Studying the effect of metformin and aerobic exercise on some biochemical factors in diabetic and healthy rats

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Abstract

Introduction: High-intensity interval training (HIIT) and moderate-intensity continuous training (MCT) in combination with metformin in diabetic patients is likely to be effective. This research aimed to investigate the possible role of HIIT and MCT training alone and also in combination with metformin on biochemical factors and lipid profiles in diabetic and healthy rats.

Materials and Methods: Sprague-Dawley rats were allocated randomly into nine groups (in each group n = 5). Streptozotocin and nicotinamide were used to induce diabetes in target rats. Special diets were given to all groups of rats and exercise protocol was performed one time per week for 8 weeks. Rats received metformin (200 mg/kg) daily by gavage. The biochemical factors and serum lipid profiles were measured. Data analysis was performed using SPSS software and the significance level was considered at $P < 0.05$.

Results: The lowest serum glucose and insulin levels among diabetic rats belonged to the diabetic group who received metformin and performed HIIT training ($P < 0.05$). Diabetic groups that performed HIIT and MCT training compared with the diabetic group that consumed metformin alone had lower HbA1c levels, which this difference was not significant ($P < 0.05$). The lowest triglyceride level among the treated diabetic groups was in the group that received metformin and performed HIIT training ($P < 0.05$). The results of HDL, LDL and cholesterol changes were similar to those found for triglyceride.

Conclusion: The study showed that both HIIT and MCT exercise, even in the absence of metformin, significantly reduce some biochemical factors and lipid profile levels as well as improve body weight in the diabetic rats under treatment with metformin compared with the diabetic control group.

Keywords: Type 2 diabetes mellitus, Metformin, MCT training, HIIT training

Introduction

Type 2 diabetes is one of the most common metabolic diseases, identified with hyperglycemia that is characterized by insulin resistance due to autoimmune responses (1). Environmental factors such as stress, lifestyle changes, and obesity increase the risk of prevalence of type 2 diabetes diseases which reduce the quality of life and increase the risk of other diseases such as heart disease, damage of eyes, retinopathy, and nephropathy (2). One of the most effective treatments for controlling blood glucose levels and delaying and preventing the progression of diabetes in diabetic patients is exercise

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(3,4). Studies have shown that aerobic and endurance training regulate blood glucose in type 2 diabetes. HIIT and MCT in diabetic patients have increased survival rates in people with type I and II diabetes (5). In addition to exercise, diet and medication are also effective factors in regulating blood glucose levels (6). One of the most effective drugs in regulating blood glucose is metformin, which has been used as the first drug line treatment for type 2 diabetes. This anti-hyperglycemic drug reduces the production of sugar in the liver and reduces intestinal absorption of glucose, and also improves insulin sensitivity by increasing the use of peripheral sugar (7). This research aimed to investigate the possible role of HIIT and MCT, and also metformin alone or in combination with exercise on biochemical factors and lipid profiles in diabetic, and healthy rats.

Materials and Methods

Animals

The protocols of the animal experiments were employed in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Sprague-Dawley rats (weighing 240 ± 20g) were randomly allocated into nine groups (in each group n = 5) including the healthy control group received distilled water (C), the healthy control group performed MCT training (C + MCT), healthy control group performed HIIT (C + HIIT), diabetic control group received distilled water (CD), diabetic group received metformin (D + M), diabetic group performed MCT training (D + MCT), the diabetic group performed HIIT training (D + HIIT), the diabetic group received metformin and performed MCT training (D + M + MCT), the diabetic group received metformin and performed HIIT training (D + M + HIIT). All rats were housed under standard laboratory conditions (temperature: 21 ± 2 °C, humidity: 60 ± 5%, 12h light, and 12h dark cycle) and received standard rodent chow and water ad libitum. The animals were trained on a treadmill for 8 days before the start of the experiment to prepare for the exercise program. After this period, for 8 weeks the main exercise was performed.

Induction of Diabetes

Hyperglycemia was induced using intraperitoneal injection of streptozotocin (STZ) 65 mg/kg BW dissolved in 300-500 mol/l normal saline, after an overnight fasting. Then, after fifteen minutes, nicotinamide (200 mg/kg) dissolved in normal saline and administrated (8). Blood glucose level was assessed after 7 days from the induction of diabetes. Animals with Plasma glucose concentration > 300 mg/dL were selected as diabetic rats.

Administration of Metformin

Rats in groups D + M, D + M +MCT, and D + M + HIIT using oral gavage were treated with metformin 200 mg/kg BW once a day for 8 weeks, while the other groups used distilled water.

Training Protocol

The Protocol of MCT training including of 6 min warm up with an intensity of 40-50% of VO2max (maximal oxygen consumption) at the beginning of the running protocol then 40-60 minutes running (65-75% VO_2 max intensity) and at the end cool-down for 6 min with an intensity of 40-50% of VO_2max. But HIIT training protocol consists of 6 min warm up with an intensity of 50-60% of VO_2max at the beginning of the running protocol and followed via 3 times intervals four minutes with 90 to 100% VO2 max intensity and finally 6 min cool down with an intensity of 50-60% of VO_2max (9).

Assessment of the Biochemical Factors

The body weight of all rats was measured by using a digital weighing machine, twenty- four hours after the last training

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session. Afterward, all of the animals were anesthetized through intraperitoneal injection of 30-50 mg/kg ketamine and 3-5 mg/kg xylazine. Blood samples were collected directly from the hearts of animals and then placed into two tubes, one of which was EDTA anticoagulant tube containing 2 ml of blood specimen using for HbA1c assay and the other anticoagulant tube containing 4 ml of the blood sample for assessment of glucose, triglycerides, HDL, LDL, cholesterol and insulin. For biochemical analyses, serum was separated and immediately stored at -20 °C.

Biochemical Assessment

Biochemical tests of serum such as triglyceride, HDL, LDL, cholesterol, and also glucose was carried out by Pars Azmoon enzyme kits (Iran) using an auto-analyzer (Hitachi, Tokyo, Japan). HbA1c levels were determined by a calorimetric method using a Pars Test kit (Iran). Insulin was determined by ELISA kit (DEVELOP, Canada) based on the manufacturer's protocols.

Statistical Analysis

The one-sample Kolmogorov-Smirnov test was applied to test the normality of all parameters. The way analysis of variance (ANOVA) and post hoc LSD one was carried out to compare the changes between the groups. SPSS statistical software v. 16.0 was conducted for statistical analyses. Differences in parameters in each group were analyzed with a paired independent student t-test. The statistically significant level was determined at $P < 0.05$. To draw graphs, Excel software was utilized.

Results

In this study, changes in body weight, biochemical parameters, and lipid profiles in diabetic and healthy rats that were affected by physical activity and drug therapy with metformin, either alone or in combination with exercise, were evaluated. Comparisons of body weight, glucose, insulin, glycosylated hemoglobin, LDL, HDL, cholesterol, and triglyceride of rats between groups before and after treatment are shown in Figures 1 and 2. Data analysis showed that the mean body weight in the diabetic control group was significantly reduced compared to the non-diabetic control groups ($P < 0.05$). Also, the mean weight of diabetic groups who did both types of aerobic exercise (HIIT and MCT) was significantly increased compared to the diabetic control group ($P < 0.05$). The diabetic group that took metformin in addition to MCT and HIIT exercise had a lower average weight than the healthy control group and the healthy group that did both types of exercise, although this amount was not significant ($P > 0.05$) but it has increased compared to the diabetic control group ($P < 0.05$) (Figure 1A) Serum glucose levels of diabetic rats increased significantly compared to other experimental groups ($P < 0.001$). Furthermore, diabetic rats that performed both HIIT and MCT training and their combination with metformin and also metformin alone showed a significant decrease in glucose level. Interestingly, serum glucose is higher in diabetic rats that perform MCT training than other diabetic rats, except for the diabetic control group (Figure 1B). Serum insulin levels showed a significant elevation in the diabetic control rats compared to healthy groups (Figure 1C). Moreover, diabetic rats that performed both types of exercise training (HIIT and MCT) showed a statistically significant increase ($P < 0.05$) in serum insulin compared to HIIT and MCT training in combination with metformin and metformin alone. The lowest level of insulin was among the diabetic group that belongs to D + M + HIIT group.

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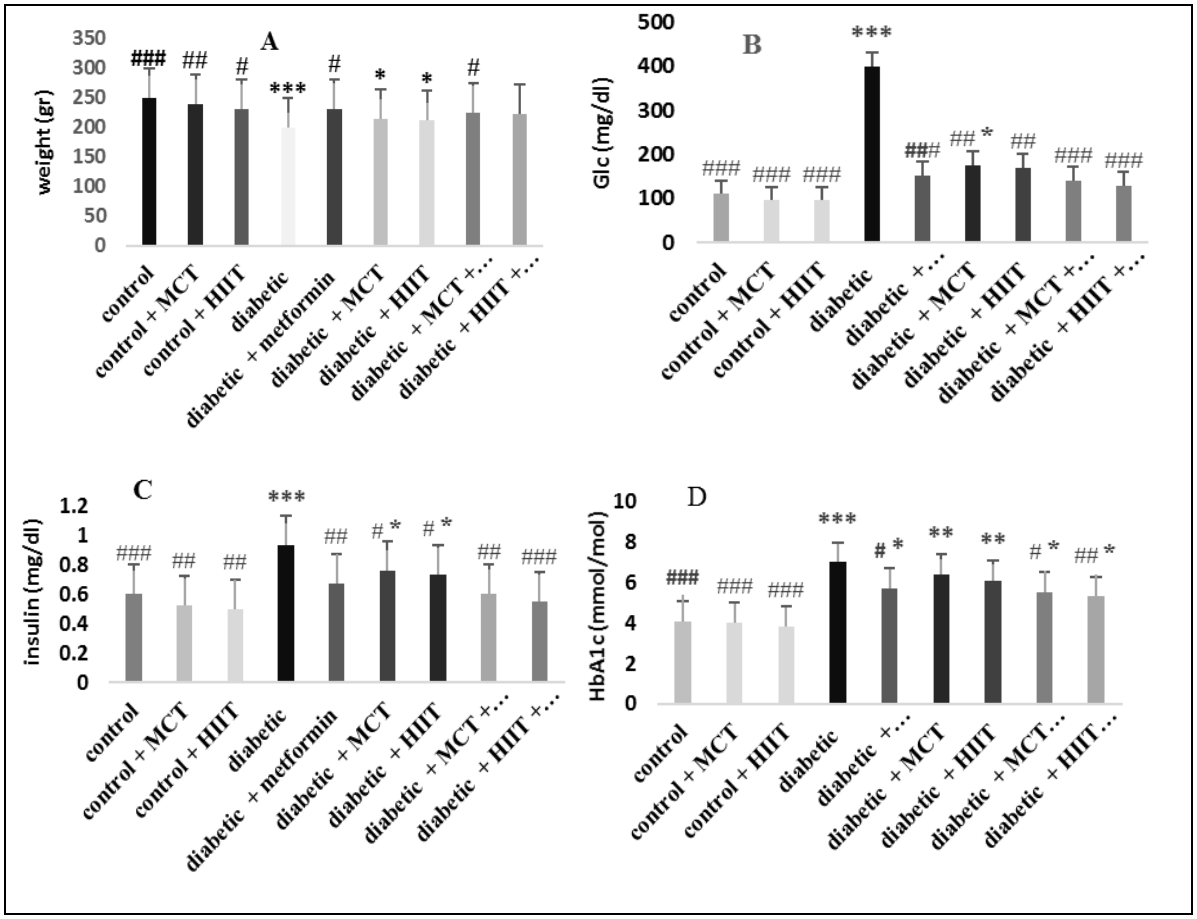


Figure 1. The changes of body weights (A), serum glucose levels (B), insulin (C) and HbA1c (D) for the 9 animal groups after eight weeks of treatment by physical activity, metformin and their combination in healthy and diabetic rats are shown (n = 5).
$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ the comparison between diabetic treatment groups and diabetic control.
$^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ the comparison between diabetic treatment and diabetic control groups with healthy control group.

Also, HbA1c concentration showed a significant increase in the diabetic control rats compared to the non-diabetic rats (Figure 1D). In addition, the levels of HbA1c in diabetic rats that performing both exercises alone ($P < 0.01$) and in combination with metformin and metformin alone significantly decreased ($P < 0.05$) when compared to diabetic control groups. The lowest level of HbA1c among the treated diabetic groups is related to D + M + MCT and especially D + M + HIIT groups. On the other hand, with comparing the treated diabetic group, the highest rate of HbA1c was related to the diabetic group doing MCT training ($P < 0.01$).

The triglyceride serum levels in all groups of rats have been shown in Figure 2A. Triglyceride levels were markedly higher in the diabetic control group than non-diabetic group. The level of TG significantly decreased ($P < 0.001$) in D + M + HIIT and D + M + MCT groups than the diabetic control group. The serum cholesterol level in the diabetic control group was significantly elevated in comparison to all experimental groups. However, this difference is not significant for D + M groups (Figure 2B). D + HIIT, D + M + MCT and D + M + HIIT were almost equally effective in lowering cholesterol of serum in comparison with diabetic control group ($P < 0.01$). LDL levels significantly increased in the diabetic control group compared to the healthy control groups. In fact, the

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induction of diabetes in the rats increased meaningfully the serum LDL levels when compared with healthy rats with the exception of the D + M + HIIT group that decreased levels of LDL even compared to healthy control rats. Among the diabetic groups, rats who did MCT training showed raiser LDL levels than the other, although this was not noticeable with the diabetic control group. The level of LDL in diabetic rats with metformin alone and exercise treatments in combination with metformin significantly declined (P < 0.05) when compared to diabetic control rats (Figure 2C). The HDL ratio was significantly higher in the diabetic control rats compared with all diabetic treated animal rats and also all healthy rats. Groups of metformin-treated diabetic rats that performed both types of aerobic exercise (MCT and HIIT) markedly reduced HDL levels is compared with the diabetic control group (P < 0.01) (Figure 2D). In addition, it should be noted that both D + M and D + MCT were equally effective in reducing HDL (P < 0.05).

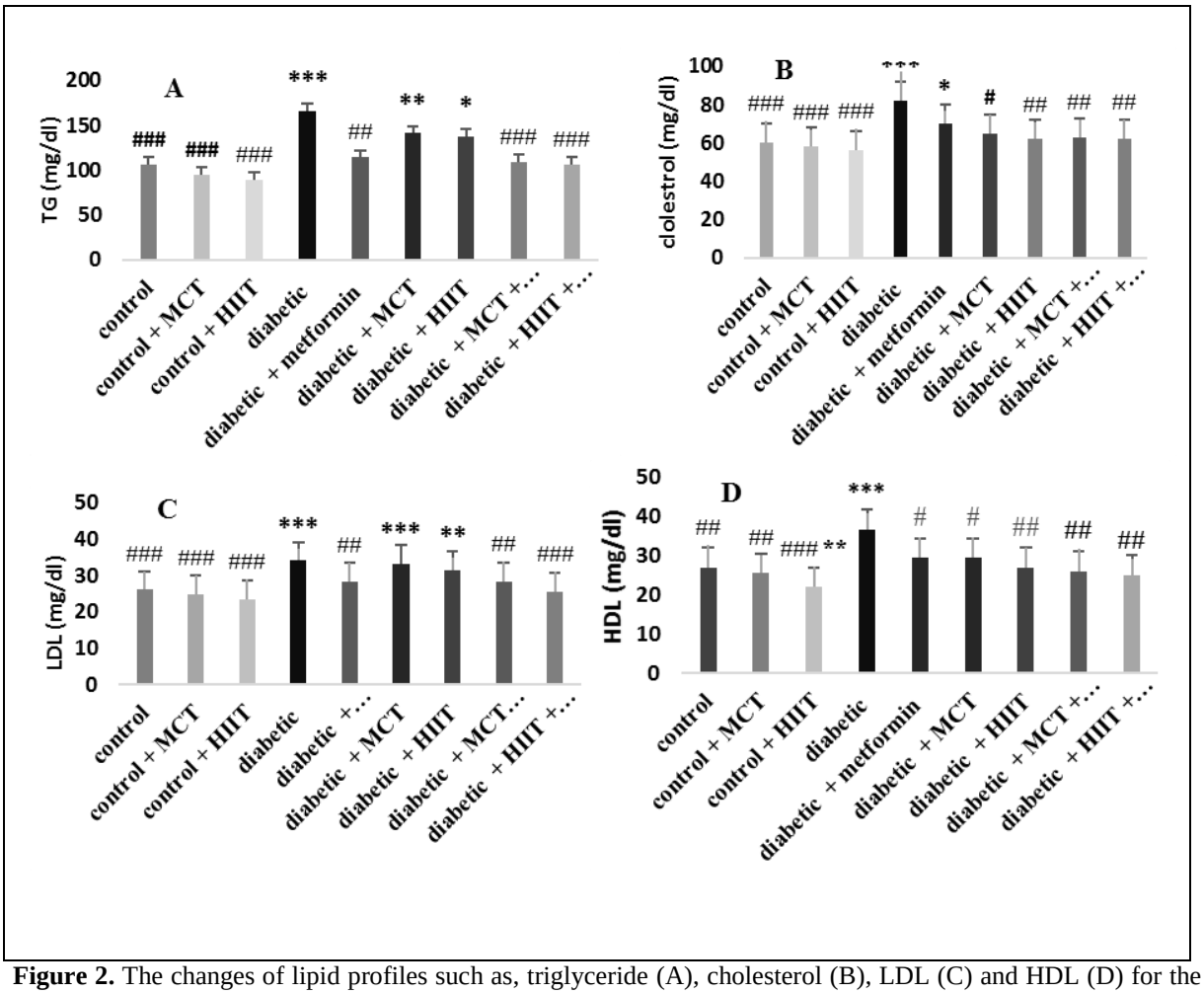


Figure 2. The changes of lipid profiles such as, triglyceride (A), cholesterol (B), LDL (C) and HDL (D) for the 9 animal groups after eight weeks of treatment by physical activity, metformin and their combination in healthy and diabetic rats are shown (n=5).

$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ the comparison between diabetic treatment groups and diabetic control.

$^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ the comparison between diabetic treatment and diabetic control groups with healthy control group.

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Discussion

The findings of the present study indicated that HIIT and MCT significantly reduced blood glucose and insulin levels compared to the diabetic control group. The effect of HIIT was slightly more than MCT, but this effect was not statistically significant. Terada et al. found that HIIT resulted in a further reduction in blood sugar levels compared with MCT in people with type 2 diabetes who had been training for 12 weeks (11). In contrast to the findings of this study, Cauza et al. did not report any significant decrease in blood glucose levels after four months of aerobic exercise on type-2 diabetic subjects, and Bello et al. reported no reduction in blood glucose levels after eight weeks of aerobic exercise (12,13). The possible mechanism of decreasing serum insulin and glucose as a result of exercises can be due to an increase in the insulin-regulated glucose transporters (GLUT4), decrease in secretion, increase in clearance of free fatty acids, increase in glucose transport to muscles, and change in increased tendency of muscles to available glucose (14). In addition to exercise, medication can also be effective in regulating blood sugar (15). The present study also indicated that using metformin in one of the diabetic groups, compared to the diabetic control group, caused a significant reduction in blood sugar and insulin. Interestingly, diabetic rats treated with exercises alone (HIIT and MCT) had significantly lower blood glucose levels than diabetic rats treated with metformin alone. However, concomitant use of metformin accompanied by exercise resulted in a significant reduction in blood glucose levels. According to this study, it can be concluded that HIIT can be a very efficient option for the management and control of type-2 diabetes due to its near-term effect with metformin alone. Several studies showed a significant reduction of serum insulin and glucose levels in diabetic groups and HIIT and diabetes and moderate-intensity exercises compared to diabetic control and diabetes and low-intensity exercise (9,16,17). In the present study, the mean insulin reduced significantly under the effect of aerobic exercise.

The HbA1c levels in groups of diabetic rats that took both types of exercise alone were significantly decreased compared to the diabetic control group. It should be mentioned that the effect of HIIT was greater than MCT, although this difference was not significant. Maiorana et al. reported a significant decrease in HbA1c after aerobic exercise (18). In contrast, Jorgea et al. reported no significant change in glycosylated hemoglobin levels after aerobic exercise in type-2 diabetic subjects (19). Groups of diabetic rats that took metformin alone showed a significant decrease in the level of HbA1c compared to the diabetic control group. However, the effect of metformin in reducing glycosylated hemoglobin was more than the effect of both types of exercise. In one group of rats that used metformin with exercise (both exercise), their HbA1c levels were significantly lower than in the other treated diabetic groups.

The diabetic control group in this study also had the lowest weight average compared to other treated diabetic groups. In addition to a decrease in blood sugar, weight loss was also observed in the HIIT group which was contrary to expectations. This is probably due to more fat burning and reduced fat mass in HIIT than MCT (20).

Based on the findings of this study, the use of metformin alone and in combination with both types of exercise and exercise alone improved the lipid profile in treated diabetic rats compared to the diabetic control group. In line with the present study, Kadoglou et al. reported a significant reduction in lipid profile (LDL, HDL, Chol, and TG) after 16 weeks of aerobic exercise (21). Interestingly, both HIIT and MCT types of exercise alone reduced triglyceride levels, even in the

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absence of metformin, although the effect of HIIT exercise was more than MCT exercise. Therefore, exercise alone and without the use of drug therapy can be effective in reducing triglyceride levels and managing diabetes, but when exercise is combined with medication, these effects are more pronounced. These results were consistent with the study by mahmoudi et al. (9). Serum cholesterol was significantly decreased compared to the diabetic control group by performing HIIT and MCT as well as metformin. Marwick et al. showed that exercise training decreases total cholesterol in patients with type 2 diabetes mellitus, which was in line with the current study (22). The results of LDL changes were similar to those found in cholesterol. Oberbach et al. also reported a significant decrease in LDL and TG after 4 weeks of aerobic exercise (23). Since HDL transports cholesterol from the blood vessels to the liver and prevents the accumulation of fats in the blood vessels, it is expected to decrease in diabetic patients (24), while the results of this study contradict this statement. The present study reported that HDL levels were significantly increased in diabetic control rats compared to healthy control rats. The reason for the increase in HDL is probably due to its secondary role in the removal and transfer of the excess cholesterol produced in the diabetic group. These findings are consistent with the results of the study by Eatemady-Boroujeni et al. which have shown that the HDL level in aerobic exercise and resistance training increased (25). As indicated in this study, the level of cholesterol in the diabetic group was significantly increased; this increased amount had to be removed by HDL, consequently requiring more HDL. The average HDL in diabetic rats performing HIIT and MCT exercise as well as the metformin-receiving group was lower than the diabetic control group, but using metformin boosted by exercise has an increasing effect on reducing HDL levels. A study reported that aerobic exercise reduces total cholesterol but has no effect on HDL and LDL (26). The general results of this study show that exercise alone and without the use of drug therapy can be effective in regulating biochemical factors and lipid profile and thus controlling diabetes.

Conclusion

Exercise alone can be effective in improving biochemical factors and lipid profiles and thus diabetes management, but when combined with metformin, it has synergistic effects.

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