The Influence of Exercise Training on Endothelial Function, Serum Irisin and Inflammatory Markers in the Elderly with Metabolic Syndrome

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SUMMARY

Background: Although exercise has beneficial effects on metabolic syndrome (Mets), the effects of exercise training on endothelial function and irisin level in the elderly with MetS remain controversial. The aim of this study is to evaluate the influence of exercise training on endothelial function and serum irisin level in the elderly with MetS.

Methods: Volunteers with MetS were recruited (n = 30). After 8 consecutive weeks of exercise training, flow-mediated dilation (FMD), colony-forming units (CFU) of endothelial progenitor cell (EPC), and serum irisin level were determined. Body mass index (BMI), Homeostasis Model of Assessment of Insulin Resistance (HOMA-IR), and inflammatory markers were detected.

Results: Exercise training program improved endothelial function, increased EPC-CFU, decreased HOMA-IR and inflammatory markers in patients with metabolic syndrome. However, exercise training had no influence on the serum irisin level. Multivariate linear regression analysis showed that change of EPC-CFU and HOMA-IR were independent prognostic risk factors for change of FMD.

Conclusions: Exercise training improved endothelial function, alleviated insulin resistance and inflammation in the elderly with MetS. The findings suggest that exercise training program is an effective means for the treatment of MetS in the elderly.

(KEY WORDS
metabolic syndrome, irisin, insulin resistance, inflammation, endothelial function

INTRODUCTION

China has become a country with an increasing aging population. It is estimated that about one-fourth of the aging population in China have metabolic syndrome (MetS) [1]. MetS refers to a cluster of medical conditions, such as central obesity, insulin resistance, dyslipidemia, and hypertension [2]. Previous studies indicated that patients with MetS had an increased risk for cardiovascular disease [3]. MetS is also associated with endothelial dysfunction, which is a systemic pathological state characterized by an imbalance between mediators of vasodilation and vasoconstriction produced by the
endothelium [4]. Although the etiology of MetS related vascular complications is not fully understood, endothelial dysfunction may be one of possible mechanisms [5]. Exercise mitigates the negative effects of metabolic diseases and releases a cascade of hormones which may increase energy consumption such as irisin. Irisin, a myokine released from the muscle, plays a key role in regulating exercise related metabolism [6]. It is reported that irisin promotes thermogenesis and energy expenditure via induction of brown adipocytes from white adipose cells and has an impact on the regulation of metabolism [7]. In this sense, exercise training may be an effective treatment to prevent endothelial dysfunction, but conflicting results have been reported in the literature regarding its clinical effectiveness [8-10]. Moreover, the effect of chronic exercise training on irisin level remains controversial [11,12]. It is more complicated in the aging population. Few studies have assessed the effects of aerobic exercise on endothelial function in the elderly. The present study was designed to find out whether regular exercise training could improve endothelial function in the elderly with metabolic syndrome. We also evaluate the effect of exercise training on irisin and inflammatory markers in these patients.

MATERIALS AND METHODS

Study population
Volunteers were recruited from the medical examination center, Qinhuangdao First Hospital. Inclusion criteria: 1. Aged 60 - 75 years. 2. Subjects with MetS. MetS was defined using the criteria [13] of the National Cholesterol Education Program Adult Treatment Panel-III (at least three criteria based on five components: waist circumference, blood pressure, blood glucose, triglycerides, and HDL-cholesterol). 3. Subjects did not exercise regularly for the previous six months. 4. Subjects had not consumed alcohol for two months prior to this study. Exclusion criteria: 1. Smoker. 2. Patients with carcinoma. 3. Patients with acute inflammation. This study was approved by the Ethics Committee of Qinhuangdao First Hospital.

Exercise training program
Thirty volunteers were recruited between January 1, 2019, to September 1, 2019. They performed a training program six days per week for 8 weeks. They performed 30 minutes running on treadmill maintaining 60% of maximum heart rate as aerobic exercise. They also had 30 minutes training wet with perspiration including squat, deadlift, and bench press as an anaerobic exercise [14]. They could start exercise at any time of day.

Laboratory measurements
Physical and anthropometric variables were measured at baseline and after 8 weeks exercise training program in volunteers. Body mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters. Waist circumference was measured at the level of the umbilicus using a flexible plastic tape with the participants in the standing position. Arterial blood pressure was measured after participants had rested for 10 minutes.

Venous blood samples were collected from all participants at baseline and after 8 weeks training. Total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), homocysteine (HCY), glucose, and insulin were measured. Insulin resistance (IR) was evaluated using the Homeostasis Model of Assessment of Insulin Resistance (HOMA-IR) and was calculated as HOMA-IR (mmol/L x µU/mL) = fasting glucose (mmol/L) x fasting insulin (µU/mL)/22.5 [14]. Serum concentrations of irisin, nitric oxide (NO), tumor necrosis factor (TNF-α), high-sensitivity C-reactive protein (hs-CRP) were analyzed using ELISA Kits (Zhuocai Biotech, Shanghai, China), following the manufacturer’s instructions for each kit.

Measurement of FMD
Flow-mediated dilation (FMD) was determined using a VIVID 8 ultrasound system (Philips Medical Systems). The brachial artery was imaged approximately 5 cm proximal to the antecubital crease using B-mode ultrasound. Baseline brachial artery diameters were measured and a forearm blood pressure (BP) cuff was inflated to 250 mmHg for 5 minutes and then deflated [15]. Ultrasound images of the brachial artery were digitally recorded 1 minute to establish end-diastolic preocclusion baseline diameter and for 2 minutes post-occlusion to determine the maximum brachial artery diameter. Flow-mediated dilation was expressed as absolute change in mm (maximum diameter - baseline diameter) and as % change \{[(maximum - baseline diameter)/ baseline diameter] x 100\}.

Endothelial progenitor cell culture and colony-forming unit assay
EPCs were isolated and cultured following our previously described protocols [16]. In briefly, peripheral blood (15 mL) was withdrawn at baseline and after exercise training program. Mononuclear cells (MNCs) were isolated and cultured on fibronectin-coated six well chamber in MCBD/F12 medium with supplements (10% FBS, VEGF10 ng/mL, bFGF 10 ng/mL, IGF 10 ng/mL, EGF 10 ng/mL, heparin 10 U/mL, and antibiotics) (Gibco) at 37°C in a 5% CO2 incubator. After changing medium on day 2, medium was replaced every 3 days. Cobblestone-shaped cells emerged 5 to 7 days after start of MNC culture. After 12 days of culture, the colony forming unit (CFU) was identified by visual inspection with an inverted microscope (Leica). A central cluster surrounded by emerging cells was recognized as a CFU. The CFU assay was performed for all the subjects.
Table 1. Clinical characteristics at baseline and 8 weeks exercise training.

<table>
<thead>
<tr>
<th></th>
<th>Baseline (n = 30)</th>
<th>8 weeks (n = 30)</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>70.34 ± 5.68</td>
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<td></td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>21/9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>168.91 ± 9.18</td>
<td>168.96 ± 9.14</td>
<td>0.125</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>88.42 ± 11.56</td>
<td>88.22 ± 12.91</td>
<td>0.125</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.95 ± 2.45</td>
<td>29.67 ± 2.23</td>
<td>0.066</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>96.75 ± 9.87</td>
<td>95.81 ± 9.32</td>
<td>0.403</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>139.51 ± 6.10</td>
<td>136.21 ± 5.86</td>
<td>0.018</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>85.54 ± 6.90</td>
<td>80.36 ± 9.13</td>
<td>0.012</td>
</tr>
</tbody>
</table>

BMI - body mass index, SBP - systolic blood pressure, DBP - diastolic blood pressure, * - p < 0.05 compared with baseline.

Statistical analysis
All statistical analyses were performed by SPSS 17 (SPSS, Chicago, IL, USA). Continuous variables were expressed as mean ± standard deviation of the mean. A paired t-test was used to analyze the significance of within group comparisons. Univariate and stepwise multiple linear regressions were performed to estimate the correlations of the mean change of FMD with changes of EPC-CFU, HOMA-IR, BMI, glucose, CRP, IL-6, and irisin levels. A probability value ≤ 0.05 was considered to indicate statistical significance.

RESULTS
Physical characteristics of baseline and 8 weeks exercise training
The baseline and 8 weeks physical characteristics are presented in Table 1. After 8 weeks exercise training, waist circumference, body weight, and BMI were decreased, although they had no statistical difference compared to baseline. Systolic blood pressure and diastolic blood pressure were both decreased after 8 weeks exercise (compared to baseline, p < 0.05).

Exercise training decreased insulin resistance and inflammation marker
After 8 weeks, volunteers had lower level of glucose and HOMA-IR than baseline. This result indicated that exercise training could decrease insulin resistance in patients with MetS (Table 2). The results also show that inflammation markers such as CRP, TNF-α, and HCY were decreased after 8 weeks exercise training. However, there were no significant differences in insulin, NO, TG, TC, LDL-C, and HDL-1 between baseline and 8 weeks.

Irisin level
As shown in Figure 1, there was no difference in serum irisin levels between baseline and 8 weeks (baseline 25.52 ± 7.08 μg/L, 8 weeks 24.64 ± 8.56 μg/L, p > 0.05).

Endothelial function
As shown in Figure 2, values of flow-mediated inflation increased significantly after 8 weeks exercise training (baseline 5.15 ± 2.12%, 8 weeks 6.73 ± 2.33%, p < 0.05). To evaluate the level of EPCs in circulation, an EPC-CFU assay was performed. EPC-CFUs can be classified as colony formed by central cluster surrounded by spindle cells (Figure 3). As shown in Figure 4, exercise training significantly increased total EPC-CFU counts (baseline 2.13 ± 1.37/well vs. 8 weeks 4.56 ± 2.13/well, p < 0.05). The correlations of changes of FMD with clinical characteristics and laboratory parameters were analyzed using univariate and stepwise multiple linear regression models (Table 3). In univariate analysis, changes of FMD were shown to be positively correlated with changes of EPC-CFU (p < 0.05) and changes of CRP (p < 0.05), but negatively correlated with changes of HOMA-IR (p < 0.05), CRP (p < 0.05), TNF-α and fasting plasma glucose (p < 0.05). Stepwise multiple linear regression models identified that the changes of EPC-CFU and HOMA-IR were independent prognostic risk factors for changes of FMD (p < 0.05).

DISCUSSION
The present study demonstrated that eight weeks exercise training could improve endothelial function, decrease insulin resistance and inflammatory markers in the elderly with metabolic syndrome.

Influence of exercise training on insulin resistance and inflammation
Metabolic syndrome is a state of chronic inflammation. It is clear that inflammatory cytokines such as TNF-α...
We found that exercise training induced a decrease of inflammation in adipose tissue due to a chronic low-level inflammation of the tissue are fundamental contributors to metabolic syndrome [17]. Previous study demonstrated that preventing inflammation in adipose tissue could prevent insulin resistance [18]. Our results pointed to the anti-inflammatory effect of regular exercise. We found that exercise training induced a decrease of hs-CRP, TNF-α, and HCY concentration. We also found a significant decrease of HOMA-IR after 8 weeks training program. These results indicated that exercise training could prevent inflammation and alleviate insulin resistance.

### Influence of exercise training on irisin level
Irisin is an exercise-related hormone. It is reported that irisin improves insulin resistance by increasing sensitivity of the insulin receptor in skeletal muscle and heart and browning of white adipose tissue [19]. Although the benefit of irisin is obvious, whether chronic exercise is a potent stimulus for irisin production is still a debate.

Table 2. Comparison of laboratory parameters between baseline and 8 weeks exercise training.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline (n = 30)</th>
<th>8 weeks (n = 30)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mmol/L)</td>
<td>4.49 ± 1.22</td>
<td>4.45 ± 1.28</td>
<td>0.618</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>2.04 ± 0.84</td>
<td>2.14 ± 0.85</td>
<td>0.256</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>2.57 ± 0.91</td>
<td>2.55 ± 0.93</td>
<td>0.488</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.05 ± 0.19</td>
<td>1.06 ± 0.13</td>
<td>0.837</td>
</tr>
<tr>
<td>HCY (μmol/L)</td>
<td>14.86 ± 5.74</td>
<td>12.72 ± 3.24</td>
<td>0.034</td>
</tr>
<tr>
<td>hs-CRP (mg/L)</td>
<td>4.01 ± 3.04</td>
<td>2.58 ± 2.32</td>
<td>0.001</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>7.66 ± 2.49</td>
<td>7.35 ± 1.67</td>
<td>0.043</td>
</tr>
<tr>
<td>Insulin (UI/mL)</td>
<td>6.54 ± 2.95</td>
<td>6.47 ± 2.35</td>
<td>0.138</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.54 ± 1.14</td>
<td>2.27 ± 1.13</td>
<td>0.026</td>
</tr>
<tr>
<td>NO (μmol/L)</td>
<td>146.91 ± 89.38</td>
<td>160.16 ± 94.36</td>
<td>0.349</td>
</tr>
<tr>
<td>TNF-α (μmol/L)</td>
<td>2.61 ± 1.21</td>
<td>2.23 ± 1.01</td>
<td>0.038</td>
</tr>
<tr>
<td>Irisin (μg/L)</td>
<td>25.52 ± 7.08</td>
<td>24.64 ± 8.56</td>
<td>0.125</td>
</tr>
</tbody>
</table>

Table 3. Linear regression of mean changes of FMD with changes of clinical characteristics and laboratory parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Univariate linear regression</th>
<th>Multiple linear regression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coefficient (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>Δ hs-CRP</td>
<td>-0.39 (-0.56 - 0.12)</td>
<td>0.036</td>
</tr>
<tr>
<td>Δ TNF-α</td>
<td>-0.17 (-0.28 to -0.11)</td>
<td>0.038</td>
</tr>
<tr>
<td>Δ HCY</td>
<td>-0.11 (-0.35 - 0.21)</td>
<td>0.127</td>
</tr>
<tr>
<td>Δ HOMA-IR</td>
<td>-0.41 (-0.65 to -0.16)</td>
<td>0.011</td>
</tr>
<tr>
<td>Δ BMI</td>
<td>-0.07 (-0.45 - 0.11)</td>
<td>0.321</td>
</tr>
<tr>
<td>Δ glucose</td>
<td>-0.18 (-0.38 - 0.05)</td>
<td>0.39</td>
</tr>
<tr>
<td>Δ Irisin</td>
<td>0.12 (-0.13 - 0.31)</td>
<td>0.135</td>
</tr>
<tr>
<td>ΔEPC-CFU</td>
<td>0.34 (0.16 - 0.75)</td>
<td>0.34 (0.16 - 0.75)</td>
</tr>
</tbody>
</table>

Clinical characteristics and laboratory parameters significantly correlated with mean changes of FMD in univariate linear regression were further analyzed in multivariate model.

FMD - flow-mediated dilation, HOMA-IR - homeostasis model assessment for insulin resistance, hs-CRP - high sensitivity C-reactive protein, HCY - homocysteine, NO - nitric oxide, TNF-α - tumor necrosis factor.

and IL-6 from adipose tissue due to a chronic low-level inflammation of the tissue are fundamental contributors to metabolic syndrome [17]. Previous study demonstrated that preventing inflammation in adipose tissue could prevent insulin resistance [18]. Our results pointed to the anti-inflammatory effect of regular exercise. We found that exercise training induced a decrease of hs-CRP, TNF-α, and HCY concentration. We also found a significant decrease of HOMA-IR after 8 weeks training program. These results indicated that exercise training could prevent inflammation and alleviate insulin resistance.
Exercise Training Improves Endothelial Function in the Elderly

Figure 1. Comparison of irisin levels between baseline and 8 weeks.
There were no significant differences in irisin levels between baseline and 8 weeks after exercise training.

Figure 2. Comparison of FMD between baseline and 8 weeks.
Eight weeks exercise training could enhance values of flow-mediated dilation (FMD).

Bostrom et al. found a two-fold increase of circulating irisin after 10 weeks of endurance training [20]. A study by Wiecek et al. showed that short-term anaerobic exercise is a factor inducing the secretion of irisin, while the increase in blood levels of irisin only occurred in women [21]. However, Huh et al. showed no increase in iri-
Figure 3. Colony of endothelial progenitor cells.

A typical colony of endothelial progenitor cells. Seven days after cell culture, a colony formed by central cluster surrounded by spindle cells. (100 x magnification).

Figure 4. Comparison of CFU-EPC between baseline and 8 weeks.

sin after 8 weeks of intermittent sprint running [22]. The results of Pekkala et al. also revealed that 21 weeks of combined endurance and strength training did not induce irisin secretion [23].

In the current study, we found that 8 weeks regular exercise could not increase serum level of irisin in the elderly with metabolic syndrome. The conflicting data revealed complexity of exercise induced irisin release. It
Exercise Training Improves Endothelial Function in the Elderly

Effect of exercise training on endothelial function
FMD in the brachial artery has been widely used for the assessment of endothelial function [15]. It has strong prognostic value in predicting cardiovascular events [15]. Values of FMD were increased after 8 weeks exercise training in this study. This result was in accordance with a study by Zguira et al. [24]. Endothelial progenitor cells are a new marker of endothelial function. They have the ability to promote endothelial repair, neovascularization, and the restoration of endothelial function [15]. An altered status of circulating EPCs represents a marker of endothelial dysfunction. Physical exercise is one of the most important physiological stimuli to mobilize EPCs in healthy individuals. Several studies had revealed that aerobic exercise could elevate circulating EPC levels [14,25,26]. But there were few studies evaluating the impact of regular exercise on circulating EPC in the elderly with MetS. Our previous study [27] indicated that objects with MetS had significantly lower level of circulating EPCs compared with healthy control. In this study, we observed that exercise training increased colonies of EPCs. Previous studies have found positive correlation between EPC-CFU and FMD response to hyperemia [15, 28]. The present study showed that changes of EPC-CFU and HOMA-IR were independent prognostic risk factors for change of FMD. This result indicated that exercise training improved endothelial function by elevation of EPC level and alleviation of insulin resistance in the elderly with metabolic syndrome.

CONCLUSION
This study found an increase of flow-mediated dilation and an increase of EPC number after 8 weeks of regular exercise program in the elderly. Exercise training also decreased insulin resistance and inflammatory factors. However, exercise could not influence circulating irisin level in these patients. These findings indicated that exercise training had beneficial effects on endothelial function and could prevent insulin resistance and inflammation in the elderly with metabolic syndrome.

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Declaration of Interest: All authors declare that there are no conflicts of interest.

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