Original research

Effectiveness of high-intensity interval training versus moderate-intensity continuous training on endothelial function of arteries in type-2 diabetes patients; a randomized double blind clinical trial

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Abstract: Background: Obesity, characterized with hypertrophy and hyperplasia of adipocytes, is a pro-
Background: Considering the importance of exercise intensity in training, the present study aimed to compare the effect of high-intensity interval training (HIIT) and moderate-intensity continuous training (MICT) on endothelial function of arteries in type-2 diabetes patients.

Methods: In the present randomized double blind parallel clinical trial, 36 T2D patients were allocated to 3 groups of control (without regular training), MICT, and HIIT. Anthropometric indices, Biochemical evaluation, peak oxygen consumption (VO\(_{2\text{peak}}\)), resting NOx, and resting ET-1, and insulin resistance index was calculated using homeostatic model assessment (HOMA-IR) method were measured and compared.

Results: Both MICT and HIIT reduced haemoglobin A1c [F (2, 33) = 80.2; p < 0.0001], insulin [F (2, 33) = 57.7; p < 0.0001], and HOMA-IR [F (2, 33) = 99.1; p < 0.0001]. However, the effect of HIIT (p = 0.004) was more than MICT (p < 0.001) in reducing the 3 mentioned factors. Both MICT (p < 0.0001) and HIIT (p = 0.0002) led to a significant increase in NOx [F (2, 33) = 57.7; p < 0.0001] in diabetic patients. This increase was significantly higher in HIIT group (p < 0.0001). In addition, HIIT intervention caused a significant increase in VO\(_{2\text{peak}}\) compared to control group (p < 0.0001) and MICT group (p < 0.0001) [F (2, 33) = 59.9; p < 0.0001]. ET-1 level was also reduced after training intervention in both MICT (p = 0.02) and HIIT (p = 0.02) groups compared to control group [F (2, 33) = 5.5; p = 0.009].

Conclusion: HIIT can lead to more improvements in endothelial function and controlling diabetes and lipid profile compared to MICT, by causing more increase in aerobic fitness, more decrease in insulin resistance, and more increase in NOx bioactivity.

Keyword: Exercise; Physical Activity; Exercise Therapy; Diabetes Mellitus, Type 2; Endothelium


1. Introduction

Diabetes, as a metabolic disorder, is a major health problem in societies. The number of those affected with diabetes has been estimated to be about 382 million people in 2013. However, it is expected to reach 592 million people by 2035 (1). More than 80% of diabetics are affected with type-2 diabetes (T2D), which is identified as hyperglycemia caused by resistance to insulin.

Hyperglycemia can lead to vascular complications (2, 3) and prevalence of coronary, peripheral, and cerebral artery diseases has been reported to be 2-4 times higher in T2D patients (3). In addition, it has been shown that

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mortality due to cardiac diseases is 4 times more frequent in T2D patients compared to other cardiac patients (4). T2D is associated with hyperglycemia, dyslipidemia, increase in inflammatory cytokines secreted from adipose tissue, and hyperinsulinemia. These factors lead to endothelial dysfunction and damage, like disorders in regulation of vasodilation and vasoconstriction, and increase in inflammatory function, which finally lead to development of cardiovascular diseases (5). One of the mechanisms that T2D leads to further cardiovascular diseases is hyperinsulinemia. Insulin is an important factor affecting modulation of vascular tone that exerts its effects via nitric oxide (NOx) and endothelin-1 (ET-1) release signalling pathways (6). NO is a strong vasodilator and plays an important role in vascular tone modulation (7). Studies have shown that baseline levels of production and bioactivity of NO significantly drop in T2D (8). ET-1 is a strong vasoconstrictor that increases in T2D, hypertension and old age, and leads to changes in smooth muscle vascular bed, and vascular dysfunction (9). It has been confirmed that insulin resistance in T2D causes a drop in production and bioactivity of NO and a rise in ET-1 production, which leads to vasoconstriction and T2D-related hypertension (6). Therefore, increase in NO and decrease in ET-1 can probably play an important role in helping treat T2D by reducing vascular tone and blood pressure. Exercise can reduce the prevalence of cardiovascular diseases risk factors such as hyperglycemia, hypertension, dyslipidemia, and insulin resistance (10). Exercise directly affects vascular health (11). Shear stress is one of the factors showing vascular health, which is accompanied by a rise in NO production. Regular exercise increases shear stress, which brings about an increase in NO bioactivity. This, in turn leads to modulation of vascular tone, and decrease in coagulation and oxidative stress response. Therefore, vascular endothelial function will improve (12). Regular physical activity, especially moderate-intensity aerobic exercise has beneficial effects in controlling T2D, like decreasing body fat mass, improving glycemic control and endothelium dependent vasodilation (13). The American College of Sports Medicine and American Diabetes Association have recommended that T2D patients exercise 150 minutes each week to decrease cardiovascular risk factors (13). However, frequency, intensity, and duration of exercise are important in diabetic and overweight patients. Reports have shown that high intensity training is more beneficial compared to moderate-intensity aerobic exercise. On the other hand, high intensity training with a long duration may be dangerous for old people with physical problems and patients with chronic illnesses such as T2D (13). Consequently, low-volume high-intensity intermittent cycle exercise (HIIT) has been introduced. Studies have shown that low-volume high-intensity intermittent cycle exercise reduces hyperglycemia and increases muscle mitochondrial capacity in T2D patients. In addition, it has been shown that HIIT leads to an increase in shear stress and NOx production and decrease ET-1 concentration in patients with cardiovascular diseases (13). Few studies have evaluated the effect of intensity and duration of training on plasma NOx and ET-1 levels in T2D patients. On the other hand, the correlation between training and bioactivity of NOx and ET-1 plasma levels has not been assessed. Considering the importance of exercise intensity in training, the present study aimed to compare the effect of HIIT and moderate-intensity continuous training (MICT) on endothelial function of arteries in type-2 diabetes patients.

2. Method

2.1. Study design and setting

The present randomized double blind parallel clinical trial was designed aiming to compare the effect of HIIT and MICT on level of nitrite/nitrate (NOx), ET-1, and aerobic fitness of T2D patients. The participants of this study were T2D patients presenting to Diabetes and Metabolic Diseases Clinic in Tehran, Iran. The study was carried out during May to September 2014. Protocol of the study was approved by the ethics committee of Tehran University of Medical Sciences. It has been registered on Iranian Registry of Clinical Trials under the number IRCT2015100423002N2. Before entering the study, a written informed consent was obtained from all the patients. The researchers adhered to the principles of the Declaration of Helsinki throughout the study. We should note that the protocol of the present study did not interfere with the patients’ treatment process. To solve ethical issues, aerobic interval training was done for the control group after the study.

2.2. Participants

In this study, T2D patients presenting to our clinic were evaluated. T2D was diagnosed based on American Diabetes Association criteria (14). Inclusion criteria were history of diabetes for more than 2 years, hemoglobin A1c (HbA1c) > 6%, not receiving insulin, not changing the medications for reducing blood sugar and blood pressure, controlled hypertension (systolic blood pressure < 140 mmHg and diastolic blood pressure <90 mmHg) and lacking history of regular (more than once a week) exercise for 6 months prior to the study. Exclusion criteria consisted of FBS >400 mg/dl, motor function limitations (such as osteoarthritis), liver and kidney disorders, history of myocardial infarction or bypass surgery of coronary arteries or angioplasty, chronic cardiac failure and cardiac arrhythmia. Sampling was consecutive and patients were allocated to 3 groups of control (without regular training), MICT, and
HIIT, using block randomization with block size 4. Patients were blind to their training group.

2.3. Training intervention

Training intervention was done 3 times a week for 10 weeks (30 sessions in a training course). The training program on leg ergometer bicycle (894E Monark Ergomedic peak bike, Varberg, Sweden) was done between 9:00 AM and 12:00 PM after eating breakfast (2 hours after eating breakfast). HIIT included 10 minutes of warm-up with 40% of maximum heart rate (HR$_{max}$) and 40 minutes of bicycle riding consisting of 8 periods, each period consisting of 3 minutes with 80% of HR$_{max}$, 2 minutes with 50% of HR$_{max}$, and 10 minutes cool-down with 40% of HR$_{max}$. MICT program consisted of 10 minutes of warm-up with 40% of HR$_{max}$, 40 minutes with 70% of HR$_{max}$, and 10 minutes cool-down with 40% of HR$_{max}$. Synchronizing the trainings was done based on heart rate (15, 16).

2.4. Measuring peak oxygen consumption

VO$_{2peak}$ was measured using incremental cycle exercise test (15) by breath-by-breath measurement using a cycle ergometer (COSMED K4, Rome, Italy) between 9:00 AM and 5:00 PM (16). One week before the exercise, all the participants performed a short-term orientation program to get familiar with the test. They were asked to avoid caffeine consumption and intense exercise for 24 hours, and not take any medication before the test (18). The initial workload was set at 30 watts for 2 minutes and increased 20 watts every 2 minutes until the participant was not able to continue and the output power reached less than 40 rpm (15).

2.6. Measuring anthropometric indices

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In summary, peak oxygen consumption (ml/min/kg), NOx (µmol/L), and ET-1 (pmol/L) were measured using Griess assay and Immunoassay, respectively, according to the guidelines of the manufacturer (R&D System Abingdon Science Park, Abingdon OX14 3NB, UK) with internal conversion coefficients of 2.5% and 4%, and external conversion coefficients of 4.6% and 7.6%, respectively. Insulin resistance index was calculated using homeostatic model assessment (HOMA-IR) method based on the following equation: HOMA-IR = [FBS (mmol/L) × fasting insulin (mU/L)] /22.5

### 2.8. Statistical analysis

In previous studies, mean and standard deviation (SD) of VO\textsubscript{peak} in control group (without treatment intervention) and in exercise training group were 26.5 ± 1.4 and 28.9 ± 1.5 ml/kg/min, respectively (15). Therefore, by considering \( \alpha = 0.05, 90\% \) power and 20\% probability of loss to follow-up, sample size was considered 12 patients in each group. SPSS (version 18.0; SPSS Inc., Chicago, IL, USA) was used for data analysis. Kolmogorov–Smirnov test showed normal distribution of data. Data were presented as mean ± SD. Using the one-way ANOVA and Tukey test, the significant difference was considered when \( \text{P} < 0.05 \). The Wilcoxon test was used for data with non-normal distribution. Chi-square test was used to compare the frequency of categorical data.

### Table 1: Demographic and baseline characteristics of the diabetic patients before training intervention

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control group (n = 12)</th>
<th>HIIT group (n = 12)</th>
<th>MICT group (n = 12)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>50.2 ± 2.0</td>
<td>50.3 ± 1.8</td>
<td>50.2 ± 1.5</td>
<td>&gt; 0.99</td>
</tr>
<tr>
<td>Gender (n, %)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>7 (58.3)</td>
<td>6 (50.0)</td>
<td>8 (66.7)</td>
<td>0.71</td>
</tr>
<tr>
<td>Female</td>
<td>5 (41.7)</td>
<td>6 (50.0)</td>
<td>4 (33.3)</td>
<td></td>
</tr>
<tr>
<td>Medication use (n, %)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diuretic</td>
<td>6 (50.0)</td>
<td>6 (50.0)</td>
<td>6 (50.0)</td>
<td>&gt; 0.99</td>
</tr>
<tr>
<td>Angiotensin converting enzyme inhibitors</td>
<td>5 (41.7)</td>
<td>3 (25.0)</td>
<td>3 (25.0)</td>
<td>0.73</td>
</tr>
<tr>
<td>Angiotensin blocker</td>
<td>2 (17.7)</td>
<td>2 (17.7)</td>
<td>2 (17.7)</td>
<td>&gt; 0.99</td>
</tr>
<tr>
<td>Metformin</td>
<td>4 (33.3)</td>
<td>6 (50.0)</td>
<td>5 (41.7)</td>
<td>0.91</td>
</tr>
<tr>
<td>Sulfonylurea</td>
<td>3 (25.0)</td>
<td>3 (25.0)</td>
<td>5 (41.7)</td>
<td>0.73</td>
</tr>
<tr>
<td>Beta blocker</td>
<td>1 (8.3)</td>
<td>1 (8.3)</td>
<td>2 (17.7)</td>
<td>&gt; 0.99</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>79.6 ± 1.4</td>
<td>77.2 ± 1.7</td>
<td>76.3 ± 1.3</td>
<td>0.29</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>170.5 ± 1.5</td>
<td>168.9 ± 1.7</td>
<td>169.9 ± 1.6</td>
<td>0.78</td>
</tr>
<tr>
<td>BMI (kg/m\textsuperscript{2})</td>
<td>27.4 ± 0.2</td>
<td>27.0 ± 0.22</td>
<td>26.9 ± 0.4</td>
<td>0.40</td>
</tr>
<tr>
<td>Waist to hip ratio</td>
<td>0.9 ± 0.01</td>
<td>0.9 ± 0.01</td>
<td>0.8 ± 0.02</td>
<td>0.53</td>
</tr>
<tr>
<td>Duration of diabetes (year)</td>
<td>6.2 ± 0.4</td>
<td>6.2 ± 0.4</td>
<td>6.2 ± 0.4</td>
<td>0.99</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>80.8 ± 4.5</td>
<td>80.1 ± 4.5</td>
<td>80.3 ± 4.6</td>
<td>0.91</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>128.8 ± 6.0</td>
<td>129.1 ± 5.0</td>
<td>127.2 ± 5.2</td>
<td>0.67</td>
</tr>
<tr>
<td>High density lipoprotein (mg/dl)</td>
<td>46.8 ± 7.2</td>
<td>46.8 ± 6.7</td>
<td>45.2 ± 8.2</td>
<td>0.82</td>
</tr>
<tr>
<td>Low density lipoprotein (mg/dl)</td>
<td>106.6 ± 21.5</td>
<td>98.9 ± 21.9</td>
<td>107.7 ± 22.4</td>
<td>0.32</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>185.0 ± 18.8</td>
<td>177.0 ± 19.4</td>
<td>181.7 ± 18.7</td>
<td>0.59</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>158.4 ± 22.3</td>
<td>156.9 ± 24.2</td>
<td>153.9 ± 22.7</td>
<td>0.96</td>
</tr>
<tr>
<td>Fasting plasma glucose (mg/dl)</td>
<td>155.8 ± 19.1</td>
<td>160.7 ± 18.1</td>
<td>156.2 ± 15.8</td>
<td>0.76</td>
</tr>
<tr>
<td>Insulin (mU/l)</td>
<td>11.1 ± 2.4</td>
<td>11.4 ± 2.5</td>
<td>12.3 ± 1.7</td>
<td>0.38</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>4.4 ± 1.4</td>
<td>4.6 ± 1.5</td>
<td>4.8 ± 1.1</td>
<td>0.79</td>
</tr>
<tr>
<td>HbA\textsubscript{1c} (%)</td>
<td>7.4 ± 1.2</td>
<td>7.4 ± 1.0</td>
<td>7.0 ± 0.8</td>
<td>0.46</td>
</tr>
<tr>
<td>Peak oxygen consumption (ml/min/kg)</td>
<td>21.2 ± 2.1</td>
<td>21.2 ± 1.9</td>
<td>20.5 ± 1.4</td>
<td>0.58</td>
</tr>
<tr>
<td>NOx (µmol/L)</td>
<td>13.8 ± 2.4</td>
<td>12.8 ± 2.5</td>
<td>11.9 ± 2.4</td>
<td>0.18</td>
</tr>
<tr>
<td>Endothelin-1 (pmol/L)</td>
<td>50.1 ± 7.0</td>
<td>48.8 ± 7.6</td>
<td>49.2 ± 4.7</td>
<td>0.88</td>
</tr>
</tbody>
</table>

**HIIT:** High-intensity interval training; **MICT:** Moderate intensity continuous training; **HOMA-IR:** Homeostatic model assessment by insulin resistance; **HbA\textsubscript{1c}:** Hemoglobin A1c.

Details of measuring anthropometric indices have been reported in previous studies (19, 20). In summary, height and weight of all the participants were measured wearing light clothes and no shoes. BMI was measured by dividing kg weight to squared height in meter (kg/m\textsuperscript{2}). Waist circumference was measured in the midline between the last rib and the iliac crest; and hip circumference was measured at the great trochanteric femoral region, and WHR was calculated by dividing waist to hip circumference.
mean and SD. To evaluate the effectiveness of training intervention, on serum insulin levels, NOx, ET-1, and VO2_peak, percentage of change in these factors before and after training intervention were calculated. The following formula was used for this purpose:

\[
\text{percentage of change} = \frac{X_1 - X_0}{X_0} \times 100
\]

\(X_0\) = before the test
\(X_1\) = after the test

Finally, to assess the difference of clinical evaluations, anthropometric and biochemical indices, before and after training intervention, one way ANOVA and Bonferroni post hoc test were used. In all analyses, \(p < 0.05\) was considered as significance level.

### 3. Result

During the study period, 100 T2D patients visited the studied clinic. Only 48 of which were eligible to participate in the study based on the inclusion and exclusion criteria. They were divided into 3 groups of 16. Four patients from each group withdrew from participating in the study. In the end, data of 12 patients in each group (a total of 36 patients) were analyzed (Figure 1). None of the studied demographic and baseline characteristics was different between the studied groups (table 1).

Table 2 shows that only LV-HIT and not MICT, causes a significant drop in DBP \([F (2, 33) = 4.1; p = 0.03]\), total cholesterol level \([F (2, 33) = 4.0; p = 0.03]\), triglyceride \([F (2, 33) = 3.6; p = 0.04]\), and fasting blood glucose \([F (2, 33) = 4.7; p = 0.01]\).

Assessment of the effect of training intervention on blood sugar regulation, level of insulin, ET-1, NOx, VO2_peak, HOMA-IR is shown in figure 2. Both MICT and HIIT reduced Ha1c \([F (2, 33) = 80.2; p < 0.0001]\), insulin \([F (2, 33) = 57.7; p < 0.0001]\), and HOMA-IR \([F (2, 33) = 99.1; p < 0.0001]\). However, the effect of HIIT \((p = 0.004)\) was more than MICT \((p < 0.001)\) in reducing the 3 mentioned factors. Both MICT \((p < 0.0001)\) and HIIT \((p = 0.0002)\) led to a significant increase in NOx \([F (2, 33) = 57.7; p < 0.0001]\) in diabetic patients. This increase was significantly higher in HIIT group compared to MICT group \((p < 0.0001)\). In addition, HIIT intervention caused a significant increase in VO2_peak compared to control group \((p < 0.0001)\) and MICT group \((p < 0.0001)\) \([F (2, 33) = 59.9; p < 0.0001]\), while the difference between MICT and control groups was not significant \((p = 0.17)\).

ET-1 level was also reduced after training intervention in both MICT \((p = 0.02)\) and HIIT \((p = 0.02)\) groups compared to control group \([F (2, 33) = 5.5; p = 0.009]\).

Figure 3 shows the relationship between NOx and ET-1. As can be seen, no correlation was detected between ET-1 reduction and NOx increase \((R^2 = 0.07; p = 0.13)\).

### 4. Discussion

The findings of the present study showed that 10 weeks of training with HIIT and MICT both significantly reduce insulin Hba1c, HOMA-IR and ET-1, and increase NOx levels. However, VO2_peak is only significantly affected by HIIT. The effect of training with HIIT in blood sugar and NOx regulation was greater than MICT. In addition, HIIT led to a decrease in lipid profile and FBS, while MICT did not.

Hyperglycemia is one of the major reasons of endothelial dysfunction in diabetes which leads to production of excess reactive oxygen species (21). In oxidative stress state, superoxide reduces NOx synthesis and its half-life by producing peroxynitrite (13). MICT in diabetic patients improves the antioxidant state and decreases oxidants (22). On the other hand, it has been shown that HIIT can increase antioxidant levels more than MICT (13). Therefore, reduction of oxidative stress will be accompanied by an increase in NO bioactivity. HIIT probably has increased NOx more than MICT by increasing the levels of antioxidants (13). In addition, high intensity training leads to more increase in vascular blood flow and therefore, increases shear stress that leads to up-regulation of NOx synthesis (13). The reverse correlation between NO and ET-1 has been
confirmed in vascular endothelium. Wisslof et al. showed that HIIT produces more shear stress. However, their results showed that HIIT and MICT did not significantly decrease ET-1 level. They believed the reason was use of bicycle training, since this type of training probably involves fewer muscles compared to running and walking due to bearing less weight, and maximum heart rate has not reached the extreme and blood flow
has not increased (13). Therefore, high intensity training that leads to an increase in NOx bioactivity and might decrease ET-1. In addition, it has been shown that insulin induces NOx release, while insulin resistance results in dysfunction in NOx production and release (23). In the present study, HIIT group showed more decrease in insulin resistance, therefore we could say that one other reason for a higher increase in NOx level is more decrease in insulin resistance in the HIIT group. Finally, the present study showed that HIIT significantly decreases total cholesterol and triglyceride levels. Thus, in addition to the beneficial effects of HIIT in controlling blood sugar and factors related to vascular endothelial regulation, it can also control the lipid profile level. The role of high cholesterol level in promoting incidence of chronic illnesses such as hypertension, atherosclerosis etc. has been reported in previous studies (24, 25). Since both diabetes and hypotension are major cardiac risk factors, using HIIT in the treatment protocol of diabetic patients might be more effective in controlling prevalence of cardiac disease and its side effects compared to MICT.

5. Limitations

Low sample size is one of the limitations of this study. It is suggested to compare HIIT and MICT protocols with larger sample size. In addition, in more long-term follow-up the results may vary. Therefore, evaluation of the long-term effects of these protocols on blood sugar regulation, lipid profile and vascular endothelial regulatory factors requires carrying out studies with at least 6-month to 1-year follow-up.

6. Conclusion

T2D patients have endothelial dysfunction. HIIT can lead to more improvements in endothelial function compared to MICT, by causing more increase in aerobic fitness, more decrease in insulin resistance, and more increase in NOx bioactivity. Finally, it was shown that HIIT is more effective than MICT in controlling diabetes and lipid profile of T2D patients.

7. Acknowledgment

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8. Conflict of interest

No conflict of interest was declared.

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10. Author contribution

All authors passed four criteria for authorship contribution based on recommendations of the International Committee of Medical Journal Editor.

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