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The Effect of Training Type on The Signaling Pathway of Ceramide-Dependent Insulin Resistance in The Flexor Hallucis Longus Muscle of Streptozotocin-Induced Diabetic Rats

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Abstract

Objective: This study aimed to compare the effect of different physical training on the mechanism of ceramide-dependent insulin resistance in the flexor hallucis longus (FHL) muscle of diabetic rats.

Materials and Methods: In this experimental study, 7 healthy as a healthy control (HC) group, and 21 diabetics (55 mg/kg Streptozotocin) Wistar rats (200-220 g; 8-10 weeks old) divided into the diabetic control (DC), moderate continuous training (MCT), and moderate intensity interval training (MIIT) groups. Both MCT (55-70% of maximal oxygen uptake (VO_{2max}), and MIIT (85% VO_{2max}) groups trained for 10-25 minutes at a speed of 10-20 m/minutes. The changes in the expression of blood glucose, insulin, insulin resistance, lipid profile and total ceramide were measured as well as ceramide synthase-1, Glucose transporter type 4 (GLUT4), Protein kinase B known as Akt, phosphorylated protein kinase B known as pAkt, protein kinase C (PKC), and tumour necrosis factor α (TNFα).

Results: Blood glucose, triglyceride (TG) and ceramide synthase-1 (CS1) expression levels in the MCT group decreased in comparison with the DC group. FHL protein expression of GLUT4 in the MCT group was higher than the DC group. FHL expression of GLUT4, pAKT, AKT/pAKT, PKC, CS1 and total ceramide in the MIIT group were higher than the DC group. Cholesterol, low-density lipoprotein (LDL), TG, and TNF-α protein expression in the MIIT group were lower than the DC group. GLUT4, PKC, pAKT, AKT/pAKT in the MIIT group were higher, and total ceramide and TNF-α were lower in the MIIT group than the MCT group.

Conclusion: It seems that both training plan MIIT and MCT have favorable effects on the metabolism of glucose, insulin, lipids, and the decrease of TNFα level in the diabetes, but in connection with the improvement of the ceramides mechanism, it seems that the MIIT training plan is more optimal than MCT training plan.

Keywords: Ceramides, Diabetes Mellitus, Exercise, Insulin Resistance, Muscle

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Introduction

Diabetes mellitus is recognized as a major global health problem that affected 463 million people worldwide to date (1). This disease includes 2 main types. In the type 1 diabetes, the body's immune system attacks and destroys the cells that produce insulin, and the body does not produce enough insulin. Type 2 diabetes leads to the compensatory hyperinsulinemia response following an insulin sensitivity reduction and/or an increase in insulin resistance (2, 3). An Insulin dysfunction in both type 1 and 2 diabetes in the long term can lead to cellular redox disorders, increased inflammatory factors, and mitochondrial dysfunction (1, 4). In the other words, a disorder in the metabolism of lipids disrupts the metabolism of sphingolipids. Sphingolipids are a class of complex lipids which is composed of a long-chain amino alcohol, and ceramide as their core structure. The ceramide disorders lead to increase insulin resistance, impair transport of fatty acids into the cell and the accumulation of ceramides (1). Also, ceramides activate protein kinase C (PKCζ), Akt/protein kinase B (PKB) inhibitor and cytosolic protein phosphatase 2 A (PP2A) activator, disrupts glucose transporter pathway (GLUT) (1, 5, 6). Increased levels of ceramid16:0 (C16:0), decrease ceramide synthase 6 (CerS6) level, impairs sphingomyelin production and sphingomyelinase enzyme function (as enzymes involved in the metabolism of cell membrane lipids) lead to decreased insulin sensitivity (5, 7).

On the other hand, increasing progress of diabetes,

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the need for non-invasive methods to prevent and treat this disease has been examined, so that the role of regular physical activity in reducing cardiovascular risks, hypoglycemia, and improved insulin in diabetic patients reported by researchers (8). Exercise can reduce the accumulation of ceramides in muscle cells by the mechanism of increasing mitochondrial biogenesis, increasing GLUT4 expression, increasing lipid metabolism, and improving sphingolipid synthase 1 and 2 (SGMS1/2) enzyme function. Also, it has been reported that 12 weeks of combined aerobic and resistance training, increased the insulin sensitivity, diacyl glycerol, ceramide, and SGMS1 in obese women, nevertheless, SGMS2 and Glut4 protein levels did not change (9). A three-hour acute training session in trained and untrained individuals was associated with increased sphingomyelin levels after exercise, nevertheless, sphingomyelinase and ceramide levels did not change significantly (7). 12 weeks of high intensity interval training (HIIT), reduced ceramide synthase 1 (Cers1), interleukin 6 (IL6), Nuclear factor kappa-light-chain-enhancer of activated B -1 (NFKB1) and tumor necrosis factor alpha (TNF-α) gene expression in skeletal muscle (10). Although, the response of ceramides to exercise depends on the duration (11), type (9), intensity of the exercise (12), the mechanism of effect of interval and continuous exercise on ceramide-dependent insulin resistance in skeletal muscle tissue is not yet fully understood.

In addition, the review of previous studies has mostly been in the field of comparative high and low intensity exercises, few studies have compared moderate continuous training (MCT) and moderate intensity interval training (MIIT). Therefore, considering the importance of the mechanism of ceramides in insulin resistance and lipid metabolism, it seems that studying the type of training on this mechanism will help researchers to obtain more information in this field. According to the published studies, it seems that the comparison of two types of MICT and MIIT exercises on the mechanism of glucose, insulin and the changes of inflammatory factors related to ceramides is one of the innovations of the current research and can provide more comprehensive information in this field to the researchers in the field of exercise and health. Therefore, the present study aimed to compare the effect of MIIT and MCT on the mechanism of ceramide-dependent insulin resistance in the flexor hallucis longus (FHL) muscle of diabetic rats.

Material and Methods

All steps of working with laboratory animals in this project were taken according to the Helsinki agreement and under the supervision of the Ethics Committee of Islamic Azad University of Marvdasht Branch, Shiraz, Iran (IR.IAU.M.REC.1400.037).

Maintenance and grouping of rats

Twenty eight Wistar rats with age range 8-10 weeks and an average weight of 200-220 g were purchased from the Laboratory Animal Breeding and Reproduction Center of Baqiyatallah University (Tehran, Iran) and transferred to the medical science laboratory of Kurdistan University (Kurdistan, Iran). The animals were kept in standard polycarbonate cages with autoclave capability, with free access to food and water as well as standard conditions (12:12 hour light-dark cycle, relative humidity of 55- 60% and a temperature of 22- 24°C). Then, after 14 hours of fasting and under anesthesia, 21 rats received peritoneally 55 mg/kg streptozotocin (572201, Sigma Aldrich, USA), dissolved in citrate buffer (P4809, Sigma Aldrich, USA) (2). After four days, blood glucose levels in rats were measured using a glucometer (DA12-B1, Delta, Taiwan), and ear punching procedure. Based on blood glucose, diabetic rats (blood glucose above 250 mg/dL) were divided into 3 groups of 7 rats as 1) diabetic control (DC), 2) MCT and MIIT as well as 7 healthy rats selected as healthy control (HC) group to review the effects of diabetes induction.

Continuous and interval training

To perform adaptation training, rats trained every day with a speed of 10 m/minutes and slope of zero for 15 minutes for one week. Then, the MCT group members were trained 10-25 minutes with speed of 10-20 m/minutes, equivalent to 55-70% of the maximum oxygen consumption, for 3 sessions per week. The MIIT group members were trained at a speed of 10-15 meters per minute, with 2-3 repetitions of 5-8 minutes at a high intensity equivalent to 85% maximum oxygen consumption and low intensity repetitions at a low intensity of 50-55% maximum oxygen consumption on a 0 to +5 slope for 3 sessions per week (Table 1).

Sampling

The rats sacrificed by injection of xylazin (10 mg/kg) (CAS 7361-61-7. Santacruz, USA) and ketamine (90 mg/kg, CAS 1867-66-9. Santacruz, USA) after 14 hours of fasting. After laboratory experts' complete ensuring of anesthesia by gently squeezing the animal's foot and testing the sensation of pain, 5 cc blood of the heart left ventricle was taken. Then the serum samples of taken bloods in this study were used to measure glycemic indices (blood glucose, insulin, insulin resistance) and lipid profile (cholesterol, triglycerides, low-density lipoproteins and high-density lipoproteins). After that the FHL muscle was carefully extracted under sterile conditions by making an incision on the dorso lateral region of the lower limb and cutting the tendon. Then, it was immediately stored at -80°C until transfer to the laboratory.

Table 1: Continuous and interval training protocol in the present study

Groups	Week							
	One	Two	Three	Four	Five	Six	Seven	Eight
MCT								
Speed (m/minutes)	10	10	12	14	16	18	20	20
Duration (minutes)	10	10	15	18	20	22	25	25
Slope (percentage)	0	0	0	0	0	0	0	0
MIIT								
Speed (m/minutes)	10	10	12	13	14	14	15	15
Duration (minutes)	2*5	2*6	2*7	2*8	2*9	3*7	3*8	3*8
Slope (percentage)	0	+1	+1	+2	+3	+4	+5	+5

MCT; Moderate continuous training and MIIT; Moderate intensity interval training.

Measurement of lipid profile, blood glucose, and insulin resistance

In this study, insulin resistance and blood glucose were measured using HOMA-IR method (13). Also, serum levels of high-density lipoprotein (HDL), cholesterol (Chol), low-density lipoprotein (LDL), and triglyceride (TG) were measured with an immunohistochemistry based method (Pars Azmoon Co., Tehran, Iran).

Western blotting Measurement

Using a lysing buffer (E-CK-A105, Elabscience, Houston, Texas, USA), protein samples were separated by page gel made of polyacrylamide and the addition of ammonium persulfate (APS) and tetramethyethylenediamine (TEMED). After the electrophoresis step, the gel proteins on PVDF paper were shaken in methanol and washed for 1 minute with distilled water and then placed in a transfer buffer. After transferring the proteins to the surface of PVDF paper, the paper was shaken with blotting solution for one hour and 15 minutes at room temperature. The paper was then exposed to the initial β-actin antibody overnight at 4°C and the next day the paper was washed 3 times each time for 15 minutes with TBS-T buffer. The paper was then incubated with Anti Rabbit secondary antibody for one hour. After incubation, the paper was washed three times with TBS-T buffer for 15 minutes each time. For exposure, the desired protein band was exposed with the ECL advanced reagent kit (GERPN2236, Sigma Aldrich, USA) and using radiology films. The blots were then washed in the stripping buffer and β-actin antibody was added to the paper and incubated again with the secondary antibody. Thus, the control β-actin was also exposed in the radiology film, and finally the exposed bands went through densiometry using Imaje J software (NIH, USA) (14).

Measurement of total ceramide and insulin levels

In this study, total ceramide levels were measured in FHL muscle tissue with a sensitivity of 1 ng/ml, and serum insulin levels were measured by ELISA kit (MBS7255105., Mybiosurse Co., Canada) with a sensitivity of 200 pg/ml.

Statistical data analysis tests

Normal distribution of data examined by Shapiro-Wilk test. Considering the normality of data distribution, one-ANOVA and Tukey's post- hoc tests were used to review the difference between groups. And statistical significance was defined as $P<0.05$. To do the statistical data analysis, SPSS software version 19 was used (IBM CO, Chicago, USA). In addition, to draw the graphs, Graphpad Prism 8.3.2 software (Dotmatic company, San Diego, California, USA) was used.

Results

Changes in the levels of serum blood glucose, insulin, insulin resistance and glucose transporter (Glut4) proteins

Our results of blood glucose serum levels showed that there was a significant difference between the DC group in comparison with the HC group ($P<0.0001$), nevertheless in the MCT group a significant decrease was observed in comparison with the DC group ($P=0.0258$, Fig.1A).

Regarding serum insulin levels, the results indicated that. Insulin levels in the DC group showed a significant

decrease in comparison with the HC group (P=0.0134) and also, it showed a significant increase in the MCT group in comparison with the MIIT group (P = 0.0237, Fig.1B).

There was no significant difference between the groups in the index of insulin resistance (Fig.1C).

There was a significant difference in the Glut4 protein concentrations in the FHL muscle of the DC group showed a significant decrease in comparison with the HC group (P<0.0001) and increased in the MCT (P=0.0343) and MIIT (P<0.0001) groups compared to DC group as well as in MIIT group increased significantly compared to MCT group (P<0.0001, Fig.1D).

Lipid profile

The results showed high-density lipoprotein (HDL) levels decreased in the DC group in comparison with the HC group (P<0.0001) and increased in the MCT group in comparison with the MIIT group (P=0.0023, Fig.2A).Also, cholesterol levels increased significantly in the DC group in comparison with the HC group (P<0.0001), nevertheless in the MIIT group decreased in comparison with the DC group (P=0.0242, Fig.2B).

LDL levels increased in the DC group in comparison with the HC group (P<0.0001) and in the MIIT group decreased in comparison with the DC group (P=0.0419, Fig.2C).

TG levels in the DC group increased in comparison with the HC group (P<0.0001) and decreased in the MCT (P=0.0005) and MIIT (P=0.0005) groups in comparison with the DC group (Fig.2D).

Total changes in ceramide and ceramide synthase-1

The results showed that there is a significant difference between total ceramide levels in the DC in comparison with the HC group (P<0.0001), while it was decreased in the MIIT group in comparison with the CD (P<0.0001) and MCT (P<0.0001) groups (Fig.3A).

The CS1 level in the DC group increased significantly in comparison with the HC group (P=0.0002), and also its level was decreased in the MCT (P=0.0060) and MIIT (P=0.0004) groups in comparison with the DC group (Fig.3B).

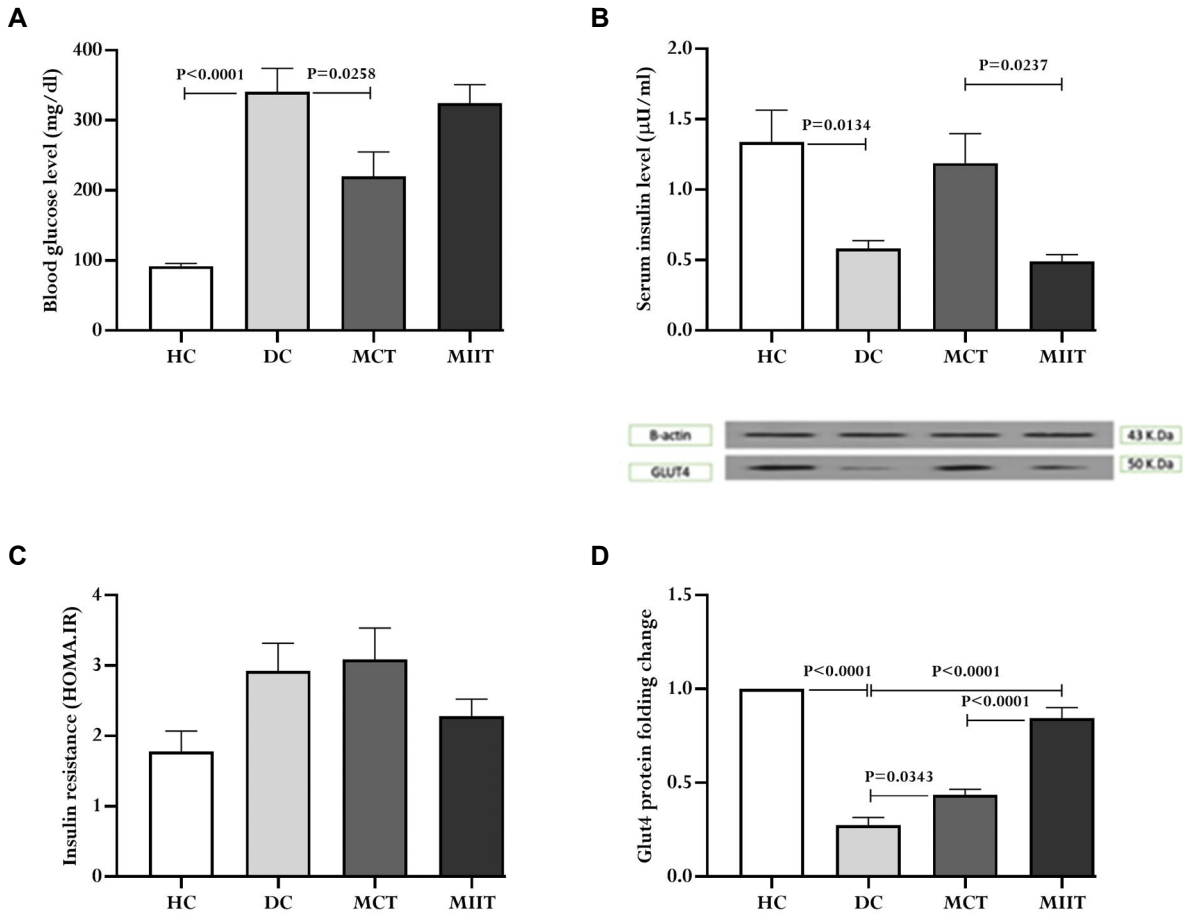

Fig.1: Glycemic indices of rats. **A.** Glucose, **B.** Insulin, **C.** Insulin resistance, and **D.** GLUT4 protein concentration in FHL muscle. HC; Healthy control, DC; Diabetic control, MCT; Moderate continuous training, MIIT; Moderate intensity interval training (n=7), and FHL; Flexor hallucis longus.

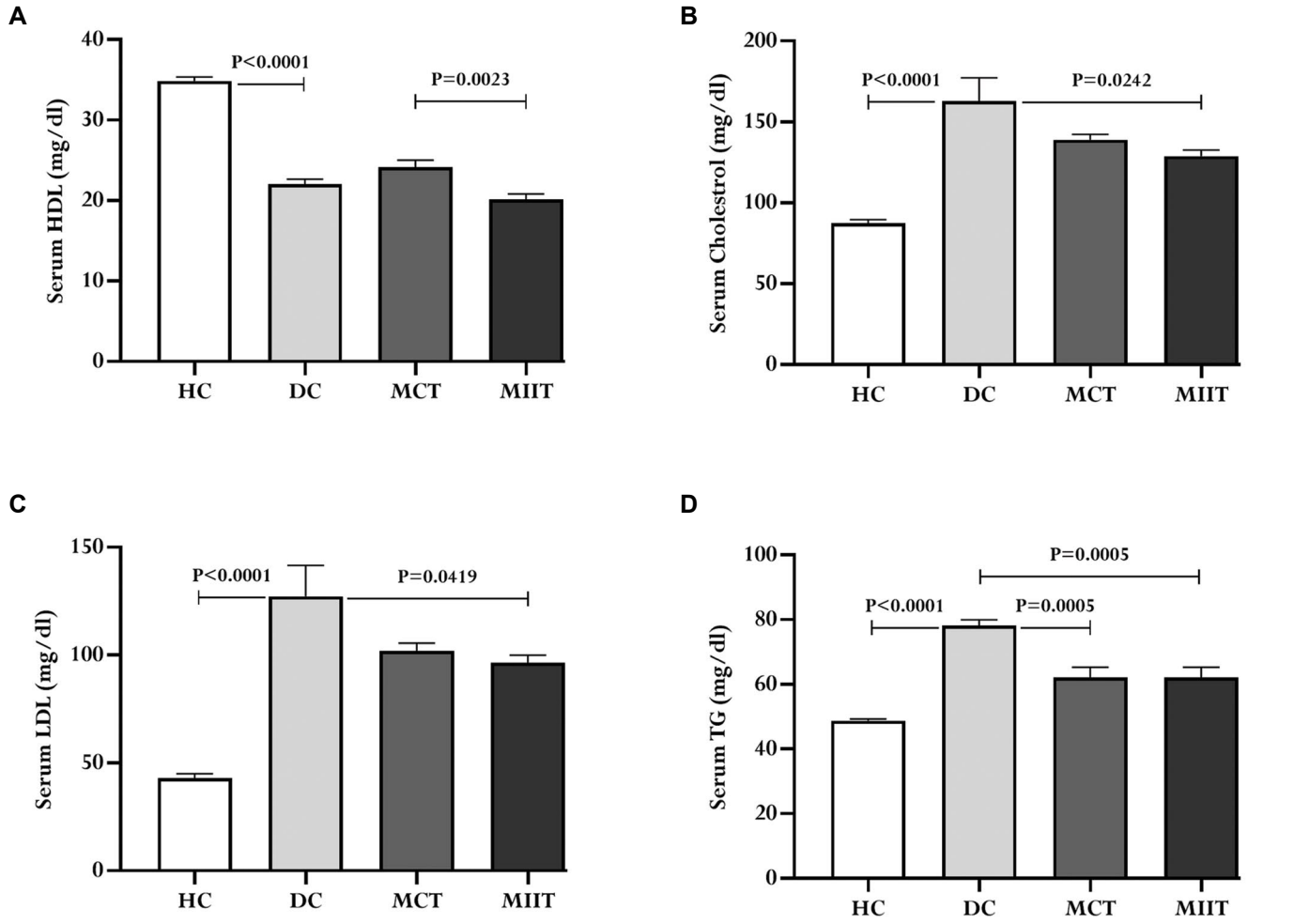

Fig.2: Lipid profile levels of rats. **A.** HDL levels, **B.** Total cholesterol, **C.** LDL levels, and **D.** TG levels. HC; Healthy control, DC; Diabetic control, MCT; Moderate continuous training, MIIT; Moderate intensity interval training (n=7), HDL; High-density lipoprotein, LDL; Low-density lipoprotein, and TG; Triglyceride.

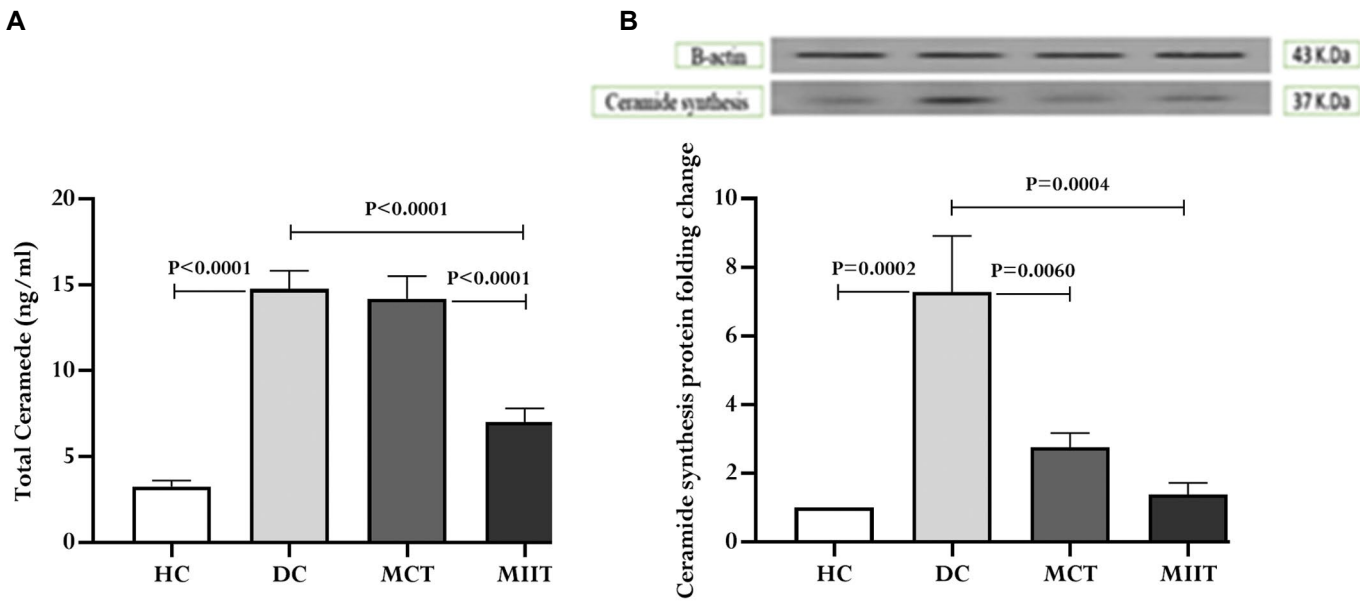

Fig.3: Total ceramide levels and ceramide synthase-1 concentrations in the FHL muscle tissue of rats. **A.** Total ceramide and **B.** Ceramide synthase concentration-1. HC; Healthy control, DC; Diabetic control, MCT; Moderate continuous training, and MIIT; Moderate intensity interval training (n=7).

Changes in insulin resistance signaling pathway proteins (PKC, pAKT, AKT and TNF-α) in the FHL muscle

Regarding Akt, although no significant difference was observed among groups (Fig.4A), its phosphorylated form (pAkt) decreased in the DC group in comparison with the HC group (P<0.0001). Also, the pAkt protein concentration in the MIIT group increased significantly in comparison with the DC (P<0.0001) group (Fig.4B). Also, the Akt / pAkt ratio in the DC group decreased significantly in comparison with the HC group (P<0.0001), while we observed a significant increase in the MIIT group in comparison with the DC (P≤0.0008), while in the MIIT group increased in comparison with MCT (P=0.0004) groups (Fig.4C).

Regarding PKC concentrations in the FHL muscle, our findings showed that the amount of this signaling protein in the DC group decreased significantly in comparison with the HC group (P<0.0001), while in the MIIT group increased in comparison with the DC (P<0.0001) and MCT (P<0.0001) groups (Fig.4D).

The amount of TNF-α inflammatory index increased in the DC group rather than the HC group (P<0.0001). However, interval training (MIIT group) caused the levels of this inflammatory protein to be lower than the DC (P=0.0004) and MCT (P=0.0041) groups (Fig.4E).

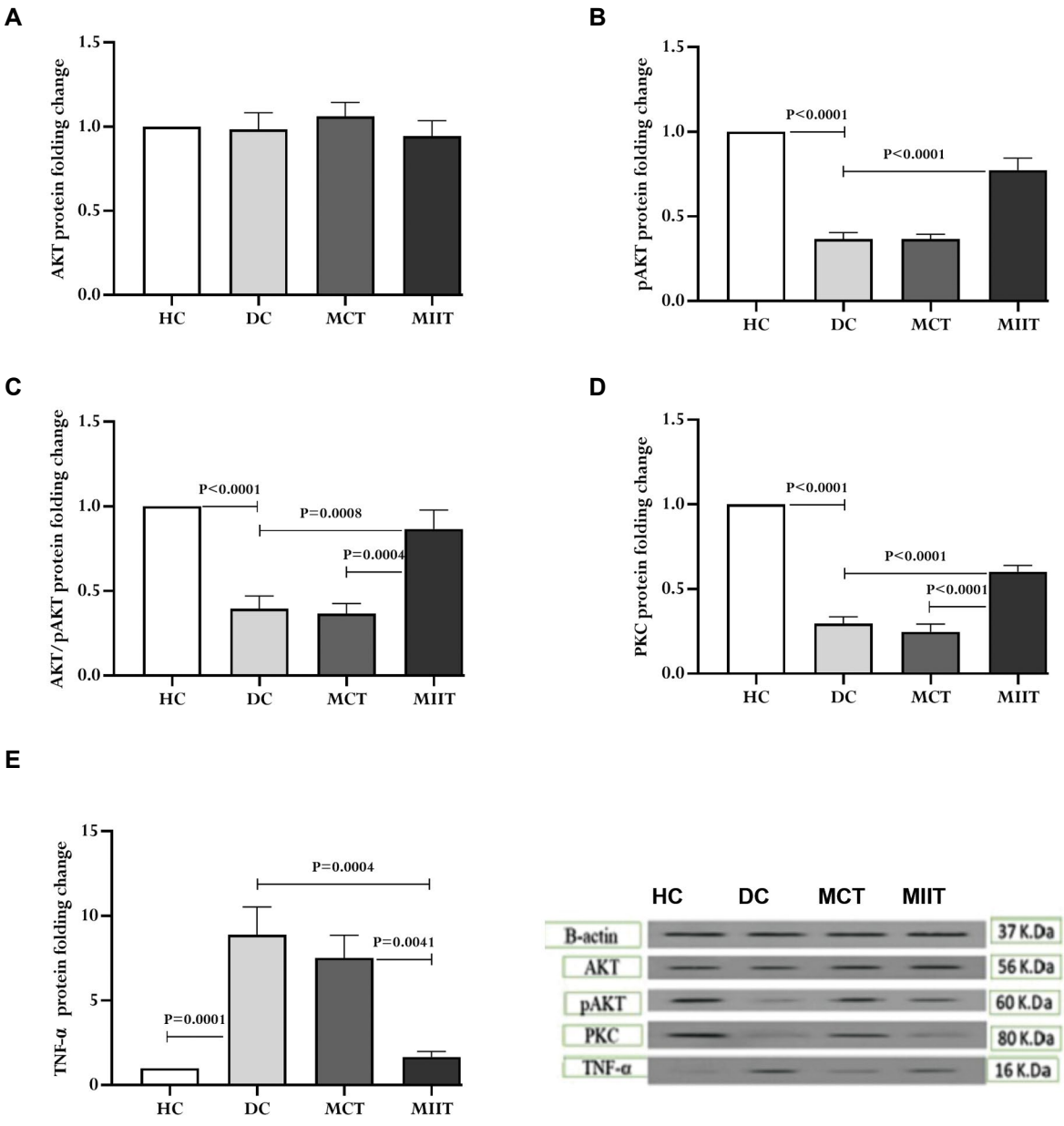

Fig.4: Concentrations of insulin resistance signaling pathway proteins in the FHL muscle tissue of rats**. A.** Akt concentration, **B.** pAkt concentration, **C.** Akt / pAkt ratio, **D.** Pkc concentration, and **E.** TNF-α concentration. HC; Healthy control, DC; Diabetic control, MCT; Moderate continuous training, MIIT; Moderate intensity interval training (n=7).

Discussion

The present study showed that MCT decreased the level of blood glucose as well as MIIT, and MIIT increased muscle GLUT4 protein concentration. The MIIT had more favorable effects on the increase of muscle GLUT4 protein concentration and decrease of insulin level in comparison with the MCT. Consistent with the present study, eight weeks of swimming and high-intensity swimming training increased the tissue GLUT4 protein and decreased insulin level in the type 2 diabetes rats, but high-intensity training had a more favorable effect on these indices such as an aerobic capacity (15). However, it seems that the effect of intensity of exercise on the dominant fuel for the type of exercise is a factor for not having a significant effect of increasing GLUT4 and decreasing blood glucose levels. In the present study, low intensity training also improved the lipid profile, decreased TG, and increased HDL. Also, in an eight-week of MICT with 50% VO_{2max} and HIIT with 90% VO_{2max} increased the protein level of the GLUT4 in the skeletal muscle of obese rats, so that both trainings had the same effect (16). According to the above studies, it can be concluded that the acute exercise with intensities above 55% VO_{2max} leads to an increased glucose uptake from non-insulin-dependent pathways, increases membrane levels of glucose carriers, and also by increasing pathways to hypersensitivity, increases energy demands, rises intracellular Ca^{2+} levels and activates phosphatidylinositol 3-kinase (PI3K). In addition, the intensity of a long-term training to select the dominant muscle fuel supply leads to the adaptation of pathways of a glucose uptake increase or lipolysis pathways (17).

In the present study, MCT decreased TG as well as MIIT decreased levels of TG, LDL and total cholesterol; also MIIT had more significant effects on the HDL level increase in comparison with the MCT. A study showed that the exercise increases diacylglycerol (DAG), and phosphoinositide 3-kinases (PI3K) (5). It has been reported that 6-8 months of endurance training and resistance training caused an increase in the levels of lipoprotein, insulin resistance index (18); therefore, the role of training duration in improving lipid and glucose metabolism. However, Mendham et al. (9) showed that 12 weeks of combined training along with resistance training improved the lipid profile and increased DAG in the skeletal muscle tissue, but had no significant effect on serum DAG levels in women with obesity. In addition, it has been shown that LDL and very-low-density lipoprotein (VLDL) levels decreased in overweight girls; however, cholesterol, C-reactive protein (CRP), and HDL levels did not change significantly after six weeks of HIIT (11). Given the consistency of the present study with Ross et al. (17), Sokolowska et al. (5) and Mendham et al. (9) researches, long-term training at moderate and high intensities seems to have a significant effect on lipid profile (5, 9, 18).

In the present study, MCT and MIIT decreased ceramide synthase-1 (CS1) levels and total ceramide levels in the MIIT group were lower than DC and MCT groups. Mandal et al. (1) have shown that increased ceramides are associated with cardiovascular disease and insulin resistance. Consistent with this study, a review study showed that same exercise reduces ceramides in the skeletal muscle (5). In the study of Kasumov et al. (19), it was suggested that 12 weeks of training with an intensity of 80-85% maximal heart rate, increases insulin sensitivity, and decreases plasma levels of C14:0, C16:0, C18:1 and C24:0 ceramide in obese women with type 2 diabetes. Also, 12 weeks of combined training along with weight resistance training, increased mitochondrial respiratory capacity and ceramide 18 (C18) levels, but it had no significant effect on the levels of total ceramide, insulin sensitivity and GLUT4 protein expression in obese women (9). Also, an acute training session for 90 minutes of exercise with an intensity of 50% VO_{2max} increased serum levels of ceramide and glycosylceramide (20). It appears that a wide range of ceramides and their function are also affected by exercise activity. In addition, the mechanism of anti-inflammatory effect of high-intensity exercise, especially in disease conditions, is still not well known. Therefore, differences in the intensity and length of a training period can be the reasons for a discrepancy in the results.

In our study, the levels of pAkt, Akt/pAkt ratio, PKC in the MIIT group increased in comparison with the DC and MCT groups. Also, the amount of TNF-α inflammatory index in the MIIT group decreased in comparison with the DC and MCT groups. In line with the present study, it has been reported that following 16 hours of an acute training, no significant changes in the levels of blood insulin and glucose were observed, however seven days of training was associated with an increase in the concentrations of PI3K, pAkt, AS160 proteins and an improvement of guanosine triphosphatase (GTPase) function as an important protein in the transport of GLUT4 to the muscle cell membrane (21). Also, eight weeks of endurance training with different intensities reduced inflammatory factors such as IL-4, IL-6, TNF-α, IL-1β and declined oxidative stress in diabetic rats. Furthermore, a MCT and MIIT exercise had a more favorable effect on reducing inflammatory factors in diabetic rats (22). Given the differences in the intensity between the present study and Kim et al. (22) study, it appears that the intensity and frequency of training are important factors in modulating or altering the balance of oxidative stress and activating inflammatory factors in diabetic conditions. However, according to the results of Mendham et al. (9), O'Gorman et al. (21), Gerosa-Neto et al. (23) and Martinez-Huenchullan et al. (24), it seems that high intensity interval training has a significant effect on inflammatory factors than moderate-intensity continuous training.

In general, lipid metabolic disorders such as disorders of glucose and insulin metabolism are caused by cytokine changes that bring an increase the serum levels of LDL, cholesterol, VLDL, TG. This increase consequently rises inflammatory and pro- inflammatory factors such as TNF-α, IL-1β, CRP and additionally disrupts the pathway

of AMP-activated protein kinase (AMPK), PI3K/Akt, that may result in an insulin sensitivity impairment and an insulin resistance increase in the skeletal muscle tissue (25, 26). In addition, disruption of the metabolism of sphingolipids, which contain ceramide nuclei, from the TLR-4 receptor-like pathway leads to an increase in inflammatory cytokines and ceramides. While, in the next step, ceramides reduce Akt/PKB ratio, decrease GLUT4 expression level, and increase insulin resistance by increasing $PK_{C\zeta}$ levels. This ceramides level increase also coincides with an oxidative stress increase, which leads to increased levels of caspases, PK_C and inflammatory factors, that results in the cell apoptosis in pancreatic beta cells and impairs the insulin secretion procedure in these cells (1). In this regard, studies have shown that an increase in the ceramides levels is associated with the incidence of cardiovascular disease (1); also a ceramide/sphingolipid catalysis increase is also associated with the insulin resistance (18). A study found that although C16-ceramide in healthy individuals is directly related to the insulin signaling pathway improvement, C16-ceramide levels increase in individuals with metabolic disease and lead to the insulin resistance increase (27).

On the other hand, an exercise with the mechanism of improving lipid metabolism, increasing GLUT4 protein expression and increasing glucose uptake from non-insulin-dependent pathways during exercise, improves the lipid metabolism and mitochondrial biogenesis. Exercise also increases the Sphingomyelin Synthase 1 & 2 (SGMS1 and SGMS2) expression by increasing the levels of sphingomyelin synthase in the Golgi apparatus, and increases the production of the DAG in the endoplasmic reticulum and the Golgi apparatus to produce phospholipids in the cell. Therefore, the increase of some ceramides (not total ceramide) along with the increase of DAG is associated with an increase in the mitochondrial biogenesis (9). Studies have shown that exercise not only decreases the level of skeletal muscle ceramide but also increases insulin sensitivity, levels of DAG, PI3K, anabolic enzymes of the Akt/PKB pathway, PP2A inhibitor, and inflammatory factors, as well as protects skeletal muscle against cell damage and death (5). However, changes in glucose and lipid metabolism pathway in skeletal muscle following exercise can be depending on duration, intensity and type of it. For example, 12 weeks of combined training improves mitochondrial biogenesis and lipid profile reduces inflammatory markers and ceramide levels (9). However, an acute training session increased serum ceramide and glycosylceramide levels and did not significantly change the levels of sphingosine-1-phosphate and the sphingomyelin, but after 30 minutes of recovery, their levels decreased significantly (20).

For example, 12 weeks of combined training improves mitochondrial biogenesis and lipid profile and reduces inflammatory markers and ceramide levels (9). Acute training session increased serum ceramide and glycosylceramide levels (20).

It seems that long-term training is preferred over short-term training, but adaptation to long-term training depends on its intensity and involvement of lipid-dependent metabolic pathways and lipid catabolism threshold in ceramide changes. For example, 12 weeks of training increase insulin sensitivity and decreases ceramide plasma levels, it was a useful plan to manage obese women with type 2 diabetic inflammatory response. The intensity of the MIIT protocol in our research and Kasumov et al. (19) study was similar. Here, we suggest that performing high-intensity interval training, especially during rest, maybe more helpful than low-intensity training.

Therefore, a possible advantage of this study is that MIIT compared to MICT has a superior effect by affecting ceramide metabolism. Considering the role of sphingolipids, sphingomyelin, TLR-4, sphingomyelin synthase, and DAG in the mechanism of ceramide-dependent insulin resistance, it seems that the lack of measurement of these variables is one of the limitations of our present study. Hence, these variables evaluations are suggested for future research.

In summary, future studies should examine the intensity and duration of exercise in type 1 and type 2 diabetes in animal models and humans.

Conclusion

According to the results of the present study on improving the metabolism of glucose, lipids and improving inflammatory factors in the muscle tissue following two types of MICT and MIIT exercises. It seems that both of the mentioned training methods in the condition of diabetes with the improvement of energy substrates decrease of the muscle tissue inflammatory factors However, the effect of MIIT on ceramides is more optimal than MICT.

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Authors' Contributions

P.R., D.Sh.-V., M.R.M.; Investigation and Writing. D.Sh.-V.; Project administration, Supervision, Writing, and Editing. S.Gh.; Conceptualization, Methodology, and Editing. M.R.M.; Resources, Data Curation, Formal analysis, and Validation. P.R.; Visualization and Software. All authors read and approved the final manuscript.

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