Exercise ameliorates serum MMP-9 and TIMP-2 levels in patients with type 2 diabetes


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Abstract

Aim. – This study assessed the impact of regular exercise on inflammatory markers (high-sensitivity C-reactive protein [hsCRP], fibrinogen), and matrix metalloproteinases (MMPs) and their inhibitors (TIMPs), in patients with type 2 diabetes mellitus (T2DM).

Patients. – Fifty overweight patients with T2DM were randomly assigned to two groups: (A) an exercise group (EXG, n = 25), with self-controlled exercise for at least 150 min/week and one additional supervised exercise session/week; and (B) a control group (COG, n = 25), with no exercise instructions. All participants were taking oral antidiabetic drugs, and none had diabetic complications. Clinical parameters, exercise capacity (VO2peak), ventilatory threshold (VT), insulin-resistance indices (fasting insulin, HOMA-IR, HOMA%S), hsCRP, fibrinogen, MMP-2, MMP-9, TIMP-1 and TIMP-2 were assessed at baseline and after 16 weeks.

Results. – No significant changes were found in body mass index, waist/hip ratio, insulin-resistance indices, MMP-2 and TIMP-1 throughout the study in either group (P > 0.05). Compared with controls, the EXG showed a significant decrease in systolic and mean blood pressure, total and LDL cholesterol, and HbA1c (P < 0.05). Also, exercise significantly suppressed levels of fibrinogen (P = 0.047), hsCRP (P = 0.041) and MMP-9 (P = 0.028), and the MMP-9-to-TIMP-1 ratio (P = 0.038), whereas VO2peak (P = 0.011), VT (P = 0.008) and plasma TIMP-2 levels (P = 0.022) were considerably upregulated in the EXG vs. COG. Standard multiple-regression analyses revealed that MMP-9 changes were independently associated with fibrinogen and HbA1c changes, while fibrinogen changes independently predicted TIMP-2 alterations with exercise.

Conclusion. – Mostly self-controlled exercise of moderate intensity ameliorated serum levels of pro- and anti-atherogenic markers in patients with T2DM, with no effects on body weight. These data offer further insight into the cardioprotective mechanisms of exercise in patients with T2DM.

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Keywords: Exercise; Type 2 diabetes; MMPs; TIMPs; Fibrinogen; HsCRP

Résumé

La pratique régulière de l’exercice physique améliore les concentrations plasmatiques de MMP-9 et de TIMP-2 chez des diabétiques de type 2.

Objectif. – Évaluer l’impact de l’exercice physique régulier sur les marqueurs de l’inflammation (CRPus ultrasensible, fibrinogène), les métalloprotéinases de la matrice (MMPs) et leurs inhibiteurs (TIMPs) chez des patients atteints de diabète de type 2 (DT2).

Patients. – Cinquante DT2 en excès pondéral traités par antidiabétiques oraux et indemnes de complications ont été repartis par randomisation en deux groupes : exercice physique (n = 25), avec 150 minutes par semaine au moins d’exercice autocontrôlé et une séance supplémentaire par semaine ; témoin (n = 25), avec aucune instruction concernant l’activité physique. Les paramètres cliniques, la capacité d’exercice (VO2peak), le seuil ventilatoire (VT), les indices d’insulinorésistance (insulinémie à jeun, HOMA-IR, HOMA%S), la CRPus, fibrinogène, MMP-2, MMP-9, TIMP-1 et TIMP-2 ont été évalués à l’inclusion puis 16 semaines plus tard.
1. Introduction

Exercise comprises the cornerstone of type 2 diabetes mellitus (T2DM) treatment, as even moderate levels of physical activity have been demonstrated to improve the glycaemic profile and reduce T2DM-associated cardiovascular risk in the diabetic population [1]. Long-term studies suggest that the modification of ‘traditional’ cardiovascular risk factors (such as dyslipidaemia and hypertension) can partly explain the cardiovascular benefits of exercise [2]. Indeed, it may be that physical activity has ‘pleiotropic’ actions on the cardiovascular system, although the precise underlying mechanisms remain elusive.

Matrix metalloproteinases (MMPs) are an ever-expanding family of endopeptidases with proteolytic activity towards components of the extracellular matrix [3,4]. Both experimental and human data suggest that MMPs – and especially MMP-2 and MMP-9 – can mediate atherogenesis, facilitate plaque disruption and enhance blood hypercoagulability [5,6]. In particular, the net proteolytic activity is determined by the balance between relative concentrations of active MMPs and their inhibitors (TIMPs) [7]. Thus, a shift in MMP/TIMP equilibrium to net proteolytic activity is closely associated with a higher incidence of cardiovascular events, especially in the diabetic population [8]. Although previous studies have investigated the pharmaceutical modulation of MMPs in atherosclerotic conditions [9], the putative influence of lifestyle interventions on MMP/TIMP homoeostasis remains obscure.

Up to now, the strong relationship between numerous inflammatory markers, such as high-sensitivity C-reactive protein (hsCRP) and fibrinogen, and cardiovascular events has been well documented [10,11]. Also, it is known that sustained exercise has the potential to inhibit the T2DM-related inflammatory milieu [12]. However, as the vast majority of data is derived from structured exercise programmes, little attention has been paid to the anti-inflammatory results of self-controlled exercise training.

In the present study, we investigated the effects of a 16-week, mostly self-controlled, exercise intervention on novel cardiovascular risk factors, such as inflammatory agents and the MMP/TIMP system, in patients with T2DM. We also hypothesized that exercise could favourably mediate MMP/TIMP balance via its anti-inflammatory properties.

2. Patient and methods

2.1. Subjects

Fifty inactive, overweight (body mass index [BMI] >25 kg/m²) Caucasian patients with T2DM (17 men, 33 women) were recruited from our institution’s diabetes outpatients department. All of the selected patients, aged 50–65 years, were following a diet and taking oral antidiabetic drugs (OADs) for at least 4 months, but still had inadequate glycaemic control (HbA1c > 7%). However, patients with ischaemic heart disease, overt diabetic vascular complications, chronic heart failure, uncontrolled hypertension (blood pressure > 170/100 mmHg), excessive hyperglycaemia (HbA1c > 10%), arrhythmias, orthopaedic problems limiting exercise, liver or renal impairment and chronic co-morbid conditions (such as cancer, immunodeficiency and autoimmune diseases) were excluded. In addition, patients using lipid-lowering, thiazolidinedione or insulin therapy were considered ineligible.

The study protocol was approved by the local ethics committee and conducted in accordance with the Helsinki Declaration. Also, written informed consent was given by all participating patients.

2.2. Clinical variables

Clinical parameters, such as BMI, waist-to-hip ratio (WHR) and blood pressure (BP), were measured at baseline (study entry) and at the end of the study by a single operator. BMI was calculated as body weight in kilogram divided by the square of height in meter (kg/m²). Waist circumference was measured at the level midway between the lowest rib margin and the iliac crest, and hip circumference was measured at the level of the greater femoral trochanters. Thus, WHR expressed waist circumference divided by hip girth. BP was measured twice, with the patient in a sitting position for 15 min and a 5-min interval between the two measurements, with the mean value calculated for the study analyses. Pulse and mean BPs were also calculated.

Antihypertensive medications remained unchanged throughout the study. Dietary habits of all patients were recorded by an experienced dietician at baseline and at the end of the study. No additional dietary instructions were given for weight control and...
patients were asked to maintain their usual eating habits. Patients were also examined for peripheral neuropathy on entering the study.

2.3. Study protocol

Eligible patients were randomly assigned to either an exercise (EXG, n = 25) or control (COG, n = 25) group, and underwent clinical examination, blood sampling and ergospirometry at baseline and after 16 weeks. Following the American Diabetes Association guidelines for cardiovascular disease prevention, patients in the EXG were given oral and written instructions to perform 30–60 min of brisk walking, distributed over at least 4 days/week, with no more than 2 consecutive days without physical activity [13]. Patients were also encouraged to increase their daily lifestyle activities (such as taking walking breaks during the workday, gardening and doing household work). The main goal for each individual was to accumulate more than 150 min/week of self-controlled, moderate-intensity (relative to their estimated ventilatory threshold [VT]) physical activity. EXG patients were asked to record all daily activities in personal diaries and to attend a meeting with an exercise trainer once a week. Each meeting comprised:

(a) records of the previous week’s activity, which were checked to ensure patients’ adherence to the prescribed exercise programme, and encouragement to the patients to achieve and/or maintain exercise targets;
(b) an exercise session, consisting mostly of a 10-min warm-up, 30–45 min of aerobic activity and a 5-min cool-down, which was supervised by the exercise trainer.

Exercise intensity was individualized and included walking on a treadmill, cycling and calisthenics. The intensity and duration of each session gradually increased to 50–70% of their peak oxygen consumption (VO2peak) and 60 min, respectively, for the first 4 weeks. After that, the exercise parameters remained constant. The COG was advised to maintain their usual habitual activities throughout the study.

2.4. Cardiorespiratory fitness assessment

Ergospirometry was performed in all participants under a physician’s supervision, using an electronically controlled ergocycle and an exercise-testing protocol, which has been described elsewhere [14]. In particular, the initial workload was set at 30 W and was gradually increased by 20 W at each 2-min stage. Electrocardiography (ECG) and BP were recorded throughout the test. Oxygen uptake and carbon dioxide output (VCO2) were also continuously measured with a gas-exchange analyzer (COSMED K4, Rome, Italy), using a facemask and a breath-by-breath technique. VT was assessed by two investigators using a combination of breakpoints in the relationship between VO2 and VCO2 (the V-slope method), as described by Beaver et al. [15]. VO2peak was considered to have been achieved if either:

1. the respiratory exchange ratio was >1.1;
2. heart rate within 10 beats/min of the age-predicted maximum heart rate;
3. no significant increase of VO2 (<1 mL kg⁻¹ min⁻¹) occurred despite the increase in work rate.

The test was terminated when the participant was no longer able to continue or maintain a constant rate of pedal revolutions. ECG abnormalities, thoracic discomfort, excessive hypertension or, conversely, a hypotensive response were indications for premature termination of the exercise challenge. All subjects were asked to refrain from caffeine, alcohol and any intensive physical activity for 48 h prior to the exercise test. To familiarize the patients to the testing procedure, preliminary ergospirometry testing of short duration and low intensity was performed a week before the study began.

2.5. Blood-sample analysis

Blood samples were drawn after overnight fasting. Patients were requested to avoid any strenuous physical activity for at least 48 h before blood sampling at baseline, and samples were also obtained 72 h after the last exercise session at the end of the study. Fasting plasma glucose (FPG) and lipid parameters were quantified using an enzymatic method and an automatic analyzer (Roche/Hitachi 912; Roche Diagnostics, Basel, Switzerland). Low-density lipoprotein (LDL) cholesterol was calculated by Friedewald’s equation. Measurements of HbA1c were taken by high-performance liquid chromatography (Menarini Diagnostics, Florence, Italy). Plasma MMP-2, MMP-9, TIMP-1 and TIMP-2 were assayed by Quantikine Immunoassay (R&D Systems Inc., Minneapolis, MN, USA), using interassay coefficients of variance (CVs) of 5.6, 7.5, 4.2 and 6.6%, respectively, while their intra-assay CVs were 5.4, 2.3, 4.4 and 5.6%, respectively. Plasma insulin was determined using a commercially available ELISA kit (DRG Diagnostics, Marburg/Lahn, Germany). The inter- and intra-assay CVs for insulin were 3 and 3.4%, respectively. Surrogates of insulin resistance, such as homeostasis model assessment for fasting insulin (HOMA-IR = fasting insulin × FPG/405), and a computer model (HOMA%S) were also calculated [16]. Ultra-high-sensitivity latex turbidimetric immunoassay (Wako Chemicals GmbH, Neuss, Germany) was used to determine hsCRP. Fibrinogen was measured immediately after sampling, using the Clauss method. The mean value of two measurements was used in the study analyses. Samples were frozen and stored (at −80 °C) until analysis in the same assay.

2.6. Statistical analysis

Results are presented as means ± S.D. Comparisons between and within groups were performed using Student’s independent t test and paired-samples t test for parametric data, respectively. Categorical variables were assessed by Chi-square analysis, and normality of distribution by the Shapiro-Wilk test. All variables were normally distributed. Pearson’s correlation coefficient was used for the univariate analysis. The relationships between
Table 1
Baseline characteristics of the study patients.

<table>
<thead>
<tr>
<th>Exercise group</th>
<th>Control group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>23</td>
<td>24</td>
</tr>
<tr>
<td>Gender (men/women)</td>
<td>8/15</td>
<td>7/17</td>
</tr>
<tr>
<td>Age (years)</td>
<td>56.8±6.76</td>
<td>60.3±9.28</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>6.5±4.64</td>
<td>7.7±4.9</td>
</tr>
<tr>
<td>Smokers [n (%)]</td>
<td>3 (13.04)</td>
<td>2 (8.33)</td>
</tr>
<tr>
<td>Antihypertensive medications [n (%)]</td>
<td>15 (65.8)</td>
<td>13 (66.7)</td>
</tr>
<tr>
<td>Oral antidiabetic drugs [n]</td>
<td>23</td>
<td>24</td>
</tr>
<tr>
<td>Sulphonylurea (n)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Metformin (n)</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Sulphonylurea + metformin (n)</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>hsCRP (mg/L)</td>
<td>4.36±2.09</td>
<td>3.89±2.73</td>
</tr>
<tr>
<td>MMP-2 (ng/mL)</td>
<td>789.2±301.3</td>
<td>746.4±213.1</td>
</tr>
<tr>
<td>MMP-9 (ng/mL)</td>
<td>574.62±218.71</td>
<td>583±141.82</td>
</tr>
<tr>
<td>TIMP-1 (ng/mL)</td>
<td>192.3±37.67</td>
<td>193.75±18.48</td>
</tr>
<tr>
<td>TIMP-2 (ng/mL)</td>
<td>34.09±10.44</td>
<td>36±11.72</td>
</tr>
</tbody>
</table>

Data are expressed as means±S.D. unless otherwise indicated; NS: not significant; hsCRP: high-sensitivity C-reactive protein.

3. Results

Twenty-five patients were initially assigned to each study group. However, two patients in the EXG and one in the COG dropped out at an early stage for personal reasons. At baseline, all clinical, biochemical and pharmaceutical parameters were similar between groups (Tables 1 and 2). It is also worth noting that no adverse events (such as hypoglycaemic episodes) or different gender-related responses to exercise were reported at any time during the study. Also, dosages of sulphonylurea agents had to be reduced in three patients in the EXG due to considerable attenuation of their hyperglycaemia. Exercise-treated patients were highly compliant to treatment: at least 85% of the structured sessions were attended and 86.96% achieved the target of 150 min/week of exercise.

Table 2
Clinical and biochemical parameters at baseline and at the end of the study in the study patients.

<table>
<thead>
<tr>
<th>Exercise group (n=23)</th>
<th>Control group (n=24)</th>
<th>16 weeks</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>16 weeks</td>
<td>p&lt;1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>31.69±4.15</td>
<td>31.56±4.47</td>
<td>0.888</td>
<td>31.3±4.91</td>
<td>31.53±4.07</td>
<td>0.908</td>
<td>0.774</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.98±0.04</td>
<td>0.95±0.05</td>
<td>0.275</td>
<td>0.94±0.13</td>
<td>0.94±0.12</td>
<td>0.912</td>
<td>0.173</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>42±4</td>
<td>41.4±3.7</td>
<td>0.559</td>
<td>41.3±3.3</td>
<td>40.8±3.7</td>
<td>0.564</td>
<td>0.211</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>134±19</td>
<td>125±18.9</td>
<td>0.014</td>
<td>133.5±17.1</td>
<td>132.3±21.6</td>
<td>0.962</td>
<td>0.022</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>81.9±10.7</td>
<td>78.1±9.6</td>
<td>0.011</td>
<td>78.5±6.9</td>
<td>80.4±9</td>
<td>0.506</td>
<td>0.008</td>
</tr>
<tr>
<td>Pulse BP (mmHg)</td>
<td>52.3±7.1</td>
<td>47.4±6.5</td>
<td>0.045</td>
<td>55.7±7.8</td>
<td>51.9±8.4</td>
<td>0.128</td>
<td>0.791</td>
</tr>
<tr>
<td>Mean BP (mmHg)</td>
<td>99.3±13.47</td>
<td>93.9±12.7</td>
<td>0.012</td>
<td>96.8±10.3</td>
<td>97.7±13.2</td>
<td>0.724</td>
<td>0.009</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.58±0.61</td>
<td>7.24±0.62</td>
<td>0.007</td>
<td>7.55±0.63</td>
<td>7.73±0.6</td>
<td>0.303</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FPG (mg/dL)</td>
<td>163.4±37.7</td>
<td>147±32.3</td>
<td>0.035</td>
<td>164.7±24.7</td>
<td>178±31.1</td>
<td>0.042</td>
<td>0.025</td>
</tr>
<tr>
<td>Insulin (mU/L)</td>
<td>11.3±4.92</td>
<td>10.89±3.51</td>
<td>0.772</td>
<td>9.87±5.58</td>
<td>10.44±6.26</td>
<td>0.739</td>
<td>0.342</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>4.56±2.4</td>
<td>3.95±2.25</td>
<td>0.592</td>
<td>4.01±1.78</td>
<td>4.59±1.8</td>
<td>0.426</td>
<td>0.100</td>
</tr>
<tr>
<td>HOMA%S</td>
<td>146.9±34.4</td>
<td>173±56.2</td>
<td>0.189</td>
<td>166.6±46.92</td>
<td>143.4±39.75</td>
<td>0.273</td>
<td>0.079</td>
</tr>
<tr>
<td>TChol (mg/dL)</td>
<td>222.6±39</td>
<td>206.3±39.6</td>
<td>0.010</td>
<td>227.9±45.4</td>
<td>229.3±52</td>
<td>0.900</td>
<td>0.012</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>53.2±15.9</td>
<td>53.4±15.4</td>
<td>0.890</td>
<td>54.4±11.2</td>
<td>53±12.5</td>
<td>0.334</td>
<td>0.321</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>141±30.5</td>
<td>125.4±30.9</td>
<td>0.016</td>
<td>143.1±41.4</td>
<td>144.8±47.7</td>
<td>0.801</td>
<td>0.026</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>142.1±60.1</td>
<td>137.6±61.5</td>
<td>0.677</td>
<td>152.2±74.6</td>
<td>159.1±78.9</td>
<td>0.747</td>
<td>0.341</td>
</tr>
<tr>
<td>Fibrinogen (mg/dL)</td>
<td>415±87.8</td>
<td>345.8±61.8</td>
<td>0.038</td>
<td>356.4±70.7</td>
<td>353.4±69.7</td>
<td>0.915</td>
<td>0.047</td>
</tr>
<tr>
<td>VO2peak (mL kg⁻¹ min⁻¹)</td>
<td>21.16±4</td>
<td>23.54±4.94</td>
<td>0.010</td>
<td>21.13±7.03</td>
<td>21.07±6.05</td>
<td>0.585</td>
<td>0.011</td>
</tr>
<tr>
<td>VT (mL kg⁻¹ min⁻¹)</td>
<td>11.73±2.1</td>
<td>13.44±2.22</td>
<td>0.025</td>
<td>11.98±3.54</td>
<td>11.89±5.04</td>
<td>0.889</td>
<td>0.008</td>
</tr>
<tr>
<td>Test duration (min)</td>
<td>8.92±1.44</td>
<td>9.59±1.51</td>
<td>&lt;0.001</td>
<td>8.81±2.21</td>
<td>8.43±1.91</td>
<td>0.363</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are expressed as means±S.D.; BMI: body mass index; BP: blood pressure; FPG: fasting plasma glucose; HOMA-IR: homeostasis model assessment for insulin resistance; HOMA%S: calculated computer model of insulin sensitivity; TChol: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; VO2peak: peak oxygen consumption; VT: ventilatory threshold; P1: changes of variables within groups; P2: changes of variables between groups.
Fig. 1. Plasma MMP-2, MMP-9, TIMP-1, TIMP-2 and high-sensitivity C-reactive protein (hsCRP) levels, and the MMP-9/TIMP-1 ratio in the exercise-treated and control patients at baseline and at the end of the study. Data are expressed as means ± S.D. \( P_1 \): changes of variables within groups; \( P_2 \): changes of variables between groups.

3.1. Clinical characteristics, insulin sensitivity, glycaemia and lipid profiles

Anthropometric parameters (BMI, WHR) and insulin-resistance surrogate indices (fasting insulin, HOMA-IR, HOMA%S) changed only slightly \( (P>0.05) \) throughout the course of the study in both patient groups. On the other hand, indices of exercise capacity, such as VO\(_{2}\)peak and test duration, were significantly improved in the EXG compared with the COG \( (P<0.05) \). In addition, the percent of VO\(_{2}\)peak at which VT occurred was significantly higher after the exercise intervention, indicating that these patients were able to exercise at higher submaximum exercise intensities before the onset of blood lactic-acid accumulation. Moreover, the 16-week exercise programme significantly reduced HbA\(_1c\) \( (P<0.001) \), FPG \( (P=0.025) \), systolic BP \( (P=0.022) \) and mean BP \( (P=0.009) \) levels compared with the usual activity in the controls. Concerning lipid parameters, the exercise intervention significantly lowered total \( (P=0.012) \) and LDL \( (P=0.026) \) cholesterol concentrations (Table 2).

3.2. New cardiovascular risk factors

The results for new cardiovascular risk factors are shown in Table 2 and Fig. 1. Those who followed the exercise protocol demonstrated a larger reduction in hsCRP \( (P=0.041) \) and fibrinogen \( (P=0.047) \) than did the controls. In addition, the differences in changes in MMP-9 \( (P=0.028) \), MMP-9/TIMP-1 ratio \( (P=0.038) \) and TIMP-2 \( (P=0.022) \) between the EXG and COG were significant. Changes in serum MMP-2 and TIMP-1 levels were negligible (and not significant) in both groups.

Pearson’s correlations were calculated for the changes in novel cardiovascular risk factors in the exercise patients. The exercise-induced reduction in MMP-9 was positively associated with changes in hsCRP \( (r=0.771, P=0.021) \) and HbA\(_{1c}\) \( (r=0.707, P=0.021) \).
(r = 0.253, P = 0.003) and, on standard multiple-regression analyses, the latter dependent variables appeared to explain 31.2% of the altered MMP-9 values (P = 0.039). The change in TIMP-2 was significantly correlated with changes in hsCRP (r = –0.108, P = 0.026) and fibrinogen (r = –0.678, P = 0.008). The latter variable retained its significant correlation with TIMP-2 on standard multiple-regression analyses (R² = 0.211, P = 0.017). No significant correlation between MMP-2 or TIMP-1 and the rest of variables, however, was detected.

4. Discussion

Despite the absence of significant alterations in body weight and insulin resistance, the 16-week, moderate-intensity intervention of mostly self-controlled exercise significantly reduced the inflammatory milieu, with significant improvements on glycaemic, lipid and cardiorespiratory parameters in patients with T2DM. To our knowledge, this was the first study to demonstrate significant exercise-related amelioration of novel atherosclerotic markers such as MMP-9 and TIMP-2 in the diabetic population. The exercise-induced beneficial effects on inflammation and glucose regulation may have mediated such changes.

Although insulin-resistance indices remained unaltered, exercise intervention yielded modest—yet significant—improvements in glycaemia and lipid profiles, and cardiorespiratory capacity. However, compared with previous studies, the present study intervention reduced HbA₁c to a lesser extent [12]. A more intensive and structured exercise intervention in patients with significantly elevated baseline values of HbA₁c may perhaps have led to better results. Although a significant proportion of our exercise-treated patients failed to achieve the glycaemia target (HbA₁c < 7%), even modest improvements in glycaemic parameters are of clinical importance. In addition, our realistic lifestyle approach considerably increased cardiorespiratory capacity, with clear benefits for T2DM management. Indeed, Brun et al. [17] have demonstrated the cost-effectiveness of 1 year of self-controlled endurance training in diabetic patients that was mainly attributed to the halting of aerobic capacity loss over time.

Furthermore, one of the most striking findings of our exercise regimen was the suppression of low-grade inflammation, as measured by hsCRP and fibrinogen. In line with other recent studies, either structured or moderate-intensity exercise interventions have been proven to suppress proinflammatory, while increasing anti-inflammatory, serum markers in sedentary diabetic patients [12,18]. As for fibrinogen, previous data have shown an either negligible or inhibitory effect of exercise on its levels [14,19,20]. Such variable results have been ascribed to different population characteristics and exercise parameters. Regarding the usefulness of hsCRP and fibrinogen as predictors of cardiovascular disease [21], reducing inflammation emphasizes the clinical relevance of moderate-intensity, self-controlled exercise in individuals who are at high cardiometabolic risk.

It is well documented that insulin resistance accelerates the risk of cardiovascular disease [22], whereas exercise training induces increased oxidative capacity, fibre-type changes and elevated glucose transport protein 4 (GLUT4) levels in skeletal muscle, adaptations that are of crucial importance for improving glucose uptake and decreasing insulin resistance [23]. However, unlike previous studies, our exercise programme did not improve insulin sensitivity [24], a discrepancy that may be mostly explained by the moderate intensity of the required physical activity [25]. It is possible that a more intensive supervised programme leading to weight loss might have shown greater benefits. In addition, our study targeted only overweight, rather than lean, diabetic patients, as it was focused on the link between obesity and low-grade inflammation, whereas the inclusion of lean patients might have revealed the net effects of the exercise intervention on insulin resistance without the interference of obesity. Furthermore, HOMA-IR and HOMA%S, as surrogates of insulin resistance, were used to reflect both hepatic and peripheral insulin sensitivity. Previous investigators have suggested that exercise training improves insulin sensitivity mostly through gains in peripheral insulin sensitivity [26]. For this reason, we hypothesized that HOMA-IR in the fasting state may not have reflected exercise-induced improvements in glucose uptake and postprandial insulin sensitivity. A hyperinsulinaemic–euglycaemic clamp test might have detected differences between the study groups. Finally, Dumortier et al. [27] demonstrated the insulin-sensitizing effects of a 2-month exercise programme similar to ours in patients with the metabolic syndrome. Their results highlighted the low validity of HOMA-IR in diabetes, the inverse relationship between changes in body weight and insulin resistance, and the different responses of diabetic and non-diabetic patients to the insulin-sensitizing effects of exercise.

Based on recent data, MMP-9 displays potent proatherogenic properties by promoting fibrous-cap degradation and plaque destabilization [8,28]. The association between coronary artery disease (CAD) and elevated serum MMP-9 levels is well established, while reducing MMP-9 levels is associated with favourable long-term cardiovascular outcomes [29,30]. This was the second study to show a marked exercise-related reduction in MMP-9 levels. Niessler et al. [31] had previously reported a significant decrease in serum MMP-9 after a 12-week supervised endurance-training programme in patients with CAD—especially statin users—and at least one cardiovascular risk factor. The present study confirms such findings in statin-free diabetic patients after a moderately intense exercise programme. Similarly, two other studies have shown significant downregulation of MMP-9 levels after a combined diet and exercise intervention in children and in men with metabolic syndrome risk factors [32,33]. Although it is difficult to distinguish the sole effects of exercise in these two studies, both underscored the beneficial influence of lifestyle modification on MMP-9 levels. On looking for the underlying mechanisms, there was an independent association of MMP-9 changes with hsCRP and HbA₁c reductions in the present study’s EXG, whereas glucose regulation and inflammatory factors have previously been found to regulate MMP expression and activity [25]. Thus, lowering levels of glucose and inflammation may have mediated the inhibitory effects of exercise on MMP-9 concentrations in our diabetic cohort, suggesting a promising atheroprotective mechanism.
To our knowledge, no previous clinical study has investigated the effects of exercise on serum MMP-2 and TIMP-1 concentrations. The present study’s physical-activity programme resulted in non-significant increases in MMP-2 and TIMP-1. As for MMP-2 concentrations, experimental and clinical evidence has underscored their contributions to atherosclerotic plaque progression and rupture [34]. However, MMP-2 has a widespread presence in almost all body tissues, so the influence of T2DM on its serum levels needs to be considered with caution. Likewise, TIMP-1 constitutes a wide-spectrum inhibitor of MMPs that is inversely associated with atherosclerotic plaque instability and a poor prognosis for cardiovascular disease [35]. Nevertheless, it is still unclear whether TIMP-1 plays a causative role or is merely a bystander in the atherosclerotic process [8]. On the other hand, the MMP-9/TIMP-1 ratio can serve as an independent predictor of CAD severity [36]. In the present study, exercise treatment significantly suppressed both MMP-9 and the MMP-9/TIMP-1 ratio. Further studies may be able to delineate the effects of exercise on TIMP-1 homeostasis and its clinical relevance.

Growing evidence supports the protective role of TIMP-2, an inhibitor of MMP-2 and MMP-9, against cardiovascular disease [28,37]. Its pharmaceutical and non-pharmaceutical modification has recently emerged as a novel therapeutic target [38]. Up to now, there have been only two studies showing either an increase or no effect on circulating TIMP-2 after acute bouts of exercise in healthy individuals [39,40]. The present study demonstrated for the first time a considerable increment in circulating TIMP-2 levels after regular exercise, which may be promising in high-risk patients [39]. In addition, TIMP-2 upregulation was independently associated with fibrinogen reduction in the EXG. However, the possibility that serum TIMP-2 does not exclusively reflect cardiovascular activity cannot be ruled out, as it has a multicellular origin. Further research should shed more light on the interplay between exercise, TIMP-2 and clinical outcomes.

The relatively small patient population was the main limitation of the present study. However, numerous selection criteria were set in the attempt to limit confounders. Another important limitation was that the main part of the exercise programme was under the patient’s control. Despite daily records, it is not known whether or not the exercise workload was similarly adequate for all patients in the EXG. A more closely supervised intervention may perhaps have produced better results. Finally, dietary habits were not monitored, and an incorporated weight-loss diet may have conferred greater benefits in combination with the prescribed exercise. On the other hand, one of the strengths of our study was the persistent encouragement given to the exercise-treated patients to incorporate more regular exercise as part of an active lifestyle. This approach is a more realistic and feasible way to permanently increase the amount of physical activity in the general population compared with the use of short-term supervised exercise programmes.

In conclusion, a moderately intense physical-activity intervention significantly ameliorated levels of inflammatory, proatherogenic and anti-atherogenic markers together with metabolic improvement. Indeed, our present study’s observations offer further insights into the underlying mechanisms of exercise-induced cardiovascular protection in diabetic individuals.

Conflicts of interest

No potential conflicts of interest relevant to this article are reported.

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