CA (P=0.001) and D+T+CA (P=0.002) were significantly higher than those in diabetic control group. The relationship between research variables and insulin levels showed that caffeine with an effect

size of 45% had the greatest effect on insulin level (P<0.001). However, there was no significant interaction between caffeine and HIIT treatments on insulin level (P=0.076).

Urea

The findings obtained from one-way ANOVA on BUN variable indicated a significant difference between the study groups (P<0.001). After 8 weeks, there was a significant reduction in the serum BUN level in D+T group compared to D group (P<0.001). On the other hand, there was a significant difference between D+T group, and D+CA and D+T+CA groups (P<0.001). The relationship between research variables and BUN levels showed that caffeine with an effect size of 66% had the greatest effect on the BUN levels (P<0.001). Additionally, there was a significant interaction between treatments regarding BUN changes (P=0.001) (Figure 1).

Creatinine

The findings obtained from one-way ANOVA on Cr variable indicated a significant difference between the study groups (P<0.001). So that, Cr level in D+T group was significantly decreased compared to that in D group (P<0.001). The relationship between research variables and Cr levels showed that HIIT with an effect size of 15% had the greatest effect on the Cr level (P=0.015). Additionally, there was no significant interaction between caffeine and HIIT on the Cr changes (P=0.144) (Figure 1).

Table 2. The initial weight (before intervention) and the second weight (after intervention) of different study groups

	C Group	D Group	D+T Group	D+CA Group	D+T+CA Group
The Initial Weight (g)	299.12±15.88	308.85±27.61	301.85±27.15	292.00±64.30	299.14±62.21
The Second Weight (g)	329.25±24.04	296.14±18.49	312.85±39.03	279.42±83.59	312.28±19.66

Values are expressed as mean ± SD (P<0.05)

Table 3. Comparative evaluation of dependent variables (mean ± SD) in different study groups

	C Group	D Group	D+T Group	D+CA Group	D+T+CA Group	P-value
Glucose (mg/dL)	80.6±5.23	373.9±89.78	141.8±50.18	235.80±48.95	150.6±29.45	0.001*
Insulin (μu/ml)	13.23±4.47	4.02±2.79	5.79±2.13	11.46±3.43	9.69±2.23	0.001*
BUN (mg/dL)	33.10±3.10	64.80±4.93	51.60±4.92	73.10 ± 6.85	77.50±8.79	0.001*
Cr (mg/dL)	0.44±0.14	1.10±0.22	0.83±0.19	1.01±0.20	0.97±0.12	0.001*
Bowman's capsule area (fold of control)	1.000±0.00	1.68±0.26	1.03±0.15	1.43±0.22	0.98±0.30	0.001*
Glomerular area (fold of control)	1.000±0.00	0.70±1.14	1.02±0.18	0.72±0.13	0.96±0.28	0.001*
Urinary tract area (fold of control)	1.000±0.00	1.38±0.35	1.01±0.38	0.77±0.18	0.91±0.31	0.001*

Values are expressed as mean ± SD.
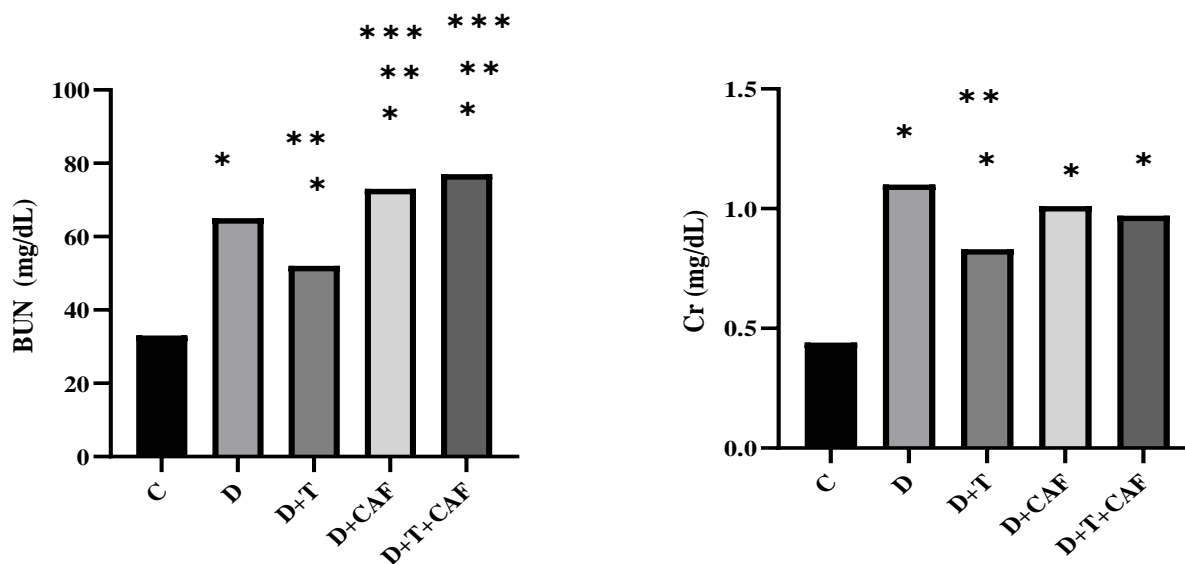
*Significant difference at P<0.05.

Figure 1. Blood urea and blood creatinine (mean ± SD) levels (mg/dL) in different study groups.

Control (C), Diabetic (D), Diabetic + Caffeine (D+CA), Diabetic + Training (D+T), and Diabetic + Training + Caffeine (D+T+CA)

*Significant difference compared to C group, **Significant difference compared to D group, ***Significant difference compared to D+T group.

Morphological changes of kidney tissue

Bowman's capsule

The findings obtained from one-way ANOVA indicated a significant difference between the study groups (P<0.001). There was also a significant difference between groups of D (P<0.001) and D+CA (P=0.001), and C group (P<0.001). There was no significant difference between D group and D+CA group (P=0.117). However, there was a significant difference between D+T and D+CA groups (P=0.001). The relationship between research variables and Bowman's capsule showed that caffeine with an effect size of 43% had the greatest effect on Bowman's capsule (P=0.015). Additionally, there was no significant interaction between caffeine and HIIT on Bowman's capsule changes (P=0.087) (Figure 2).

Glomerular area

The findings obtained from one-way ANOVA indicated a significant difference between the study groups (P<0.001). There was a significant difference between C and D groups (P=0.005). There was also a significant difference between D and D+T groups (P=0.002). However, there was no significant difference between D and D+CA groups (P=0.117). The

relationship between research variables and glomerular area showed that caffeine with an effect size of 30% had the greatest effect on the glomerular area (P=0.001). Additionally, there was no significant interaction between caffeine and HIIT on the glomerular area changes (P=0.665) (Figure 2).

Urinary tract area

The findings obtained from one-way ANOVA indicated a significant difference between the study groups (P<0.001). There was no significant difference between D group and D+T (P=0.043) and D+T+CA (P<0.001) and D+CA (P<0.001) groups. The relationship between research variables and urinary tract area showed that HIIT with an effect size of 15% had the greatest effect on the urinary tract area (P=0.015). Additionally, there was a significant interaction between caffeine and HIIT on the urinary tract area changes (P=0.002) (Figure 2) (Table 4).

In general, examination of kidney tissue under the optical microscope indicated that in D+T group, glomerular area was larger than that in diabetic models, and the Bowman's capsule was smaller and reduced compared to that in the urinary tract area (Figure 3).
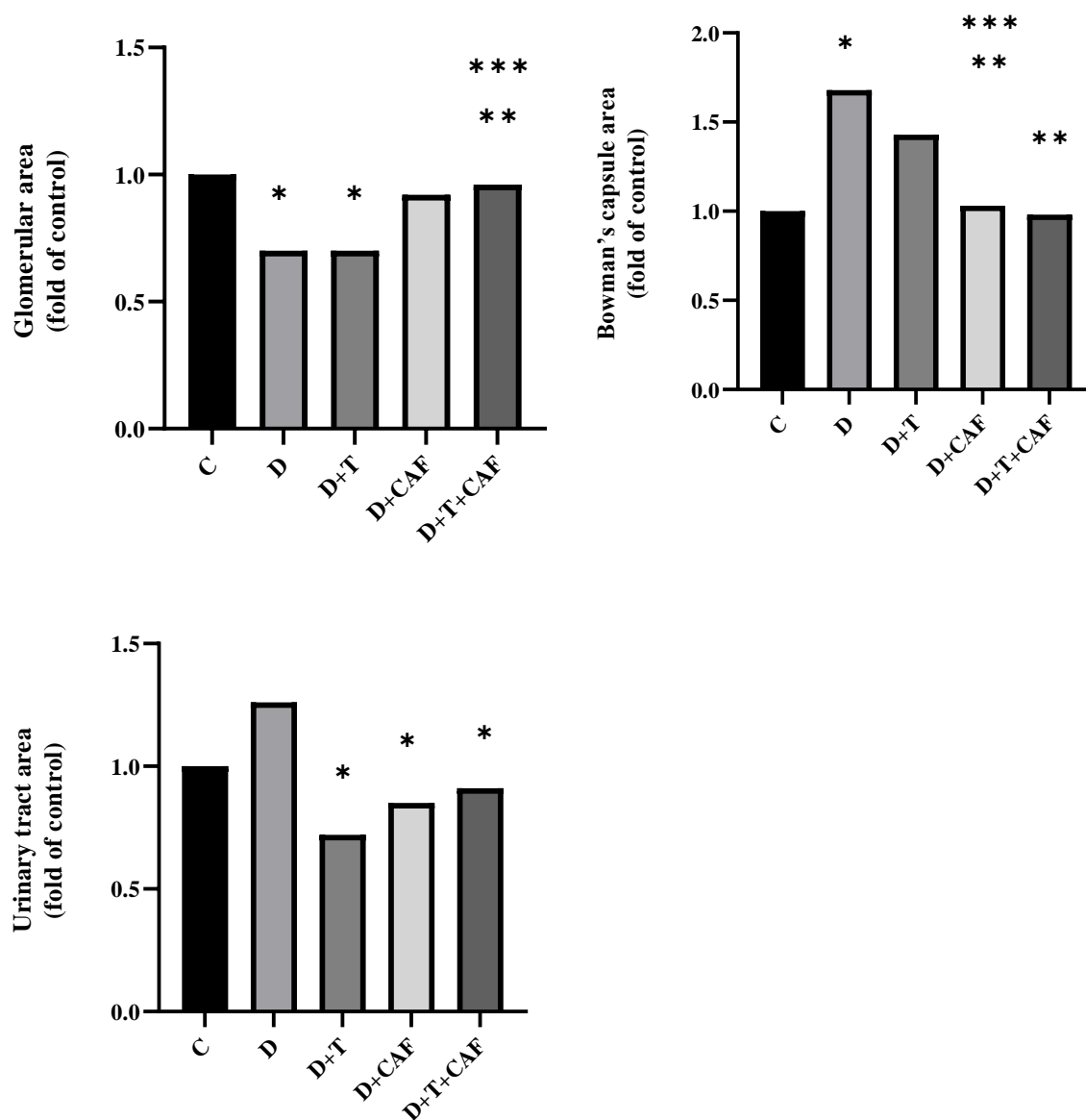
Figure 2. The average Bowman's capsule, urinary tract area, and glomerular factors in different study groups.

Control (C), Diabetic (D), Diabetic + Caffeine (D+CA), Diabetic + Training (D+T), and Diabetic + Training + Caffeine (D+T+CA)

*Significant difference compared to C group, **Significant difference compared to D group, ***Significant difference compared to D+T group.

Table 4. Effect size of HIIT and caffeine on dependent variables in diabetic rats

|		F	P	Eta Squared
Blood glucose (mg/dl)	Training	11.19	0.002*	0.47
	Caffeine	16.75	0.001*	0.63
	Training × Caffeine	2.64	0.14	0.18
Insulin (µu/ml)	Training	5.12	0.04*	0.21
	Caffeine	9.13	0.002*	0.45
	Training × Caffeine	3.12	0.076	0.17
BUN (mg/dL)	Training	6.889	0.013*	0.161
	Caffeine	71.848	0.001*	0.666
	Training × Caffeine	19.130	0.001*	0.347
Cr (mg/dL)	Training	6.492	0.015*	0.153
	Caffeine	0.721	0.401	0.020
	Training × Caffeine	2.234	0.144	0.058
Bowman's capsule area (fold of control)	Training	1.865	0.181	0.049
	Caffeine	27.714	0.001*	0.435
	Training × Caffeine	3.089	0.087	0.079
Glomerular area (fold of control)	Training	0.026	0.872	0.001
	Caffeine	15.210	0.001*	0.297
	Training × Caffeine	0.191	0.665	0.005
Urinary tract area (fold of control)	Training	6.584	0.015*	0.155
	Caffeine	1.402	0.244	0.037
	Training × Caffeine	10.569	0.002*	0.227

*Significant difference at P<0.05
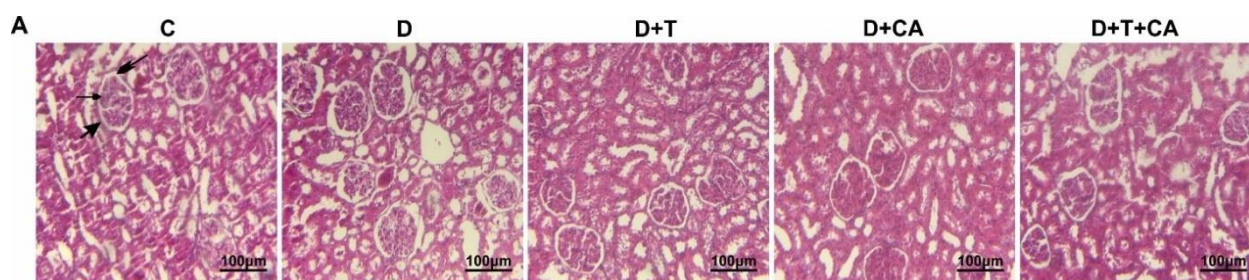
Figure 3. Illustration of kidney tissue under the optical microscope.

Control (C), Diabetic (D), Diabetic + Caffeine (D+CA), Diabetic + Training (D+T), and Diabetic + Training + Caffeine (D+T+CA).

Discussion

The present study investigated the effect of 8-week caffeine supplementation and HIIT on the serum BUN and Cr levels and morphological changes of kidney tissue in diabetic rats. Regarding glucose level, the results showed that 8-week HIIT and caffeine supplementation significantly reduced the blood glucose level in diabetic male rats. In this regard, HIIT and caffeine supplementation led to a significant decrease in the blood glucose level around 36.93% and 62% respectively. Although most previous studies have shown that resistance and aerobic exercises improve glycemic index (GI) in patients with diabetes (31, 32), there are few studies on the effect of the HIIT on these parameters. Consistent with the results of the present study, Alvarez et al. reported that HIIT can be used as a useful therapeutic intervention in patients with diabetes (33). The important points about the effect of different exercises on the GI are the volume, duration, and intensity of training. Recent studies, in which the HIIT programs were used in patients with diabetes, have suggested that the intensity of exercise may play a key role in the management of diabetes. In this regard, HIIT programs resulted in the substantial improvement in HbA1c (34), fasting insulin (35), and blood sugar (34) levels in patients with diabetes compared to the continuous training programs.

Consequently, the mechanisms related to the effect of HIIT on the GI in patients with diabetes are not completely and accurately defined.

On the other hand, due to a 36.93% reduction in fasting blood glucose level following 8-weeks HIIT in the present study, improvement of the exercise-induced hepatic glucose output is not unexpected. On the other hand, HIIT has a greater capability to use muscle fibers and deplete muscle glycogen faster (8), so after training, it may lead to a further increase in insulin sensitivity of muscle cells. Although hypersensitivity to insulin occurs even about 24 to 48 hours after a session of exercise (8), it can be seen that the effect of HIIT on insulin sensitivity has been established to be long-lasting compared to continuous training protocols (especially over a long period of 2 to 4 months) in terms of increasing insulin sensitivity and lower blood glucose in patients with diabetes. Moreover, Furthermore, the results of the present study showed that caffeine supplementation alone reduced blood glucose level by approximately 62% in diabetic rats. Consistent with the results of the present study, Urzua et al. reported that long-term high-dose caffeine supplementation (93 mg/kg/day) reduced blood glucose level, improved glucose tolerance, and delayed the onset of diabetes (36).

Caffeine appears to increase glucose transport in the absence of insulin in rodent skeletal muscle (37) and enhance the expression of the glucose transporter (GLUT4) gene in the established muscle cells (38). Also, Egawa et al. claimed that caffeine mainly activates 5'AMP-activated protein kinase (AMPK) by non-insulin-dependent mechanisms and increases glucose transport (39). However, Beaudoin et al. reported that short-term and acute caffeine consumption, even at low doses, increased blood glucose level and decreased insulin sensitivity in healthy men and women (40). which is not consistent with the results of the present study. It seems that the most important reasons for the discrepancy between the results are the duration of caffeine consumption and the subjects.

The findings of the study demonstrated that caffeine supplementation and HIIT had a significant effect on the serum BUN and Cr levels after eight weeks. According to the comparison of the study groups in terms of BUN and Cr indices, the application of HIIT had the most effect on the serum BUN and Cr levels. Regarding caffeine effect on diabetes, Santos and Lima concluded that the risk of type 2 diabetes is reduced in people who drink 3-4 cups of coffee a day regularly. They claimed that these effects are likely from chlorogenic acid (with antioxidant property) and caffeine in coffee (41). In fact, chlorogenic acids are the most common bioactive compounds in herbal foods such as coffee (42). Consistent with this result, several studies indicated that caffeine has led to weight loss in patients with diabetes due to the increased thermogenic (43) and ergogenic (44) properties. However, caffeine has been known as a diuretic substance and meta-analysis studies have demonstrated the general effects of caffeine on the increased volume of urine (45). Nevertheless, some studies have shown that the diuretic effect of caffeine

depends on the consumption volume (46). Moreover, some other studies have reported that the diuretic effect of caffeine can be seen only at the first day of its consumption, but it is not sustainable continuously and cannot be seen in a typical consumption (47). Recent studies have represented that the diuretic effect of oral caffeine consumption emerges in the consumption of more than 300 mg of caffeine. Although, consumption of 300 mg dose by habitual consumers of caffeine showed the same effects (48). In some studies, it was suggested that increased glomerular filtration arises from caffeine consumption (44, 49). On the other hand, excessive consumption of caffeine may lead to nephrotoxicity and some kidney diseases, while low-dose consumption of this substance is beneficial. Additionally, the effects of caffeine on healthy people are somewhat different from those in patients with kidney diseases. So, it seems that caffeine has no significant effect on kidney function in healthy people and even has preventive effects on some kidney diseases in healthy people. On the contrary, caffeine may exacerbate the severity of some kidney diseases. Nonetheless, an exact mechanism affecting the beneficial and adverse effects of caffeine is not still presented or even is unknown. Thus, there is a need to conduct more widespread research on the exact mechanism of caffeine effects in kidney diseases (21). A research by Smavati (2015) the effects of aerobic exercises on patients with type 2 diabetes and its different feedbacks on the improvement of kidney function, which is one of the important indices in determining the diabetes complications, were investigated. They also investigated the effects of aerobic exercises on the glomerular filtration and serum level of biochemical factors of BUN, Cr, and uric acid in patients with type 2 diabetes, and found that 10-week aerobic exercises improved the level of BUN level, but it

had no significant effect on the uric acid level (50). In general, it was concluded that aerobic exercises can be helpful in improving the kidney complications of the patients. Another study by Bijeh and Farahati showed that there were no significant changes in the glomerular filtration and BUN levels after six months of aerobic exercises, however, aerobic exercises can improve the kidney functions if they are performed in enough time and with appropriate intensity (51). Kakhak et al concluded in another study that aerobic exercises did not affect the kidney function indices in fat girls, one needs to have an intense training program and a diet in order to improve these effects (52).In a study by Chiasera et al. the effects of aerobic exercises on the functional changes and a diabetic structure in type 2 diabetic rats, were investigated. The exercises had no effects on morphometric indices. They found that the aerobic exercises interventions significantly affects metabolic indices control and albuminuria reduction in type 2 diabetic rats (53). They also found that exercise interventions led to a significant weight loss and albumin density compared to the control group. Moreover, the exercises improved the metabolism control and albuminuria reduction in type 2 diabetic rats (53). Another study by Suzuki et al. demonstrated the improvement of diabetic nephropathy by caffeine consumption alone or with exercises which includes increased creatinine clearance, urinary sodium excretion, reduced urinary protein excretion and kidney morphological changes. According to our study, there is no other studies suggesting that caffeine inhibits diabetic nephropathy's development (54). Also, the reverse effects of caffeine on people with chronic kidney failure have been studied and the findings have not been submitted for humans yet and further studies on this issue are needed (55).

To the best of our knowledge, this is the first study that was conducted on the effect of high-intensity interval training (HIIT) and caffeine supplementation on the serum creatinine (Cr) and blood urea nitrogen (BUN) levels and morphological changes of kidney tissue in diabetic animal models, and further confirmation or rejection of the results of this study requires further studies. There seems to be a discrepancy in the literature regarding this subject which may be explained, to some extent, with dose, duration and methods of caffeine supplementation as well as the selected subjects (healthy or non-healthy). The findings of the present study raise the question of whether exercise training has any interaction with caffeine consumption in patients with diabetes. Moreover, longer duration of caffeine consumption may be required to observe its positive or negative effects on these factors in patients with diabetes. This question need to be answered in the future studies. The present study had also some limitations, which need to be addressed. The main limitation is the lack of dosage and different protocols to administrate caffeine supplementation in diabetic laboratory animals. Another limitation is that the other indicators in this regard were not evaluated.

Conclusion

Generally, according to the studies on the caffeine properties including diuretic property, as well as its toxic effects, different results have been reported. So that the effect size varies based on the given doses, time and way of consumption, genetic, enzymatic and metabolic status of individuals. According to the results of kidney tissue morphology in diabetic rats in the present study, the caffeine group exacerbated the tissue condition, which is opposite to the training group condition. But there were no reasons determine

whether or not the caffeine consumption is beneficial for kidney. Furthermore, there were no evidences suggesting increased risk of nephropathy with consuming 3-4 cups of coffee containing 350 mg caffeine. According to the results of the present study and previous studies, it seems that HIIT in diabetic patients has a significant contribution to improving renal function, which can be measured by changes in BUN and Cr levels. Obviously, definitive comment on the effects of caffeine consumption and HIIT in patients with diabetes requires further and more detailed research.

Ethical Considerations

Conflict of Interests

The authors declare that there is no conflict of interest.

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