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The Effect of Exposure to Hypoxia during Resistance Training on the Concentration of PAX7 and PGC-1α Proteins in Fast-Twitch Muscle Tissue of Diabetic Rats Fed a High-Fat Diet

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ABSTRACT

Introduction: Myopathy caused by diabetes can accelerate the disease process in diabetic people. Myopathy has important indicators in muscle tissue related to regeneration and intracellular metabolism in skeletal muscles. The current study aimed to evaluate the effect of eight weeks of resistance training in hypoxia on the content of PAX 7 and PGC-1α proteins in the gastrocnemius muscle of type 2 diabetic rats.

Methods: This experimental study was conducted on 24 male Wistar rats for six weeks after induction of type 2 diabetes. Rats were divided into three groups: healthy control (HC), diabetic control (DC), and hypoxia group (HPX). Resistance training was applied for eight weeks under oxygen deficiency conditions in the groups of resistance training in hypoxia. The tissue sample was taken from the biceps muscle after finishing the exercises and evaluated to measure the concentration of PAX7 and PGC-1α proteins. The results were analyzed by one-way analysis of variance at a significance level $\alpha \leq 0.05$.

Results: There was a significant difference in PAX7 and PGC-1α proteins between the research groups (P=0.0001). Induction of diabetes led to a significant decrease in PAX7 compared to the control group. PGC1-α protein levels also decreased significantly in the diabetes induction group compared to the control group (P=0.0001). However, exposure to hypoxia did not change the gene expression values of this variable compared to the diabetic patient group (P=0.451).

Conclusion: Exposure to temporary and passive hypoxia can be considered as a suggested strategy to improve indicators related to type 2 diabetes in humans.

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Introduction

As the primary site of insulin-stimulated glucose absorption, skeletal muscles are the primary driver of insulin resistance in the whole body. The disruption of muscle tissue renewal, the largest tissue in the body that controls energy metabolism, is one of the driving factors of type 2 diabetes. Although insulin resistance in skeletal muscle is reversible, β-cell destruction is not. Satellite cells are skeletal muscle stem cells responsible for muscle tissue renewal. Age-related slowing of muscle tissue renewal is associated with disturbances in energy metabolism. Physiologically, this issue relates to satellite cell and mitochondrial disruption (1). Paired Box 7 (Pax7) is one of the well-known and influential markers in the activation and function of satellite cells (2). Transcription factors such as my-5, PAX7, and myogenic differentiation 1 (MyoD1) participate in muscle cell development after activating satellite cells. First, PAX7 and MyoD1 are activated in muscle progenitor cells, and the simultaneous expression of these two factors is known as an indicator of satellite cell activation. Then, myogenin is produced in differentiated cells (2, 3).

As a protein, PAX7 is located upstream of MyoD and other myogenic indicators, and the decrease in PAX7 expression leads to the termination of MyoD expression. Myf5 is also expressed without PAX7, but the result is apoptosis of the resulting myotubes (4). Therefore, PAX7 seems to be a key regulator of satellite cells and myogenesis process. Satellite cells differentiate into fat or

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fibrotic tissue when PAX7 is absent (5). This issue is essential because PAX7 maintains or increases the pool of satellite cells (the number of satellite cells) (6). A diet restricting caloric intake for three weeks also increases PAX7 levels, indicating the relationship between cellular energy metabolism and the renewal of satellite cells and muscle tissue (7).

On the other hand, studies have shown that the disorder in the metabolism of fats, caused by the reduction of mitochondrial function, is considered one of the essential possible factors in the occurrence of type 2 diabetes (8). PGC-1α protein is one of the most important regulators of mitochondrial biogenesis, which is significantly reduced in type 2 diabetes patients (9). Increasing PGC-1α significantly improves the metabolism of fat and sugars and increases mitochondrial function. A common signaling pathway between increased PGC-1α phosphorylation and decreased insulin resistance appears to be AMPK, MAPK, and SIRT1 (1). On the other hand, PGC-1α increases significantly under the influence of physical activity, which can lead to improved insulin resistance and mitochondrial function (10). Less attention has been paid to the regeneration and metabolism of muscle tissue in type 2 diabetes in exercise physiology. In recent years, sports activities and exercises have been mentioned as factors to control diabetes. For example, some evidence shows that physical activity is influential in preventing and treating type 2 diabetes (5, 11). A ten-week resistance activity program has been shown to reduce fasting glucose and insulin resistance in people with diabetes. Therefore, exercise can be non-pharmacological in controlling type 2 diabetes (12). On the other hand, exposure to hypoxia without physical activity led to the improvement of indicators related to type 2 diabetes, including the improvement of insulin sensitivity (13). The mortality rate due to heart disease at an altitude above 1500 meters among diabetic men and women is lower than people living in lower areas (14). Some studies have revealed that hypoxia activates satellite cells and increases mitochondrial biogenesis (15). There are few studies regarding the combination of these two factors. For example, four weeks of aerobic exercise on a treadmill and exposure to hypoxia during the day (separate from exercise) improve glucose and fat metabolism in people with type 2 diabetes, which is significantly more than the group. The control and exercise groups were normoxic (6). However, the mechanisms of such an effect are still not well understood. These mechanisms appear to involve the activation of satellite cells and mitochondrial biogenesis, so the present study examines the effect of resistance training in hypoxia conditions on indicators of these two variables, namely, the degree of satellite cell activation and mitochondrial biogenesis in Wistar rats.

Materials & Methods

Samples

The current research population consisted of male rats from which the samples were randomly selected. An experimental design with a control group included more than 24 rats aged about six weeks and an approximate weight of 180g. Then, they were transferred to the sports physiology laboratory. A week was spent in the laboratory under completely standard conditions to get acquainted with the environment, and one week was spent adapting to the environment. Next, the samples were divided into three groups, each containing eight samples, and all four samples were placed in a polycarbonate cage. The groups included the health control group, the diabetic control group, and the hypoxia group. Two groups were induced with diabetes and hypoxia using a high-fat diet and streptozocin (STZ) injection. The induction of diabetes lasted for about three weeks. The laboratory environment had a temperature of 22±2°C, the humidity level was 45 to 55%, and the samples were asleep for 12 hours and awake for 12 hours.

Inducing Diabetes in Rats

The samples were first given a high-fat diet containing 59% fat for three weeks to induce diabetes in the DC and HPX groups. About 59% fat, 14% protein, and 27% carbohydrates were freely available to the samples during these three weeks, leading to metabolic disorders and insulin resistance in mice. At the end of 3 weeks, 35mg of streptozocin per kilogram of the weight of the samples was injected intraperitoneally. Samples with fasting blood sugar above 300 mg/dL were identified as subjects one week after the induction of diabetes. Two diabetic control groups were used to control the results: those who only became diabetic and healthy control groups who were not subjected to any changes.

Hypoxia was applied in the hypoxia groups, and the resistance training group used normobaric hypoxia conditions with an oxygen level of 14.4%, equivalent to an altitude of 3000 meters, consistently in all the weeks of the research.

Sampling and Measuring Variables

The samples were first anesthetized according to ethical standards 48 hours after the last training session, and their tissues were taken after dissection of their gastrocnemius muscle tissue to measure PAX 7 and PGC-1α protein concentrations. The plantar muscle tissue was washed in physiological serum and frozen by liquid nitrogen at -80°C until the experimental protocol was performed. Specific amounts of soleus muscle tissue were homogenized in Phosphate-buffered saline (PBS) solution as an antiprotease to extract PAX 7 and PGC-1α proteins. The homogenized tissue was centrifuged with a force of 5000g for 5 minutes at 4°C, and the resulting supernatant was evaluated by the ELISA kit of the Chinese American company Cusabio with the catalog number CSB-EL018425RA.

Statistical Methods

The Shapiro-Wilk test was used to determine the normality of data distribution. A one-way analysis of variance was used to measure the difference between groups. Bonferroni's post hoc test was used to compare the differences between groups. The significance level was 5%. SPSS version 21 software was used for data analysis, and 2021 software was used to prepare graphs.

Results

The data analysis showed that the PAX7 protein concentration in the research groups was significantly different (P=0.0001). Bonferroni's post hoc test was used to compare the origin of the difference between the groups (Figure 1). The results indicated that the concentration of PAX7 protein in the muscle tissue of the diabetic control group and hypoxia group decreased significantly compared to the control group (Figure 1). However, no difference was observed between the two diabetic control groups and the hypoxia group (P=0.171). In addition, the data analysis showed that the concentration of PGC1-α protein in the muscle tissue of the research groups was significantly different (P=0.0001), so the amount of PGC1-α protein was reduced in the diabetic group. Exposure to hypoxia did not cause a significant change in its concentration (P=0.439).

Table 1. One-way analysis of variance results for two variables PAX7 and PGC1-α

varible	sum of squares	average of squares	df	F	p Value
PAX7	610.298	152.572	2	31.145	0.0001
PGC1-α	461.284	131.145	2	44.179	0.0001

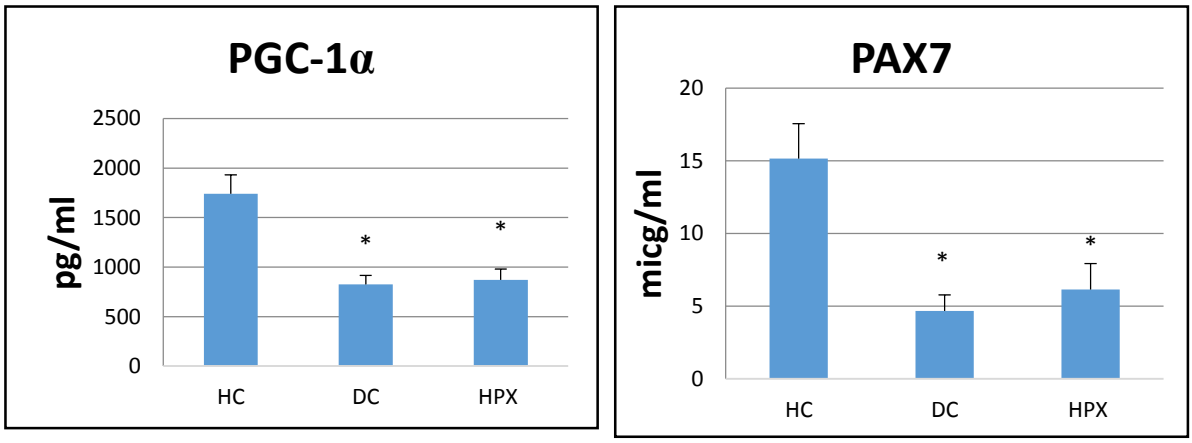

Figure 1. Comparison of PGC1-α and PAX7 protein concentrations in the calf muscle of samples in different research groups
(*) Significant difference with healthy control group at 0.05 level

Discussion

This research showed that type 2 diabetes with the HFD-STZ method in rats led to a decrease in the content of PAX 7 protein in the quadrilateral muscle of rats. At the same time, exposure to hypoxia could partially compensate for this

decrease. Diabetes mellitus is one of the most common metabolic diseases worldwide, resulting in hyperglycemia due to decreased insulin secretion, insulin action, or both. Different studies have shown that type 1 and type 2 diabetes cause muscle myopathy with other mechanisms and activate its mechanisms (15). The function and ability of stem cells in diabetic conditions were weakened in several tissues, including skeletal muscle tissue, and diabetes mellitus causes atrophy (Kamei, 2004). Fiber type transitions from oxidative to glycolytic (16) and energy metabolism is disrupted in skeletal muscles (17, 18). These changes lead to skeletal muscle dysfunction, such as muscle weakness and exercise intolerance (19). Previous studies have shown that diabetes impairs the function of satellite cells. Satellite cells derived from STZ-induced diabetic mice cannot form myotubes and consequently show poor regeneration in skeletal muscle after cardiotoxin-induced muscle damage (17). Diabetic Akita mice also showed impaired muscle regeneration following damage caused by weak macrophage infiltration and satellite cell recruitment to degenerative muscle fibers (Krause, 2013). In addition, the expression of MyoD and myogenin transcription factors decreased in soleus muscle from STZ-induced diabetic rats (Fujimaki, 2017). The findings of the present study confirmed these results. In contrast, exposure to hypoxia increased the mRNA levels of several satellite cell markers in young men, including MyoD and Mrf4 mRNA levels, after exposure to hypoxia (14% FiO2). However, this response is not sufficient for hypoxia-induced muscle hypertrophy. Although the short-term anabolic response to hypoxia is not significantly increased, the trend of increased expression of several myogenic markers suggests that hypoxic conditions may lead to the activation and differentiation of satellite cells such as PAX 7 (20). Myogenesis may be mediated by the secretion of inflammatory markers interleukin-6, interleukin-8, and TNF-α in skeletal muscle and immune cells (21). Factors such as IL-6 and TNF-α, as well as mRNA levels of CD 68 and CD 197, both markers of macrophages/neutrophils, increase after exposure to hypoxia more than the same conditions performed in normoxia. CD68 and CD197 also regulate skeletal muscle regeneration, which may involve activating satellite cells (21). Therefore, resistance training in hypoxia can increase PAX 7 protein and other muscle regeneration indicators in diabetic people through other mechanisms. Inflammation-induced by activation of the IL-6/STAT3 pathway may lead to the activation, proliferation, and differentiation of satellite cells, as measured by concomitant increases in MyoD and Mrf4 mRNA levels (21). In addition, the IL-6/STAT3 pathway may also help repair exercise-induced muscle tissue damage by recruiting lymphocytes, monocytes, and neutrophils, thereby promoting myogenesis (Tierney, 2014). Finally, HIF-1α stabilization and nuclear translocation under acute hypoxic conditions in vitro was associated with increased STAT3 protein expression in human primary muscle cells, underscoring the importance of this pathway in adaptation to hypoxic conditions in skeletal muscle (21). In practice, the results emphasized the importance of a moderate and well-controlled inflammatory response to induce myogenic adaptations after exposure to hypoxic conditions.

Another result was the reduction of PGC1-α protein in diabetic rats compared to the healthy control group, as shown in other studies (22). Some studies have reported that diabetes impairs mitochondrial function and dynamics. In contrast, proper mitochondrial function is essential in regulating metabolic pathways and is critical for maintaining proper energy balance (8, 15, 24, 25). The oxidative capacity of mitochondria in the skeletal muscle of insulin-resistant individuals is significantly lower than that of healthy individuals, leading to increased fat accumulation in skeletal muscles (Petersen, 2003). Mitochondrial dysfunction has also been observed in cultured myocytes from the skeletal muscle of patients with T2DM (10). These findings indicated that PGC1-α protein content in skeletal muscle is reduced under diabetic conditions (15). These findings are in line with the results of the present study. However, exposure to hypoxia alone could not increase the content of PGC1-α protein in the muscles of diabetic rats. Hypoxia in the environment created in the present study was not enough to activate the essential mechanisms of mitochondrial metabolism activation. Nevertheless, some studies have shown the effect of hypoxia in reducing the intracellular PGC1-α protein concentration and mitochondrial biogenesis (26). The examined tissues and the

pathological condition of the samples in these studies differed from the present study, which is the most crucial reason for the contradictions in this field. Hypoxia-inducible factor (HIF-1α) and its activation due to tissue oxygen deficiency seem to be the most essential cause of intracellular responses to hypoxia exposure. The effect of HIF-1α was different in different tissues and caused mitochondrial biogenesis to decrease in most tissues with the increase of glycolytic processes. However, this effect seemed to be different in muscle tissues. Muscle tissue was resistant to hypoxia, and the expression of HIF-1α in skeletal muscles occurred under hypoxia conditions, which does not decrease the expression of PGC1-α proteins and downstream signaling factors in short-term hypoxia with low degrees (26). Since HIF-1α leads to an increase in vascular NO, vasodilation in tissues, and renal erythropoietin (27), the increase in PGC1-α protein may be an adaptive response of muscles to hypoxic conditions independent of HIF-1α. (26). Overall, exposure to hypoxia equivalent to passive hypoxia created at an altitude of 3000 meters (14% FiO2) could not significantly change PGC-1α and PAX7 protein concentrations in diabetic samples with a high-fat diet. Hypoxia was applied during resistance activity for the first time in the country and has not been observed in previous studies. As a new method of exercise for diabetics, it helps prevent and slow down the process of injuries caused by diabetes and aging, as well as for older adults at risk of diabetes-related myopathy and sarcopenia. Considering the slight increase in both variables, applying this variable with more intensity will probably lead to its greater effectiveness, so future studies will clarify more facts. The effect of the mentioned variables on muscle tissue in humans and the generalization of the results of this research to humans should be conducted with caution. However, exposure to temporary and passive hypoxia has also been suggested as a solution to improve type 2 diabetes indices.

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References

1. Sergi D, Naumovski N, Heilbronn LK, Abeywardena M, O'Callaghan N, Lionetti L and Luscombe-Marsh N. Mitochondrial (Dys) function and Insulin Resistance: from pathophysiological molecular mechanisms to the impact of diet. Front Physiol 2019; 10:532.
2. Bazgir B, asgari A. The Interactive role of exercise and satellite cells in skeletal muscle regeneration and hypertrophy. EBNESINA. 2015; 16 (4) :47-63
3. Went Y, Bi P, Liu W, Asakura A, Keller C, Kuang S. Constitutive Notch activation upregulates Pax7 and promotes the self-renewal of skeletal muscle satellite cells. Molecular and Cellular Biology. 2012; 32(12):2300-11.
4. Evans PL, McMillin SL, Weyrauch LA, & Witczak CA. Regulation of skeletal muscle glucose transport and glucose metabolism by exercise training. Nutrients. 2019; 11(10), 2432.
5. Li J, Li Y, Atakan MM, Kuang J, Hu Y, Bishop DJ, Yan X. The molecular adaptive responses of skeletal muscle to high-intensity exercise/training and hypoxia. Antioxidants. 2020; 9(8):656.
6. Nguyen TH, Conotte S, Belayew A, Declèves AE, Legrand A, Tassin A. Hypoxia and hypoxia-inducible factor signaling in muscular dystrophies: cause and consequences. International Journal of Molecular Sciences. 2021; 22(13), 7220.
7. Nishimura A, Sugita M, Kato K, Fukuda A, Sudo A, Uchida A. Hypoxia increases muscle hypertrophy induced by resistance training. Int J Sports Physiol Perform. 2010; 5(4):497-508.
8. Fan W, Evans R. PPARs and ERRs: Molecular mediators of mitochondrial metabolism. Curr Opin Cell Biol. 2015; 33: 49–54.
9. Krako Jakovljevic N, Pavlovic K, Jotic A, Lalic, K, Stoiljkovic M, Lukic L, Milicic T, Macesic M, Stanarcic Gajovic J, Lalic NM. Targeting mitochondria in diabetes. Int J Mol Sci. 2021; 22, 6642.
10. Gaster M, Rustan AC, Aas V, Beck-Nielsen H. Reduced lipid oxidation in skeletal muscle from type 2 diabetic subjects may be of genetic origin: Evidence from cultured myotubes. Diabetes. 2004; 53:542–8.
11. FallahpourNooshabadi S, Kazemzadeh Y, Gorzi A. The effect of eight weeks of daily normobaric hypoxia (60 minutes) on PGC1α content of soleus muscle, insulin resistance, and fasting glucose in type 2 diabetic rats. Ebnesina. 2021; 22 (4):89-94.
12. Tashakori Zade, M, Mogharnasi M. A Study of the Effect of 10 Weeks of Resistance Training on HSP70 and Insulin Resistance in Type 2 Diabetic Women. Journal of Sport Biosciences. 2016; 8(3): 341-351.
13. Faramoushi M, Amir Sasan R, Vahid S S, Karimi P. Effect of simulated intermittent altitude on the metabolic and

hematologic parameters in streptozotocin induced diabetic rats. J Ardabil Univ Med Sci. 2016; 16 (1):53-64.
14. Woolcott OO, Ader M, Bergman RN. Glucose homeostasis during short-term and prolonged exposure to high altitudes. Endocrine Reviews. 2015; 36(2), 149–73.
15. Fujimaki, Shin, Tomoko Kuwabara. "Diabetes-induced dysfunction of mitochondria and stem cells in skeletal muscle and the nervous system. International Journal of Molecular Sciences. 2017; 18(10): 2147.
16. Bachman JF, Klose A, Liu W, Paris ND, Blanc RS, Schmalz M, Knapp E, Chakkalakal JV. Prepubertal skeletal muscle growth requires Pax7-expressing satellite cell-derived myonuclear contribution. Development. 2018; 145(20): dev167197.
17. Krause MP, Al-Sajee D, D'Souza DM, Rebalka IA, Moradi J, Riddell MC, Hawke TJ. Impaired macrophage and satellite cell infiltration occurs in a muscle-specific fashion following injury in diabetic skeletal muscle. PLoS ONE. 2013; 8: e70971.
18. Oberbach A, Bossenz Y, Lehmann S, Niebauer J, Adams V, Paschke R, Schon MR, Bluher M, Punkt K. Altered fiber distribution and fiber-specific glycolytic and oxidative enzyme activity in skeletal muscle of patients with type 2 diabetes. Diabetes Care. 2006; 29: 895–900.
19. Kamei D, Yamakawa K, Takegoshi Y, Mikami-Nakanishi M, Nakatani Y, Oh-Ishi S, Yasui H, Azuma Y, Hirasawa N, Ohuchi K, Kawaguchi H, Ishikawa Y, Ishii T, Uematsu S, Akira S, Murakami M, Kudo I. Reduced pain hypersensitivity and inflammation in mice lacking microsomal prostaglandin e synthase-1. J Biol Chem. 2004; 279(32):33684-95.
20. Ruas JL, White JP, Rao RR, Kleiner S, Brannan, KT, Harrison BC, Greene NP, Wu J, Estall JL, Irving BA, Lanza IR, Rasbach KA, Okutsu M, Nair KS, Yan Z, Leinwand LA, & Spiegelman B M. A PGC-1α isoform induced by resistance training regulates skeletal muscle hypertrophy. Cell. 2012; 151(6), 1319–31.
21. Britto FA, Gnimassou O, De Groote E, Balan E, Warnier G, Everard A, Cani PD, Deldicque L. Acute environmental hypoxia potentiates satellite cell-dependent myogenesis in response to resistance exercise through the inflammation pathway in human. Faseb J .2020; 34(1):1885-900.
22. Tierney MT, Aydogdu T, Sala D, Malecova B, Gatto S, Puri PL, Latella L, Sacco A. STAT3 signaling controls satellite cell expansion and skeletal muscle repair. Nat Med. 2014; 20(10):1182-6.
23. Ryen SvDd T, Francaux M, Deldicque L. Regulation of satellite cells by exercise in hypoxic conditions: a narrative review. European Journal of Applied Physiology. 2021; 121(Suppl 1).
24. Joseph AM, Joanisse DR, Baillot RG, Hood DA. Mitochondrial dysregulation in the pathogenesis of diabetes: Potential for mitochondrial biogenesis-mediated interventions. Exp. Diabetes Res. 2012; 2012: 642038.
25. Boushel R, Gnaiger E, Schjerling P, Skovbro M, Kraunsoe R, Dela F. Patients with type 2 diabetes have normal mitochondrial function in skeletal muscle. Diabetologia. 2007; 50: 790–6.
26. Van Thienen R, Masschelein E, D'Hulst G, Thomis M and Hespel P. Twin resemblance in muscle HIF-1α responses to hypoxia and exercise. Front Physiol. 2017; 7:676.
27. Pircher T, Wackerhage H, Aszodi A, Kammerlander C, Böcker W and Saller MM. Hypoxic signaling in skeletal muscle maintenance and regeneration: a systematic review. Front. Physiol. 2021; 12:684899.