Original Article

Vibration exercise decreases insulin resistance and modulates the insulin signaling pathway in a type 2 diabetic rat model

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Abstract: Vibration exercise (VE) is a new type of physical training, but little is known about its effects on insulin resistance at the molecular level. A Sprague-Dawley rat model of type 2 diabetes with insulin resistance was induced with a high-fat diet and low-dose streptozotocin. Animals were then subjected to 8 wk of VE consisting of placing the rats on a vibration stand bracket (8 cm × 8 cm × 20 cm) with a maximum vertical vibration displacement of 52 mm for 15 min twice a day, 6 d each week. Various metabolic markers and the phosphorylation and expression of proteins within the PI3K/AKT insulin signaling pathway were assessed. The high-fat diet and low-dose streptozotocin increased food intake, body weight, and levels of blood glucose, triglycerides, total cholesterol, and free fatty acids, while ketone rate, 2-deoxyglucose uptake, and glycogen levels were decreased. These effects were ameliorated in animals receiving VE. VE treatment activated the PI3K/AKT insulin-signaling pathway, and also increased the expression of GLUT4. In conclusion, VE improved the metabolic issues associated with the diabetic state by suppressing the reduction of IRS1, AKT2, and GLUT4 in the diabetic condition, indicating that VE could be used as a therapeutic intervention for insulin resistance and type 2 diabetes.

Keywords: Diabetes, GLUT4, Insulin resistance, PI3K/AKT insulin signaling, metabolism, phosphorylation, vibration exercise

Introduction

Type 2 diabetes mellitus (T2DM) is a metabolic disease characterized by insulin resistance and relative β-cell dysfunction resulting in hyperglycemia and subsequent complications in many tissues and organs [1, 2]. Although there is a strong genetic influence on its development, environmental factors, including obesity and physical inactivity, also hasten the development of diabetes in humans [3-5]. As such, pharmacological interventions, in addition to weight management and exercise, are being explored to enhance glycemic control in type 2 diabetes patients [6]. Insulin resistance or reduced insulin sensitivity prohibit muscle cells from taking up and storing glucose and triglycerides. As a result, higher levels of glucose and triglycerides circulate in the blood instead of cells. Improving insulin resistance and promoting its intake and utilization are critical for the treatment and management of T2DM.

Many studies have focused on the role of exercise for T2DM treatment [7-10]. Previous reports indicate that exercise preserves pancreatic β-cell function in animals [11-13] and humans [14, 15]. In addition, increased physical exercise improves glucose homeostasis and enhances insulin sensitivity. It has been shown that after acute exercise, sensitivity to insulin is enhanced in skeletal muscle, adipose tissue, the liver, and the hypothalamus [16-18].

In general, both insulin and exercise promote translocation of the glucose transporter, GLUT4, to the plasma membrane in skeletal muscle. The binding of insulin to its receptor leads to autophosphorylation of its tyrosine residues and phosphorylation of insulin receptor sub-
strate (IRS)-1 and IRS-2. These proteins act as the docking site for phosphoinositide 3-kinase (PI3K), which once activated, produces lipid second messengers that stimulate downstream proteins, including the serine/threonine kinase Akt and glycogen synthase kinase 3β (GSK3β) [19, 20]. These signaling cascades trigger the translocation of GLUT4 to the cell surface and ultimately promote glucose uptake into cells, increased glycogen and protein synthesis, and long-term transcriptional effects [21, 22]. Additional studies have indicated that exercise also enhances the phosphorylation and expression of molecules downstream of PI3K, enhancing insulin sensitivity in skeletal muscle [23] and increasing insulin receptor protein levels [24].

Vibration exercise (VE) is a novel type of physical training that increases muscle power. Compared to traditional training regimens, VE requires less time and reaches higher compliance in inactive patients. This point is vital given that most patients with T2DM are obese, follow a lifelong sedentary lifestyle, and are difficult to motivate for longer-lasting physical activities [25]. Thus, choosing an appropriate, time-efficient and persistent exercise style is very important for T2DM patients. Unfortunately, little is known about the effectiveness of VE on insulin resistance. In the present study, the impact of VE on insulin resistance and glucose control was analyzed. A Sprague-Dawley rat model of type 2 diabetes with insulin resistance was induced using a high-fat diet and low-dose streptozotocin to evaluate the effect of VE on insulin resistance. The molecular mechanisms underlying insulin resistance were also evaluated.

Materials and methods

Ethics statement

Healthy Sprague-Dawley rats weighing 250-270 g used for the current study were supplied by the Experimental Animal Center of Liaoning Medical College (certificate number SCXK [Liao] 2009-0004). The Ethics Committee for the Use of Experimental Animals approved all procedures and the instructions for animal care and usage provided by the institution were followed.

Animals and diet

Rats were housed in standard polypropylene cages at a temperature of 22 ± 2°C with humidity of 50 ± 10%. Control animals were fed a diet consisting of 57.3% carbohydrate, 19.4% protein, 18.8% cellulose, and 4.5% fat. Treatment animals received a high-fat diet (HFD) consisting of 57% normal diet, 18% lard, 20% sucrose, 2.5% cholesterol, and 2.5% egg yolk powder. Animals were fed the diets for eight weeks before additional treatment. The diet was provided by the Animal Center of Liaoning Medical College, China.

Chemicals and reagents

Streptozotocin (STZ) was purchased from Sigma-Aldrich (St. Louis, MO, USA). The primary antibodies IRS-1, P-IRS-1-Ser307, PI3Kp110, AKT2, P-AKT2-Ser474, GLUT4 and P-GSK3β-Ser9, and secondary antibodies were purchased from Wuhan Boster Biological Engineering Co., Ltd. (Wuhan, China).

Establishment of a type 2 diabetic rat model and VE

Rats were randomly assigned to the following groups (n = 10 per group): control diet (Con); HFD and STZ (HFD+STZ); HFD and STZ with high-frequency (35 Hz) VE (HFD+STZ+HE); HFD and STZ with medium-frequency (25 Hz) VE (HFD+STZ+ME); and HFD and STZ with low-frequency (15 Hz) VE (HFD+STZ+LE). STZ injections (35 mg/kg, i.p.) were administered after 8 wk of HFD intervention; Con rats were injected with an equal volume of citrate buffer solution (pH 4.4, 1 mL/kg). One week after STZ injection, the blood sugar level was measured by the glucose oxidase method. The induction of diabetes was considered successful in rats when the levels of fasting blood glucose levels ≥ 7.8 mmol/L and postprandial blood glucose was ≥ 11.1 mmol/L.

Rats in the HFD+STZ+HE, HFD+STZ+ME, and HFD+STZ+LE groups were put on a vibration stand bracket (8 cm × 8 cm × 20 cm) with a vertical vibration at a maximum displacement of 52 mm. In the vibration stand bracket, rats were kept standing and lower limbs received stimulation under vibration training. Rats in all vibration exercise groups received training twice a day for 15 min, 6 d/wk for a total of 8 wk.

Blood sample collection and analysis

Blood was obtained from tail veins the morning following an overnight fast at 0, 2, 4, 6, and 8
wk after VE training was initiated, and the glucose levels and high levels of body mass were measured. Twenty-four hours after the last VE, all rats were anesthetized by an i.p. injection of 20% urethane (0.3 mL/100 g). Blood samples were collected and centrifuged at 120 × g for 10 min. Serum glucose, triglyceride, total cholesterol, and free fatty acid concentrations were determined using an auto-analyzer. Serum insulin concentrations were determined using a radioimmunoassay. In addition, soleus muscle tissues were dissected and prepared for Western blot analyses.

Table 1. Effects of vibration exercise (VE) on metabolic characteristics and biochemical parameters

<table>
<thead>
<tr>
<th>Metabolic parameter</th>
<th>Control</th>
<th>HFD+STZ</th>
<th>HFD+STZ+HE</th>
<th>HFD+STZ+ME</th>
<th>HFD+STZ+LE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food intake (g/kg/d)</td>
<td>64.2 ± 5.8</td>
<td>83.4 ± 6.1*</td>
<td>77.3 ± 5.3</td>
<td>78.3 ± 6.1</td>
<td>78.4 ± 4.3</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>482.4 ± 8.2</td>
<td>360.8 ± 6.6*</td>
<td>369.2 ± 3.1</td>
<td>366.7 ± 7.0</td>
<td>363.9 ± 8.8</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>67.1 ± 5.4</td>
<td>79.4 ± 6.5*</td>
<td>72.3 ± 4.1*</td>
<td>74.3 ± 4.2*</td>
<td>78.7 ± 5.4</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>0.59 ± 0.05</td>
<td>2.65 ± 0.54*</td>
<td>1.78 ± 0.67*</td>
<td>1.89 ± 0.51*</td>
<td>2.34 ± 0.54*</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>2.48 ± 0.14</td>
<td>4.52 ± 1.16*</td>
<td>2.89 ± 0.87*</td>
<td>3.45 ± 1.04*</td>
<td>4.36 ± 1.09*</td>
</tr>
<tr>
<td>Free fatty acid (mmol/L)</td>
<td>0.35 ± 0.04</td>
<td>0.59 ± 0.06*</td>
<td>0.47 ± 0.08*</td>
<td>0.52 ± 0.07*</td>
<td>0.56 ± 0.09*</td>
</tr>
<tr>
<td>Kitt (%/min)</td>
<td>3.38 ± 0.09</td>
<td>2.13 ± 0.04*</td>
<td>2.79 ± 0.13*</td>
<td>2.64 ± 0.08*</td>
<td>2.25 ± 0.18*</td>
</tr>
<tr>
<td>Muscle 2DG uptake (μmol/g/h)</td>
<td>1.85 ± 0.11</td>
<td>1.48 ± 0.12*</td>
<td>1.61 ± 0.14*</td>
<td>1.58 ± 0.10*</td>
<td>1.51 ± 0.13*</td>
</tr>
<tr>
<td>Muscle glycogen (μmol/g wet weight)</td>
<td>25.6 ± 0.3</td>
<td>23.5 ± 0.3*</td>
<td>25.4 ± 0.1*</td>
<td>24.8 ± 0.2*</td>
<td>23.9 ± 0.3*</td>
</tr>
</tbody>
</table>

HE, high-frequency VE; HFD, high-fat diet; LE, low-frequency VE; ME, medium-frequency VE; STZ, streptozotocin; 2DG, 2-deoxyglucose. Values are presented as mean ± SE; n = 10; *P < 0.05 vs. Con group; **P < 0.05 vs. HFD+STZ.

Insulin tolerance tests were performed after blood sample collection. Briefly, rats were fasted for 12 h, anesthetized, and given 1.5 IU/kg artificial insulin (Sigma-Aldrich). To determine blood glucose concentrations, blood samples were collected and centrifuged at 120 × g for 10 min. Serum glucose, triglyceride, total cholesterol, and free fatty acid concentrations were determined using an auto-analyzer. Serum insulin concentrations were determined using a radioimmunoassay. In addition, soleus muscle tissues were dissected and prepared for Western blot analyses.

Insulin tolerance test and serum insulin quantification

Insulin tolerance tests were performed after blood sample collection. Briefly, rats were fasted for 12 h, anesthetized, and given 1.5 IU/kg artificial insulin (Sigma-Aldrich). To determine blood glucose concentrations, blood samples were collected at 0, 5, 10, 15, 20, 25, and 30 min after insulin injection, centrifuged at 1100 × g for 15 min at 4°C, and stored at -20°C. Kitt, the rate constant for plasma glucose decline, was calculated using the formula 0.693/biological half-life. The plasma glucose half-life (t 1/2 ) was calculated from the slope of least squares of the plasma glucose concentration during the linear phase of the decline [23].

Skeletal muscle [3H]-2-deoxyglucose (2DG) uptake

Ex vivo muscle glucose uptake was performed using methods adapted from Hinkley et al. [26]. Briefly, the extensor digitorum longus muscles were cleaved and placed for 60 min in gassed, 37°C Krebs-Ringer bicarbonate (KRB) solution containing the following (in mmol/L): 117 NaCl, 4.7 KCl, 2.5 CaCl 2 ∙2H 2 O, 1.2 KH 2 PO 4 , 1.2 MgSO 4 ∙7H 2 O, and 24.6 NaHCO 3 , and 2 pyruvate. Muscles were then incubated in KRB containing 600 mU/mL insulin (Roche Diagnostics) for 20 min, then in KRB buffer containing 1.5 mCi/mL [3H]-2-deoxyglucose, 1 mmol/L deoxyglucose, 0.45 mCi/mL [14C]-mannitol, and 7 mmol/L mannitol with or without insulin. Muscles were frozen in liquid N 2 , solubilized in 1N NaOH at 80°C for 10 min, followed by neutralization with 2 mol/L HCl. The solution was heated by water bath at 85°C for 2 h and neutralized with 1N HCl. Samples were centrifuged at 11,000 × g for 1 min. Aliquots were removed for scintillation counting of the [3H] and [14C] labels, and 2DG uptake was calculated.

Skeletal muscle glycogen content

Frozen samples weighing 5-10 mg were hydrolyzed in 1 mol/L NaOH (1:9 wt/vol) at 80°C for 10 min, followed by neutralization with 2 mol/L HCl. The solution was heated by water bath at 85°C for 2 h and neutralized with 5 mol/L NaOH, and glycogen content was measured using a glucose hexokinase assay kit (Sigma-Aldrich).

Western blot

Skeletal muscle tissue protein concentrations were determined using the Bradford method. After boiling for 5 min, the protein samples were loaded onto a polyacrylamide gel, separated by SDS-PAGE, and transferred to a PVDF membrane (EMD Millipore, Billerica, MA, USA). The membranes were blocked with milk powder for 2 h at room temperature, then incubated overnight at 4°C in the following primary anti-
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Figure 1. The effect of vibration exercise (VE) on blood glucose levels at different time points (A) and glucose changes during the insulin tolerance test (B). Blood glucose levels were measured from tail veins at 0, 2, 4, 6, and 8 weeks with a blood glucose meter. Con, Control; HFD, high-fat diet; STZ, streptozotocin; HE, high-frequency VE; ME, medium-frequency VE; LE, low-frequency VE.

Figure 2. Expression analysis of proteins within the insulin signaling pathway after vibration exercise (VE). A, B: Western blot analysis of IRS-1 and phosphorylated IRS-1Ser307 protein expression. C, D: Western blot analysis of the expression and phosphorylation of PI3K, AKT, and GLUT4. β-actin served as the loading control. Values are presented as mean ± SE; n = 10; For each panel: a, control group; b, high-fat diet (HFD) and streptozotocin (STZ); c, HFD and STZ with high-frequency VE; d, HFD and STZ with medium-frequency VE; e, HFD and STZ with low-frequency VE. *P < 0.05, **P < 0.01 vs. a; #P < 0.05, ##P < 0.01 vs. b.
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antibodies (anti-rabbit, anti-mouse, or anti-goat IgGs). Detection of positive bands was performed with enhanced chemiluminescence reagents (ZSGB-BIO, China). Quantification of band intensities was performed using ImageJ2x analysis of the digital images.

Statistical analysis

All data are expressed as mean ± SE. Statistical analysis was performed using one-way analyses of variance followed by Bonferroni’s post hoc tests. Differences were considered statistically significant when \( P < 0.05 \).

Results

**VE improves fasting glucose and insulin action**

In order to determine the effects of HFD and STZ treatments in the rat model, food intake, blood glucose, triglyceride, total cholesterol, and free fatty acid levels were analyzed. As shown in Table 1 and Figure 1, each parameter was increased relative to the control group, while the Kitt rate and glycogen content were lower in the HFD+STZ group. These results indicate that HFD+STZ treatment induces a diabetic state in this rat model. Compared with the HFD+STZ group, rats in HFD+STZ+HE and HFD+STZ+ME groups showed significantly lower levels of insulin, blood glucose, triglyceride, total cholesterol, and free fatty acids, and had higher body weights and muscle 2DG uptake (all \( P < 0.05 \)). Food intake also decreased, but the difference was not significant. Conversely, Kitt rate and glycogen content were significantly increased in the HFD+STZ+HE and HFD+STZ+ME groups compared to HFD+STZ.

**Effect of VE on expression of IRS-1, PI3K, AKT, and GLUT4**

We next examined the phosphorylation status of proteins within the PI3K/AKT insulin-signaling pathway following the various experimental treatments using Western blot analysis. As shown in Figure 2A and 2B, treatment with HFD+STZ increased P-IRS-1-Ser\(^{307}\) expression 1.47-fold, while the IRS-1 expression was decreased compared to the control group. Following VE, P-IRS-1-Ser\(^{307}\) expression was decreased in the HFD+STZ+HE group, while the IRS-1 expression increased compared to the HFD+STZ group. No difference in P-IRS-1-Ser\(^{307}\) expression was observed between HFD+STZ and either HFD+STZ+LE or HFD+STZ+ME treatment groups.

In order to further investigate the insulin pathway, the phosphorylation and expression of PI3K, AKT, and GLUT4 was also evaluated by Western blotting. Compared with control group, the expressions of PI3Kp\(^{110}\) and P-AKT2-Ser\(^{474}\) were decreased in the HFD+STZ group, while in the HFD+STZ+HE and HFD+STZ+ME groups, expressions of PI3Kp\(^{110}\) and P-AKT2-Ser\(^{474}\) were increased (Figure 2C, 2D). GLUT4 expression was reduced with HFD+STZ treatment relative to the control group. However, with high- and medium-frequency exercise, a dramatic increase in GLUT4 was observed. These results suggest that VE influences the PI3K/AKT insulin-signaling pathway and contributes to improvement of insulin resistance at the molecular level.
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Effect of VE on phosphorylation and expression of GSK3β

Previous studies have shown that acute, selective GSK3β inhibition enhances insulin signaling in pre-diabetic, insulin-resistant rat skeletal muscle [26]. We therefore also examined the effect of VE on phosphorylation of GSK3β. As shown in Figure 3, phosphorylation of GSK3β decreased with HFD+STZ relative to the control, but increased significantly with high-frequency VE. Medium- and low-frequency exercise did not have a significant effect on GSK3β phosphorylation.

Discussion

VE is a new type of exercise that evokes muscle contraction through the monosynaptic stretch reflex [27]. It has been shown to be an effective treatment for the prevention of muscular atrophy and osteoporosis [28, 29]. Compared to other types of exercises, vibrations activate a higher number of motor units at the same time. Specifically, VE enhances glycemic control, improves the glycemic profile, lipid-related cardiovascular risk factors, and functional capacity [30], and reduces peripheral neuropathic pain [31] in T2DM patients. These benefits exceed the general health-related effects of exercise on skeletal muscles, such as improvement in endothelial function [32] and increased enzyme capacity in energy metabolism [6]. However, the molecular effects of VE on the insulin resistance pathway were not clearly known. In the present study, the results demonstrate that VE improves insulin resistance and lowers fasting glucose levels in a diabetic rat model, in part through modulation of the IRS/PI3K/Akt/GSK3β signaling pathway.

Insulin resistance is defined as a decrease in the peripheral tissue response to insulin-mediated cellular actions and an overall total body reduction of glucose uptake in response to physiological levels of insulin [33]. In the present study, we observed significantly elevated food intake, blood glucose, triglyceride, total cholesterol, and free fatty acid levels, but decreased KITT and glycogen content in animals treated with a HFD and STZ. The results indicate that those experimental conditions produced abnormalities in glucose and lipid metabolism and created metabolic characteristics that are known to be common with T2DM. VE, especially that of high frequency, reduces blood glucose, improves lipid metabolism, and increases the glucose disappearance rate and glycogen content, but has little effect on body weight. These results are consistent with a previous study [23]. This report is distinctly different from previous studies that only focused on one pattern of exercise, namely swimming or treadmill usage [34, 35]. Though it has been established that these types of exercise are effective for T2DM [36, 37], only a negligible amount of patients choose to take advantage of any sport activity. Overall, VE exhibited a positive effect on blood control and insulin resistance, and may represent an alternative form of exercise for patients.

However, reports have been conflicting on the effect of exercise on insulin signaling. A few studies have suggested that neither exercise nor muscle contraction can induce an increase in insulin receptor autophosphorylation or tyrosine phosphorylation, IRS-1 phosphorylation, or PI3K activation [38-40]. In contrast, other reports indicate an increase in insulin-stimulated PI3K activity in rat skeletal muscle after acute exercise [23]. In the present study, we observed an increase in the phosphorylation of IRS-1, PI3K, and AKT in animals that received high-frequency VE compared to those that only received the HFD and STZ. These results indicate that VE has a positive effect on the insulin-signaling pathway in the T2DM rat model.

In order to investigate the mechanism underlying the effect of VE on insulin resistance, we examined GLUT4 expression. Similar to the effect of VE on insulin signaling, the expression of GLUT4 was significantly increased with a concomitant decrease in glucose levels in animals that received high-frequency VE, thus providing evidence for an effect on insulin resistance. We observed an effect of VE on GSK3β phosphorylation as well; others have shown its increase in skeletal muscle of obese humans with T2DM [41] and its association with decreased insulin sensitivity [42]. High-frequency VE increased glycogen content and increased GSK3β phosphorylation levels relative to the HFD+STZ-treated group, confirming previous reports. However, the mechanism by which frequency affects these parameters remains unknown. It is assumed that vibrations with a frequency of 15-35 Hz evoke muscle contractions via the monosynaptic stretch reflex [27, 43].
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In conclusion, we investigated the effect of VE on glucose control and insulin signaling in a rat T2DM animal model. The results indicate that VE is an effective method of controlling the levels of glucose, triglycerides, cholesterol, and fatty acids. Furthermore, VE was shown to modulate components of the insulin-signaling pathway. Understanding the cellular and molecular mechanisms of insulin resistance is essential to establishing a sound theoretical basis for recommending VE as a therapeutic intervention for insulin resistance and T2DM.

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Disclosure of conflict of interest

None.

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References

[17] Pauli JR, Ropelle ER, Cintra DE, Carvalho-Filho MA, Moraes JC, De Souza CT, Velloso LA, Carvalheira JB and Saad MJ. Acute physical ex-


[34] Farias JM, Maggi RM, Tromm CB, Silva LA, Luciano TF, Marques SO, Lira FS, de Souza CT and Pinho RA. Exercise training performed simultaneously to a high-fat diet reduces the degree of insulin resistance and improves adipor1/2/APPL1 protein levels in mice. Lipids Health Dis 2012; 11: 134.


[44] Farias JM, Maggi RM, Tromm CB, Silva LA, Luciano TF, Marques SO, Lira FS, de Souza CT and Pinho RA. Exercise training performed simultaneously to a high-fat diet reduces the degree of insulin resistance and improves adipor1/2/APPL1 protein levels in mice. Lipids Health Dis 2012; 11: 134.


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