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COMBINED EFFECTS OF CAFFEINE AND AEROBIC EXERCISE ON LEPTIN LEVELS AND SOME INDICES OF INSULIN RESISTANCE IN DIABETICS

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Abstract The study aimed to examine the combined effects of caffeine and aerobic exercise on leptin levels and some indices of insulin resistance in diabetics

Thirty-two males with type 2 diabetes participated in a quasi-experimental and double-blind design. All participants were divided into four homogeneous groups of 8 individuals, including placebo (P), caffeine supplementation (C), aerobic training (AT), and aerobic training and caffeine (AT + C). The design protocol included eight weeks of aerobic exercise and caffeine consumption. Blood samples were taken to measure serum levels of leptin, glucose, insulin, HbA1c, HOMA-IR, and insulin sensitivity (QUICKI) indices at two phases. Data were analyzed by repeated measure ANOVA, Bonferroni posthoc, and independent T-test at a significant level of $\alpha \leq 0.05$.

The results showed that the levels of leptin, glucose, insulin, HbA1c, and HOMA-IR in the three intervention groups significantly decreased compared to the placebo group ($P = 0.001$). In addition, QUICKI was significantly increased in the three groups of caffeine (C), aerobic training (AT), and aerobic training + caffeine (AT + C) compared to the placebo group ($P = 0.001$). Also, the AT+C group has double effects on the investigated indices compared to the caffeine (C) group ($P = 0.001$).

Regular aerobic exercise and caffeine supplementation may be more effective treatments for improving insulin resistance indicators associated with type 2 diabetes.

Key words: caffeine, aerobic exercise, type 2 diabetes, leptin, insulin resistance

Introduction

The worldwide prevalence of type 2 diabetes (T2D) rises with obesity. Insulin resistance (IR), defined as a disorder in the ability of insulin to stimulate glucose uptake from surrounding tissues, is a prominent feature in metabolic disorders (Talukder, Hossain, 2020). It has been well established that individuals with IR are usually diagnosed with a decrease in glucose transportation and increased plasma-free fatty acid (FFA) concentrations with metabolic disorders of substrates (Kawada, 2013; Bergman, 2007). The sensitivity and function of insulin and beta-cell function significantly affect carbohydrate, fat, and protein metabolism; impaired regulation of these factors leads to metabolic syndrome and diabetes (Kawada, 2013).

Obesity and diabetes are accompanied by alterations in adipose tissue biology, rooted in the dysregulation of adipokines and cytokines (Frühbeck et al., 2018; Ayina et al., 2017). In summary, hypertrophy of adipocytes increases secretion of chemokines and penetration of macrophages. Hypertrophy reduces the expression of some adipokines like adiponectin and increases pro-inflammatory cytokines and other hormones secreted by white adipose tissue, such as leptin. Also, as an adipokine secreted from white adipose tissue (WAT), leptin has a role in regulating energy homeostasis and fat metabolism in interaction with carbohydrates by affecting the brain (Ayina et al., 2017).

On the other hand, the amount of free leptin in the bloodstream is proportional to the amount of body fat and reflects the state of long-term energy reserves (Frühbeck et al., 2018; Ayina et al., 2017). Therefore, the results of several studies point out the anti-obesity effects of leptin as one of its most important and prominent properties. High circulating leptin level in obese individuals is relatively common and defined as leptin resistance (Frühbeck et al., 2018). Also, changes in visceral and total fat mass and increased energy expenditure following regular exercise can adequately regulate the IR indicators (Tayebi et al., 2016). For example, it has been shown that 8 weeks of aerobic training significantly reduced weight, BMI, body fat percentage, insulin resistance, glucose and insulin compared to baseline conditions (Gharakhanlou & Bonab, 2022). It has been reported that 12 weeks of aerobic training reduced plasma leptin levels (Akbarpour, 2013). Also, it was stated that 12 weeks of aerobic training did not affect serum leptin levels (Saremi et al., 2012).

Today, however, medical-sports researchers benefit from combining physical activity with some pharmacological interventions to control obesity and the symptoms of T2D (Alshawi, 2020). As the most popular and common beverage globally, coffee indicates weight loss and improvement of T2D symptoms (Farajpour et al., 2017). Thus, some existing literature claims that the beneficial effects of coffee are caused by caffeine which is the most crucial and active compound of it (Alshawi, 2020). Caffeine (1, 3, 7-Trimethylxanthine) is a methylated purine alkaloid derived from the methyl-xanthine family (with the chemical formula C8H10N4O2). Since caffeine has full potential for altering energy metabolism and affects glucose homeostasis in individuals with diabetes and obesity, it has been investigated in both epidemiological and experimental studies (Alshawi, 2020; Farajpour et al., 2017; Jafari, et al., 2014). Chronic consumption of caffeinated compounds, such as coffee, has also been associated with a significant reduction in the risks of T2D in cohort studies (Alshawi, 2020). For instance, in a prospective cohort study following the consumption of 5 cups of coffee, it has been noted that coffee consumption increased glucose tolerance, and insulin sensitivity compared to the control group (Wedick et al.,2011).

In addition, Sacramento et al. (2015) examined the effects of single-dose caffeine consumption on whole-body insulin sensitivity. The results showed that acute caffeine consumption reduced insulin sensitivity through a concentration-dependent effect (Sacramento et al., 2015).

Also, it is stated that acute consumption of caffeine at the rate of 1.5 mg/kg of BW with 40% HR max physical activity protocol for 40 minutes on a treadmill significantly improved peripheral glucose reduction during activity in people with T2D (da Silva et al., 2014). These contradictory findings suggest that acute and chronic caffeine administration or acute administration combined with physical activity have opposite pharmacological effects. Therefore, the positive effects of combined exercises on many different biological aspects in human studies of healthy individuals and animal studies have been studied and identified. However, the interaction of similar exercises with fat-burning supplements, such as caffeine, on glycemic index response has not been thoroughly studied in patients such as individuals with diabetes. Therefore, the present study aimed to investigate and determine the effects of long-term caffeine supplementation (3 mg/kg of BW for eight weeks) with aerobic training (three days a week walking on a treadmill with an intensity of 60–70% of heart rate reserve for 40 minutes) on levels of IR markers (leptin and adiponectin, glucose, insulin, HbA1c, HOMA-IR, and QUICKI) in men with T2D. However, sports medicine coaches and clinicians should answer some of the ambiguities regarding the interactive effects of exercise, bodybuilding, and caffeine supplementation based on the obtained data and determine whether combinations of these therapeutic interventions can reduce diabetes complications and avoid high treatment costs or not.

Materials and Methods

This study is a quasi-experimental, double-blind clinical trial with a pre-and post-test design. The study was performed on 32 men with T2D. Participants were selected from 65 patients (with a history of more than one year of diabetes) and were randomly divided into four groups of 8 individuals by available and purposive sampling methods. Groups included three experimental and one control group, with an age range of 50–55 years and body mass index range of 32–35 kg/m^2 (Table 1). The entire research was conducted according to the Declaration of Helsinki. Accordingly, all participants were first guided about cooperation and signed informed consent; before starting any physical activity, the participants answered the International Physical Activity Questionnaire (IPAQ) to be screened (Shirali et al., 2016). The amount of caffeine consumption of the subjects was evaluated and controlled using a 24-hour nutrition recall questionnaire. (Farajpour et al., 2017).

The main inclusion criteria of subjects in this study include: being in the age range of 40–60 years, fasting blood glucose between 125–250 mg/dL, no history of diseases related to diabetes (such as neuropathy, nephropathy, retinopathy), no history of using other supplements to lower blood glucose and blood pressure higher than 140/90 mm Hg, lack of regular sports activities that are more than one session per week during the last three months and having a history of diabetes for more than one year. In addition, none of the subjects were under insulin treatment and all patients were on metformin and glibenclamide orally during the research period. Also, exclusion criteria included: having complications of diabetes such as diabetic foot, history of cardiovascular diseases, uncontrolled arrhythmia. Finally, individuals who were non-compliant with the study protocol and were smoking and consuming alcohol were excluded.

In addition, the daily diet of subjects was analyzed using a 24-hour nutritional recall questionnaire to check the amount of calories and the percentage of energy received from macronutrients based on the database of the Nutritionalist IV[1] software (Table 1).

[1] Nutritionist IV. Copyright 2004. N-Squared computing and First DataBank Inc. The Hearst Corporation 1111 Bayhill DR, San Bruno, CA 94066.

The participants' body composition, height and BW were measured with Seca217 gauge (made in Germany) with a sensitivity of one millimeter and Seca digital scale with an accuracy of 0.1 kg, respectively. Body fat percentage was also determined by Body Composition Analyzer (InBody-570, made by south korea) (Swain, 2014).

Caffeine supplement contract

Participants in the supplement group (C) equally consumed a daily dose of caffeine-containing capsules made by German Merck Company and licensed by the Ministry of Health (registration number of 0225/02584/1) according to their weight (3 mg/kg of BW). Also, individuals in the placebo (P) group consumed dextrose in the same amount (3 mg/kg of BW), similar to the supplement group.

During two months, each subject took one caffeine or dextrose capsule with 250 ml of water an hour before the activity on training days (three days a week) and half an hour after breakfast on other days without exercise (three days a week). All participants were unaware of the capsules' content (double-blind plan). According to the results of previous studies, the amount of caffeine consumed in the present study was considered in the effective range (3 to 6 mg/kg of BW) required to improve athletes' plasma levels and performance (Jafari et al., 2014). Also, during the intervention, patients were investigated regarding possible unwanted side effects and adherence to the protocol by daily calls.

Exercise protocol

The exercise protocol of the present design included walking on a treadmill (on a zero-degree slope with an intensity of 70–60% of the reserve heart rate). The baseline heart rate of participants was recorded after 10 minutes of resting (sitting) with a polar heart rate monitor made in Finland. The maximum heart rate of the subjects was calculated using the Karvonen formula given at the end of the section. In order to control the activity intensity of 60–70% of the maximum heart rate, the Karvonen method or the reserve heart rate, which is equal to the percentage of maximum oxygen consumption (aerobic capacity), was used. Based on the protocol, subjects walked on a zero-degree slope treadmill with a 60% reserve heart rate for 25 minutes from the first to the third week, a 65% reserve heart rate for 30 minutes from the fourth to the sixth week, and with a 70% reserve heart rate for 40 minutes from seventh to the eighth week. Therefore, a researcher controlled the subjects' heart rate and treadmill speed until the end of the training protocol. Participants performed stretching exercises to warm up for 10 minutes before performing the exercise protocol and executed cooling down training for 10 /minutes at the end of each training session (Shirali et al., 2016).

Method of preparing blood samples

Blood sampling was performed in two stages (first stage: 24 hours before supplementation and training protocol; and second stage: 24 hours after the last training session and eight weeks of supplementation). The samples were drawn at a rate of 5 ml from the left forearm vein (Antecubital vein) after 8–10 hours of fasting. Then, the blood samples were placed at the average laboratory temperature for clot formation and then placed in a centrifuge made by Behdad-Iran Company for serum separation at 4000-35000 rpm (RPM). All measurements were performed at 9–12 am, 26-28 °C temperature, 50–55% humidity, and the same ventilation and ambient light condition. In addition, before the test, subjects refrained from taking any dietary supplements affecting glucose and

diabetic parameters for 48 hours while maintaining a regular diet. The participants also avoided strenuous exercises and activities (such as massage, sauna, anti-inflammatory, and anti-diabetic drugs) that might affect injury, cell inflammation, and blood glucose.

Laboratory analysis

The enzymatic colorimetric method was used to measure fasting serum blood glucose based on the glucose oxidase reaction (Pars Azmoun Company Kit; Iran) with a sensitivity of 5 mg/dl and employing model 902 auto-analyzer (Hitachi; Germany). Also, fasting serum insulin was measured by the competitive and sandwich ELISA method with a sensitivity of 0.5 micro-units per milliliter (μU/ml) and the coefficient of internal and external changes of 6.45% made by ELISA Reader (DIARJ; Germany). Additionally, IR and insulin sensitivity indices were calculated using the homeostatic model assessment (HOMA) and the quantitative insulin sensitivity check index (QUICKI), respectively, with the following equation (Farajpour et al.2017; Torabi & Mirzaei, 2022):

HOMA-IR = fasting plasma glucose (mmol/l) × fasting serum insulin (mU/l) / by 22.5

QUICKI = 1/ (log (fasting insulin μU/ml) + log (fasting glucose mg/dl))

Leptin was measured based on the double-antibody sandwich ELISA using a kit made by the Germany BINDER Company with RD191001100 serial number. All measurements were simultaneously performed according to the instructions using the standard curve and control at a specific time. This method has a sensitivity of at least 0.05 ng/ml, the daily coefficient of change of 0.13 in 0.32 ng/ml, and %5.8 in 2.8 ng/liter. In order to measure the serum level of adiponectin, the ELISA method was performed using a commercial kit by BINDER company (tracking code: RD195023100) with a sensitivity of 0.5 μg/ml. Also, HbA1c index was measured using a biosystem kit made in Spain and by spectrophotometric method.

Statistical methods

All data were expressed as Mean ± SD. Shapiro-Wilk (S-W) test was used to determine the normality of the initial data distribution. Then, 2 × 4 (group × steps) repeated measures Analysis Of Variance (ANOVA) was used to calculate the mean changes of each variable in the dual stages of measurement, the interaction of the groups (supplement and placebo), and blood sampling stages. In case of differences between time stages, the Bonferroni post hoc test was used. The independent t-test was used to determine the difference between groups. All the statistical analyses were performed by SPSS software version 22 at a significant level of 5%.

Results

The anthropometric and physiological characteristics of the participants (Age, VO2 MAX, Energy Received, Fat Percentage, BMI, and WHR) of each group are presented in Table 1.

The present study showed that basal serum leptin level is high in men with diabetes. Eight weeks of caffeine supplementation (C) in individuals with diabetes significantly reduced the leptin concentration by 17.87% compared to the placebo group (P) (P = 0.001). Additionally, leptin levels in the other two experimental groups, aerobic training (AT) and aerobic training + caffeine supplementation (AT + C), were significantly reduced by 22.79% and 31.82% compared to the baseline, respectively (P = 0.001). The simultaneous intervention of aerobic training and caffeine

supplementation (AT + C) had far more modulatory effects compared to the separate application of aerobic training (AT) and caffeine supplementation (C) (P = 0.001) (Figure 1).

Also, the analysis showed that serum levels of glucose, HbA1c, insulin, and HOMA-IR in men with diabetes significantly decreased by about 13, 7, 17, and 10% compared to the placebo group after eight weeks of caffeine intake with aerobic training (AT + C), respectively (P = 0.001) (Figure 2 and Table 2). The further reduction in the indices related to IR in the group of aerobic training with caffeine (AT+C) was statistically significant compared to the caffeine group (C) ($P \leq 0.05$). Changes in insulin sensitivity index (QUICKI) in all intervention groups (aerobic training, caffeine and training + caffeine combination) were significantly increased compared to the placebo group (P = 0.001). The mentioned increase was significant in the aerobic training (AT) and aerobic training + caffeine groups (AT + C) compared to the caffeine group (C) (P = 0.001) (Table 2).

Discussion

The present study showed that serum leptin concentrations in diabetes were significantly higher than normal values (2.5–21.8 ng/ml). Also, the present study showed that caffeine consumption in diabetes significantly reduced serum leptin by about 18%. The existing literature has also shown that caffeine supplementation reduces leptin resistance in diabetes by modulating leptin-dependent signaling pathways. In this regard, consistent with the present study, Hosoi et al. (2014) and Yamashita et al. (2012) suggested that caffeine supplementation in overweight men had a significant lowering effect on the investigated leptin resistance indices (Yamashita et al., 2012; Hosoi et al., Leptin upregulates transcription of anorectic neuropeptides and downregulates transcription of appetite neuropeptides through phosphorylation of the JAK2/STAT3 pathway proteins. It also controls glucose homeostasis by suppressing the glycogenic gene expression in the liver and stimulating phosphorylation of the insulin receptor substrate-1 (IRS-1) (Kempf et al., 2010).

Additionally, pharmacological activation of β-adrenergic receptors, which is coupled to the stimulated guanosine protein (Gs), has been shown to reduce gene expression and leptin secretion. Therefore, it is inferred that caffeine may be a beneficial therapeutic agent to reduce leptin secretion by increasing stimulation of the PKA/cAMP signaling cascade. The cascade is stimulated through increasing levels of stress hormones (catecholamines and cortisol), inhibition of the cyclic nucleotide phosphodiesterase (PDE), and the guanine protein-coupled receptors (GPCRs), especially A1 and A3 isoforms of adenosine (Rice et al.,2000).

In contradiction with the present study, the results of some researchers indicated that caffeine and physical activity did not have any effects on the levels of leptin resistance (Kim et al., 2016; Rasaei et al., 2019; Shirali et al., 2016; Hongu & Sachan, 2000). It seems that the circumstances of participants, such as being with diabetes or healthy, the habitual status of caffeine intake, exercise protocol, and baseline levels of the measured indicators, are among possible reasons for the differences and contradictions between the present study and the results of the mentioned studies.

As seen in the present study, co-administration of caffeine with aerobic training caused a significant reduction in leptin levels, which is 4 and 6 ng/ml more than the caffeine supplementation alone and aerobic training intervention, respectively. In this regard, Kazemi et al. (2015) studied rats with T2D and stated that eight weeks of aerobic training with an intensity of 65–75% VO2max reduced leptin resistance (Kazemi & Saleh, 2015). Also, Amani et al. (2018) declared that performing advanced aerobic training for six weeks was associated with decreasing serum leptin levels (Amani et al., 2018).

Also, discoveries in other investigations have indicated an inverse relationship between circulating leptin concentrations and IR. It has been shown that leptin receptors in pancreatic beta cells inhibit insulin biosynthesis and secretion by pancreatic cells (Talukder & Hossain, 2020; Kawada, 2013).

Insulin secretion may be suppressed by leptin through a variety of mechanisms directly affecting β-cells. For β-cells to release insulin it is crucial that β-cell membrane be depolarized by closing adenosine triphosphate (ATP)–sensitive potassium (K_{ATP}) channels as a reaction against glucose or other insulin secretagogues. The cell membrane is hyperpolarized by leptin opening K_{ATP} channels, which results in a decline in intracellular Ca^{2+}, known to be involved in the β-cell releasing insulin vesicles. Additionally, insulin biosynthesis and gene expression are assumed to be affected by leptin through a transcriptional process. This seems to be independent of K_{ATP} channels activation as the K_{ATP} channel opener diazoxide does not affect the suppression of insulin gene transcription in β-cells which is brought about by leptin. So, the inhibitory action of leptin on insulin gene expression and insulin secretion has to be exerted through different signal transduction pathways (Cited in Yong-ho et al.,2011).

On the other hand, insulin also stimulates leptin secretion from adipose tissue. This hormone-regulating feedback loop has a fundamental Adipo-insular axis (Frühbeck et al., 2018), so dysfunction of the mentioned message axis plays a vital role in developing hyperinsulinemia. Another possible mechanism for modulating leptin secretion induced by physical activity can be the improvement of insulin sensitivity. Exercise improves glucose transport into adipocytes by increasing GLUT4 in the plasma membrane; glucose acts as an intracellular messenger and stimulates leptin secretion from fat cells (Saremi et al.2012).

The present study indicates a significant decrease in serum levels of glucose, insulin, HOMA-IR index, and an increase in insulin sensitivity index (QUIKI) due to long-term caffeine consumption, which is consistent with the results of some researchers (Gharakhanlou & Bonab, 2022; Conde et al., 2012; Guarino et al., 2013).

Many studies have expressed that the PI3K activation provides a central message for stimulating the transport of the GLUT4 by insulin. On the other hand, caffeine acts as an inhibitor of the PI3K, inhibiting insulin-induced GLUT4 transport (Yamashita et al., 2012). These findings suggest that inhibition of glucose uptake by caffeine may occur by stopping the IRS-PI3K-Akt/AS160 cascade (Sacramento et al., 2015; Yamashita et al., 2012; Conde et al., 2012; Kolnes et al., 2010). Other literature has shown that adenosine antagonists reduce insulin sensitivity in adipose tissue and heart muscle and, on the other hand, increase insulin sensitivity in skeletal muscle. According to a recently published study, Sacramento et al. (2015) reported that acute caffeine consumption (0.001-5 μmol) reduced insulin sensitivity (insulin resistance) (Sacramento et al., 2015). The present study results showed that daily consumption of 3 mg/kg of BW for eight weeks reduced serum glucose and insulin levels of diabetes by 13.5% and 7%, respectively.

However, caffeine consumption and aerobic training with synergistic effects (enhancement) decrease glucose index (da Silva et al., 2014; Shirali et al., 2016; da Silva et al., 2014). Researchers have claimed that chronic caffeine consumption improves the probability of glucose excretion from the bloodstream by activating GLUT4 expression pathways through increasing intracellular calcium concentration and AMPK enzyme expression (Sacramento et al., 2015; Jensen et al.,). Also, caffeine stimulates insulin secretion by pancreatic beta cells, increases cascade activity, and enhances the path of improving insulin secretion by stopping ATP-sensitive potassium channels in the pancreas and increasing calcium concentration (Wedick et al., 2011). On the other hand, Zaharieva et al. (2016) showed that caffeine with a dose of 6 mg/kg of BW 60 minutes before exercise (with the intensity of 60% VO2max for 45 minutes)

in people with type1 diabetes (with HbA1C equal to 7.4%) modulated hypoglycemia during exercise compared with placebo (Zaharieva et al., 2016).

Improvements in HbA1C due to caffeine consumption and aerobic training are associated with a decrease in inflammatory markers such as adiponectin and leptin secreted by adipose tissue. HbA1C is an indicator that shows the level of blood sugar in the last 8–12 weeks (Yousefipoor et al., 2015). In the present study, the levels of HbA1C decreased significantly by 10.5% following caffeine consumption. In contrast, following a pilot study, James (2011) stated that three-month avoidance of caffeine and caffeine compounds reduced HbA1C levels by 0.56% and improved long-term glucose control in 12 coffee-addicted consumers (consuming more than two cups of coffee per day) who had T2D (James, 2011). As observed in the present study, aerobic training with a higher effect than caffeine consumption reduced glucose, insulin, IR index, and HbA1c by 19.6, 15.6, 32.5, and 10% compared to the baseline, respectively. In this regard, Yousefipoor et al. (2015) noted a significant decrease in HbA1c and fasting blood glucose levels following a quasi-experimental study examining the effects of aerobic training, including three sessions of running per week with an intensity of 60–80% of maximum heart rate for eight weeks (Yousefipoor et al., 2015). A decrease in erythrocyte glycosylation improves oxygen delivery to muscle cells and increases maximal oxygen consumption (VO2max) in patients with T2D during exercise. Thus, with an increase in HbA1c, the patients with T2D develop chronic hypoxia resulting in compensatory polycythemia, which may eventually lead to systolic hypertension in individuals with diabetes (Yousefipoor et al., 2015). The present study showed that combining caffeine supplementation with aerobic training was involved in reducing HbA1c by 16.86%, which was a more effective method than applying each of them alone.

Moosavi et al. (2016) reported that glucose concentration and IR in women with T2D participating in an 8-week exercise program (with an intensity of 50 to 70% of maximum heart rate) were reduced (Moosavi et al., 2016). Exercise decreases blood glucose and improves insulin function. Researchers suggested that the beneficial effect of exercise can be mediated by mechanism as follows:

increase in insulin receptor signaling, glucose transporter relocation from intracellular stores to plasma membranes, glycogen synthase and hexokinase enzyme activity, improvement in muscle capillary function, changes in the muscle composition for glucose uptake and decrease in the release or clearance of FFAs (Frühbeck et al., 2018; Ayina et al., 2017; Farajpour et al., 2017; Torabi & Mirzaei, 2022). However, the failure to measure the upstream and downstream pathways of insulin messaging is one of the most critical limitations of the present study. Physical activity may increase the exocytosis of GLUT4 to the cell membrane for 48 hours after activity and improve insulin sensitivity by improving the proximal insulin signaling pathway (IR tyrosine kinase activity, PI3K activity, or IRS-1 tyrosine phosphorylation) and affecting the distal parts such as AS160 (Rasaei et al., 2019; Torabi & Mirzaei, 2022). Therefore, it is recommended that there should be no break for more than two days between physical activities to maintain sensitivity to insulin transmitters (Rasaei et al., 2019).

Conclusion

Based on the result, caffeine supplementation for two months and chronic aerobic training may prevent adverse changes in the risk indices in men with T2D by improving glucose homeostasis and other anti-diabetic properties. Therefore, considering the precautionary aspects, it can be suggested to patients with pre-diabetes and diabetes and even active individuals to use caffeine supplementation and regular aerobic training to prevent and reduce undesirable glycemic levels.

Ethical approval Code of ethics in research number IR.TBZMED.REC.1398.1179 was obtained from the Vice-Chancellor for Research and Technology of Tabriz University of Medical Sciences, and the trial was registered in Iran Clinical Trial Center (IRCT- 20170116031982N2).

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Table 1. Anthropometric and physiological characteristics of the participants

Variables	Groups N	P 8	C 8	AT 8	AT+C 8	Intergroup p-value
Age (Year)	Pre-test	50.23 ±2.42	49.78 ±3.56	52.10 ±1.74	51.7 ±2.43	0.46
VO_2 Max ($ml.kg^{-1}.min^{-1}$)	Pre-test	30.04 ±5.11	31.29 ±7.09	33.51 ±2.67	32.03 ±3.42	0.28
Energy received (kcal/day)	**Pre-test**	2525.3 ±115	2550.6 ±210	2610.5 ±167	2575.2 ±185	0.87
BMI (kg/m^2)	**Pre-test**	27.56 ±3.92	28.12 ±3.40	29.11 ±1.16	28.45 ±2.58	0.74
	Post-test	28.11 ±2.15	27.45 ±2.31	28.01 ±3.32	27.13 ±2.32	0.036
	within group p-value	0.54	0.036	0.02	0.01	
WHR	**Pre-test**	1.02 ±0.05	0.98 ±0.003	1.01 ±0.02	0.99 ±0.001	0.87
	Post-test	1.003 ±0.004	0.92 ±0.002	0.91 ±0.001	0.89 ±0.002	0.03
	within group p-value	0.37	0.03	0.02	0.01	
BF (%)	**Pre-test**	30.12 ±3.16	31.42 ±2.13	31.1 ±2.62	32.43 ±4.12	0.33
	Post-test	31.23 ±2.45	30.00 ±3.32	29.14 ±3.56	29.00 ±2.10	0.03¥
	within group p-value	0.45	0.051	0.003#†	0.001#†	

The values are expressed in standard deviation ± mean. AT, Aerobic Training, C, Caffeine, P, Placebo.
¥ Independent t-test
Paired t-test
† Significance compared to the placebo group

Table 2. Mean ± standard deviation of glucose and insulin resistance indices in men with type 2 diabetes, in Pre- and Post-test

Variables	Groups	Sampling steps		Percentage of changes	Within group p-value	Intergroup p-value
		Pre-test	Post-test			
FBS (mg/dl)	**P**	150.3 ±16.15	151.54 ±15.54	0.8	0.32	0.001¥
	C	148.16 ±14.12	128.13 ±4.08	–13.5	0.023‡	
	AT	151.85 ±18.81	122.01 ±10.96	–19.65	0.001‡	
	AT+C	148.14 ±19.45	117.17 ±4.48	–20.90	0.001‡	
HOMA-IR	P	4.55 ±0.53	4.64 ±0.63	1.9	0.13	0.001¥
	C	4.64 ±0.41	3.72 ±0.27	–19.82	0.03‡	
	AT	4.65 ±0.96	3.14 ±0.41	–32.47	0.001‡	
	AT+C	4.28 ±1.15	2.70 ±0.34	–36.91	0.001‡	
QUICKI	P	0.304 ±0.005	0.301 ±0.006	–0.98	0.08	0.001¥
	C	0.305 ±0.002	0.308 ±0.003	0.98	0.034‡	
	AT	0.303 ±0.010	0.318 ±0.006	4.95	0.001‡	
	AT+C	0.306 ±0.012	0.328 ±0.005	7.18	0.001‡	
HbA1c (%)	P	6.91 ±2.21	6.93 ±2.28	0.28	0.11	0.001¥
	C	6.89 ±2.01	6.15 ±1.81	–10.74	0.04‡	
	AT	6.93 ±2.32	5.92 ±2.42	–14.57	0.001‡	
	AT+C	6.88 ±2.31	5.72 ±1.87	–16.86	0.001‡	

The values are expressed in standard deviation ± mean. AT, Aerobic Training, C, Caffeine, P, Placebo.
‡ Significance within the group ($P < 0.05$).
¥ Significance between the groups ($P < 0.05$).

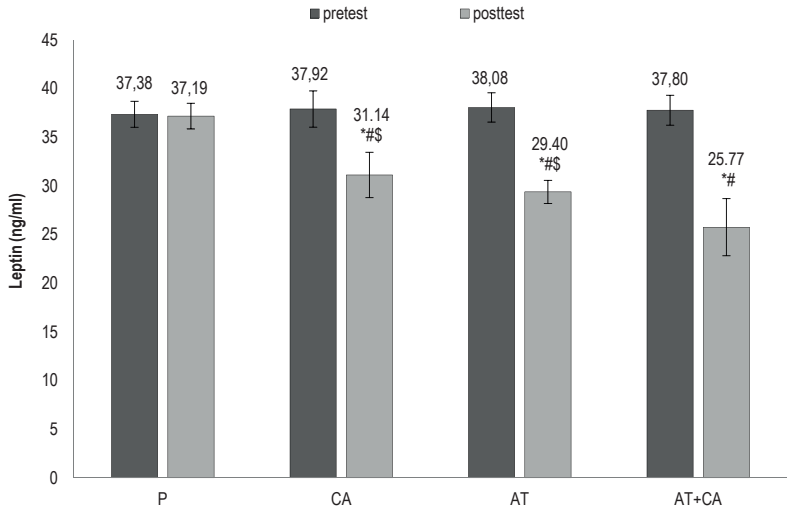

Figure 1. Serum leptin levels in men with type 2 diabetes

* Significance within the group (P <0.05). # Significance compared to the placebo group (P) (P < 0.05). $ Significance compared to the combined group (AT + CA) (P < 0.05).

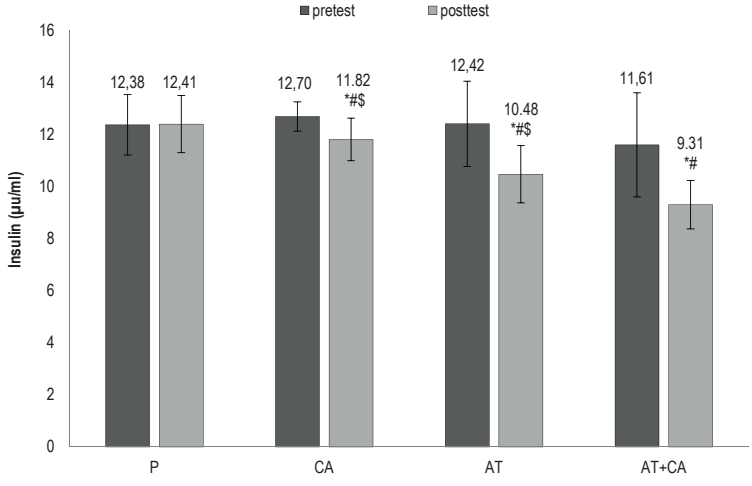

Figure 2. Serum insulin levels in men with type 2 diabetes

* Significance within the group (P < 0.05) # Significance compared to the placebo group (P) (P < 0.05). $ Significance compared to the combined group (AT + CA) (P < 0.05).

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