Clinical Research

Acute High-Intensity Intermittent Aerobic Exercise Reduces Plasma Angiopoietin-Like 2 in Patients With Coronary Artery Disease

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ABSTRACT

Background: Circulating levels of angiopoietin-like 2 (ANGPTL2), a proinflammatory and proatherogenic protein, are elevated in patients with coronary artery disease (CAD). We hypothesized that high-intensity intermittent exercise (HIIE), known to be beneficial in patients with CAD, would reduce circulating ANGPTL2 levels.

Methods: Plasma levels of ANGPTL2 were measured before and 20 minutes, 24 hours, and 72 hours after an acute exercise session in a crossover study comparing HIIE to moderate-intensity continuous exercise (MICE) in 14 patients with CAD and 20 age-matched and 20 young healthy controls.

Results: Pre-exercise ANGPTL2 levels were 3-fold higher in patients with CAD than in age-matched controls (P < 0.005) and correlated negatively with VO2max/lean body mass (P < 0.0001). In healthy

Regular physical training is known to decrease all-cause mortality,1-4 reduce the risk of cardiovascular disease (CVD),2,3 slow the progression of coronary artery disease (CAD), and improve cognitive function.2 Globally, regular exercise increases life expectancy, even in patients with CVD.5,6

Multiple mechanisms appear to underlie the beneficial effects of exercise training: exercise delays the age-related increase in blood pressure and aortic stiffness,7 improves the lipid profile and endothelial function,1,4,8,18 reduces oxidative stress,10 and promotes an anti-inflammatory effect by lowering the production of proinflammatory cytokines and proteins, such as interleukins, adhesion molecules, fibrinogen, and C-reactive protein (CRP).1,3,10-13 Thus, the mechanisms of benefit from exercise involve interconnected systems, but the pathways for these effects are not fully understood.

Angiopoietin-like 2 (ANGPTL2), a circulating proinflammatory protein belonging to the angiopoietin-like family,7 has recently been reported to contribute to chronic inflammation associated with atherosclerosis,17,18 insulin resistance, and obesity.14 ANGPTL2 is produced by adipocytes,21 endothelial cells,21 and monocytes/macrophages and promotes chronic inflammation.21 Accordingly, circulating levels of ANGPTL2 are elevated in patients with
controls, ANGPTL2 levels were low and not affected by HIIE or MICE. In patients with CAD, ANGPTL2 levels decreased significantly by 41% after 20 minutes of HIIE, a reduction that was maintained after 24 and 72 hours (P < 0.05). In contrast, although ANGPTL2 levels decreased by 47% after 20 minutes of MICE, they increased by 104% after 24 hours and returned to baseline values after 72 hours (P < 0.05). A negative correlation was observed between this increase in ANGPTL2 levels and the mean rate-pressure product (heart rate × systolic blood pressure; index of myocardial O₂ consumption) measured during MICE, suggesting that subclinical ischemia might promote ANGPTL2 expression.

Conclusions: In patients with CAD, circulating ANGPTL2 levels are acutely reduced after HIIE and transiently increased after MICE. A sustained reduction in circulating ANGPTL2 levels could contribute to the chronic beneficial cardiometabolic effects of HIIE in patients with CAD.

Cad, diabetes, insulin resistance, and obesity. Interestingly, a study performed in overweight but otherwise healthy Japanese men showed that a 3-month lifestyle intervention combining nutritional and physical activity counselling reduced ANGPTL2 plasma levels in parallel with weight loss and an improvement in lipid metabolism. In this latter study, however, the modalities of exercise were not mentioned, and the effect of exercise on ANGPTL2 levels could not be deciphered from the effect of the diet. The effects of physical training on circulating levels of ANGPTL2 in patients with risk factors for CVD have never been reported. We hypothesized that the known beneficial effects of exercise in patients with CAD would be associated with a reduction in circulating ANGPTL2. Our objective was to measure ANGPTL2 levels in patients with CAD before and after acute aerobic exercise; we compared the effects of high-intensity intermittent exercise (HIIE) and isocaloric moderate-intensity continuous exercise (MICE)—2 approaches with known beneficial effects in patients with CVD.

Methods

Participants

In the context of a crossover study comparing HIIE with MICE, fit patients with stable CAD (n = 13 men and 1 woman), fit age-matched healthy controls (n = 10 men and 10 women), and fit young healthy controls (n = 10 men and 10 women) who provided written informed consent were recruited at the cardiovascular prevention center of the Montreal Heart Institute. Blood samples from patients with CAD were obtained from 14 of 19 patients included in a previous study in which inclusion and exclusion criteria have been described. Young and older healthy controls were recruited starting in January 2012, and the study protocol was registered at controlled-trials.com/ISRCTN46169845. Controls were

Results: Les concentrations d’ANGPTL2 avant l’exercice étaient 3 fois plus élevées chez les patients souffrant de MC que chez les témoins appariés selon l’âge (P < 0.05) et corrélaient négativement avec le VO₂max/masse maigre (P < 0.0001). Chez les témoins en santé, les concentrations d’ANGPTL2 étaient faibles et non affectées par l’EIHI ou l’ECIM. Chez les patients souffrant de MC, les concentrations d’ANGPTL2 diminuaient significativement de 41 % après 20 minutes d’EIHI, une réduction qui était maintenue après 24 heures et 72 heures (P < 0.05). En revanche, bien que les concentrations d’ANGPTL2 diminuaient de 47 % après 20 minutes d’ECIM, elles augmentaient de 104 % après 24 heures et revenaient aux valeurs initiales après 72 heures (P < 0.05). Une corrélation négative était observée entre cette augmentation dans les concentrations d’ANGPTL2 et le produit moyen de la fréquence et de la pression (fréquence cardiaque × pression artérielle systolique; indice de consommation du myocarde en O₂) mesuré durant l’ECIM, ce qui montre que l’ischémie sous-clinique favoriserait l’expression d’ANGPTL2.

Conclusions: Chez les patients souffrant de MC, les concentrations circulantes d’ANGPTL2 sont réduites à court terme après l’EIHI et augmentées de manière transitoire après l’ECIM. Une réduction soutenue des concentrations circulantes d’ANGPTL2 pourrait contribuer aux effets cardiométaboliques bénéfiques à long terme de l’EIHI chez les patients souffrant de MC.

Experimental design

On the first visit, patients and healthy controls underwent a complete medical evaluation and a maximal continuous graded exercise test, which allowed the measurement of anthropometric parameters, maximal oxygen uptake (VO₂max, mL·min⁻¹·kg⁻¹ of lean body mass), blood pressure, and heart rate. During 2 subsequent weeks, all participants performed in random order the 2 isocaloric exercise sessions (HIIE and MICE) under the supervision of an exercise physiologist, a nurse, and a cardiologist. All tests were conducted on an electromechanically braked bicycle ergometer; all experimental details concerning the exercise sessions have been previously described. Briefly, HIIE consisted of a 10-minute warm-up and 2 10-minute blocks of intermittent exercise (15 seconds at 100% of maximal aerobic power/15 seconds of passive recovery) separated by a 4-minute passive recovery. MICE duration was 28 minutes at 70% maximal aerobic power. Exercise sessions were randomly performed within a 1-week interval.

Laboratory analyses

Venous blood samples were collected 4 times for each exercise session (10 minutes before and 20 minutes, 24 hours, and 72 hours after exercise) and centrifuged, and plasma was stored at −80°C. Nonfasting circulating levels of ANGPTL2 were
Table 1. Baseline characteristics of participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients with CAD (n = 14)</th>
<th>Age-matched healthy controls (n = 20)</th>
<th>Young healthy controls (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>62 ± 3</td>
<td>61 ± 2*</td>
<td>28 ± 1</td>
</tr>
<tr>
<td>Men</td>
<td>13 (93%)</td>
<td>10 (50%)</td>
<td>10 (50%)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27.3 ± 1.0*</td>
<td>23.8 ± 0.5</td>
<td>21.6 ± 0.4</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>28.4 ± 1.9</td>
<td>25.2 ± 1.6</td>
<td>17.2 ± 1.5</td>
</tr>
<tr>
<td>Glucose (mM/L)</td>
<td>5.5 ± 0.2*</td>
<td>5.0 ± 0.1</td>
<td>4.8 ± 0.1</td>
</tr>
<tr>
<td>Triglycerides (mM/L)</td>
<td>1.1 ± 0.2</td>
<td>1.0 ± 0.1</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>Total cholesterol (mM/L)</td>
<td>3.9 ± 0.2</td>
<td>4.9 ± 0.2</td>
<td>4.2 ± 0.1</td>
</tr>
<tr>
<td>HDL cholesterol (mM/L)</td>
<td>1.4 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td>LDL cholesterol (mM/L)</td>
<td>2.0 ± 0.1</td>
<td>2.8 ± 0.2</td>
<td>2.3 ± 0.1</td>
</tr>
<tr>
<td>Cholesterol-to-HDL ratio</td>
<td>3.0 ± 0.3</td>
<td>3.2 ± 0.2</td>
<td>2.6 ± 0.1</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>1.51 ± 0.47</td>
<td>0.99 ± 0.20</td>
<td>0.89 ± 0.25</td>
</tr>
<tr>
<td>Hemoglobin A1C (×10⁷)</td>
<td>5.95 ± 0.25</td>
<td>5.40 ± 0.06</td>
<td>5.29 ± 0.04</td>
</tr>
<tr>
<td>Medication (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Statins</td>
<td>11 of 14 (79)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antiplatelet agents</td>
<td>9 of 14 (64)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Blockers</td>
<td>6 of 14 (43)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>3 of 14 (21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AR antagonists</td>
<td>4 of 14 (29)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diuretics</td>
<td>1 of 14 (7)</td>
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</tbody>
</table>

Data are mean ± standard error of the mean of (n) patients. One-way ANOVA and the Tukey post hoc test were used for analysis. ACE, angiotensin-converting enzyme; ANOVA, analysis of variance; AR, angiotensin receptor; CAD, coronary artery disease.

* P < 0.05 for patients with CAD vs age-matched healthy controls.

† P < 0.05 for age-matched controls vs young healthy controls.

Statistical analysis

Standard statistical methods were used for the calculation of means and standard error of the mean. Normal Gaussian distribution of the data was verified by the d’Agostino and Pearson omnibus normality test (Prism; GraphPad, San Diego, CA). If the data were not normally distributed, nonparametric tests were used. Baseline characteristics of the patients (Table 1) were compared using a 1-way analysis of variance (ANOVA) followed by a Tukey test. The changes in ANGPTL2 levels between the 4 time points, within 1 group of participants, were compared using nonparametric ANOVA (Friedman test) with repeated measures followed by a Dunn post hoc test. The changes in ANGPTL2 levels between groups (CAD vs age-matched healthy controls and age-matched controls vs young controls) were compared using a 2-way ANOVA (time × group) followed by Bonferroni post-tests. P < 0.05 was considered statistically significant.

Results

Pre-exercise ANGPTL2 levels

Baseline circulating ANGPTL2 levels were higher in patients with CAD (5.74 ± 0.75 ng/mL) than in age-matched healthy controls (2.16 ± 0.35 ng/mL; P < 0.05), in agreement with previous reports from our group and others. Pre-exercise ANGPTL2 levels in young healthy volunteers (1.72 ± 0.3 ng/mL) were similar to levels measured in age-matched healthy controls. Levels measured before MICE (Fig.1A) and measured at the 4 time points using a commercial ELISA kit (No. ABIN415096; antibodies-online.com), as previously described.17

Figure 1. Baseline pre-exercise nonfasting plasma angiopoietin-like 2 (ANGPTL2) levels measured in patients with coronary artery disease (CAD) (n = 11-12), in age-matched healthy controls (n = 18-20), and in young healthy controls (n = 18-20). ANGPTL2 levels were measured in participants before they randomly performed (A) a moderate-intensity intermittent exercise session and 1 week later (B) a high-intensity intermittent exercise session. Data are mean ± standard error of the mean of (n) patients. PP, pulse pressure. *P < 0.01 vs aged-matched healthy controls (Mann-Whitney test).

Vo_{2max} and ANGPTL2 levels

As expected, Vo_{2max} was lower in patients with CAD than in age-matched healthy controls, and Vo_{2max} was lower in healthy age-matched controls vs young controls (P < 0.05) (Fig. 2A). Interestingly, when all participants were considered, Vo_{2max} correlated negatively with baseline ANGPTL2 levels: the fitter the participant, the lower the plasma ANGPTL2 levels (Fig. 2B). In addition, the slimmer the participant and the lower his/her blood and pulse pressure, the lower the basal ANGPTL2 level (Supplemental Results; Supplemental Tables S1 and S2; Fig. 2C). These relationships are not independent of each other: when performing a forward stepwise multiple regression, only the negative correlation between...
Due to the increase in exercise intensity, circulating levels of ANGPTL2, a single bout of exercise reduces ANGPTL2 levels after 20 minutes to levels observed in age-matched controls; (2) in patients with CAD who have high (3-fold) circulating levels of ANGPTL2, a single bout of exercise reduces ANGPTL2 levels after 20 minutes to levels observed in age-matched controls; (3) in contrast, after 24 and 72 hours, the effect of 1 bout of exercise on ANGPTL2 levels differs according to the type of exercise—although HIIE induces a short-term (72 hours) reduction in ANGPTL2 levels, MICE leads to a transient increase in ANGPTL2 levels; (4) the slimmer and fitter the participant and the lower his/her blood and pulse pressures, the lower the basal circulating ANGPTL2 levels. To the best of our knowledge, this is the first report describing the relation between ANGPTL2 and cardiovascular function in patients with CAD at rest and after acute intermittent vs continuous exercise.

Our study is observational, and the mechanisms underlying our data remain to be investigated. Hypotheses will be proposed. Physical activity is known for its chronic anti-inflammatory properties, which are highlighted by an exercise-induced reduction in cytokines, chemokines, adhesion

**Mean rate-pressure product measured during HIIE or MICE**

The mean rate-pressure product (RPP) (mean RPP = mean heart rate × mean systolic blood pressure), which is an index of myocardial O2 consumption, was calculated in all participants during MICE and HIIE (Table 2): in young and older controls and in patients with CAD, mean RPP was significantly higher in patients with CAD than during HIIE, suggesting higher myocardial O2 consumption during continuous vs intermittent exercise. In addition, a negative correlation was observed between the increase in ANGPTL2 levels after 24 hours of MICE in patients with CAD and their mean RPP measured during MICE (Fig. 5): the higher the increase in ANGPTL2 levels, the lower the RPP. This correlation was noted only in patients with CAD and was not observed for the other time points or during HIIE (data not shown).

**Discussion**

The major findings of this study are that (1) in young and older fit healthy individuals, plasma ANGPTL2 levels are low and not affected by an acute bout of either HIIE or isocaloric MICE; (2) in patients with CAD who have high (3-fold) circulating levels of ANGPTL2, a single bout of exercise reduces ANGPTL2 levels after 20 minutes to levels observed in age-matched controls; (3) in contrast, after 24 and 72 hours, the effect of 1 bout of exercise on ANGPTL2 levels differs according to the type of exercise—although HIIE induces a short-term (72 hours) reduction in ANGPTL2 levels, MICE leads to a transient increase in ANGPTL2 levels; (4) the slimmer and fitter the participant and the lower his/her blood and pulse pressures, the lower the basal circulating ANGPTL2 levels. To the best of our knowledge, this is the first report describing the relation between ANGPTL2 and cardiovascular function in patients with CAD at rest and after acute intermittent vs continuous exercise.

Our study is observational, and the mechanisms underlying our data remain to be investigated. Hypotheses will be proposed. Physical activity is known for its chronic anti-inflammatory properties, which are highlighted by an exercise-induced reduction in cytokines, chemokines, adhesion
molecules, and CRP. Acute exercise, in contrast, induces a transient up to 100-fold increase in circulating interleukin (IL)-6. IL-6 is an inflammatory cytokine, but it also promotes a strong anti-inflammatory response by increasing the production of anti-inflammatory cytokines (such as IL-10, IL-1-RA) and by inhibiting the release of proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α); altogether, this reduces systemic inflammation in the long term. We did not measure the changes induced by 1 bout of exercise in inflammatory markers such as IL-6, TNF-α, or CRP, but the reported rise in IL-6 caused by acute exercise could inhibit TNF-α. Because TNF-α is known to promote proinflammatory ANGPTL2 expression and production in cultured adipocytes, and because adipocytes are probably the major source of ANGPTL2, it is possible that acute exercise, such as that performed in our study, could induce a rise in IL-6, which in turn reduces ANGPTL2 levels. In addition, epinephrine, which is massively released during physical exercise, has been shown to inhibit ANGPTL2 expression and production in cultured adipocytes; thus, it is also plausible that the reduction in ANGPTL2 levels after 20 minutes of HIIE or MICE is related to the rise in sympathetic activity during exercise. Interestingly, it has been reported that epinephrine itself inhibits TNF-α production; thus, the rise in both IL-6 and epinephrine could independently explain the acute reduction in ANGPTL2 after either HIIE or MICE in patients with CAD.

We also observed that 24 hours after MICE, ANGPTL2 levels rose significantly in patients with CAD. Because this was not observed after HIIE, the type of exercise may explain this difference. What differs between a single bout of MICE and HIIE? Both are strong physiological stimuli, but MICE may promote prolonged rather than intermittent periods of myocardial O2 demand and thus the possibility of ischemic stress in patients with CAD.
during isocaloric HIIE in all participants, including patients with CAD (Table 2), suggesting higher myocardial O2 consumption during MICE. Because ischemia is known to promote ANGPTL2 expression and production,\textsuperscript{15,21} it is possible that acute subclinical ischemic periods induced by MICE lead to a rise in ANGPTL2 levels, a hypothesis supported by the negative correlation observed between the increase in ANGPTL2 levels after 24 hours of MICE in patients with CAD.

Figure 4. Nonfasting plasma angioptielin-like 2 (ANGPTL2) levels 10 minutes before (pretest) and 20 minutes, 24 hours, and 72 hours after a single bout of moderate intensity continuous exercise (MICE) in (A) and (B) patients with CAD (n = 8-11), (C) age-matched healthy controls (n = 18-20), (D) young healthy controls (n = 18-20), and (E) patients with CAD vs age-matched healthy controls. Data are mean ± standard error of the mean. *P < 0.05 vs pretest value (nonparametric 1-way analysis of variance [ANOVA] with repeated measures followed by a Dunn post hoc test). \textsuperscript{1}P < 0.05 vs controls (2-way ANOVA followed by Bonferroni post hoc test).

Table 2. Mean heart rate, mean systolic blood pressure, and mean rate-pressure product measured during a session of MICE or HIIE

<table>
<thead>
<tr>
<th>Variable</th>
<th>MICE</th>
<th>HIIE</th>
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<tbody>
<tr>
<td></td>
<td>Young controls (n = 20)</td>
<td>Aged controls (n = 20)</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>155 ± 3</td>
<td>134 ± 3\textsuperscript{*}</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>153 ± 3</td>
<td>162 ± 3\textsuperscript{*}</td>
</tr>
<tr>
<td>Mean RPP (Heart rate × SBP)</td>
<td>23,677 ± 819</td>
<td>21,646 ± 486\textsuperscript{*}</td>
</tr>
</tbody>
</table>

Data are mean ± standard error of the mean (SEM) of (n) participants.
BP, blood pressure; HIIE, high-intensity intermittent exercise; MICE, moderate-intensity continuous exercise; RPP, rate-pressure product.
\textsuperscript{*}P < 0.05 vs young healthy controls (unpaired t test).
\textsuperscript{1}P < 0.05 vs age-matched healthy controls (unpaired t test).
\textsuperscript{1,1}P < 0.05 vs MICE (paired t test).
CAD, and the mean RPP measured during MICE. This would represent a transient adaptive response in patients with CAD exposed to an effort near the ischemic threshold. Indeed, we observed that the highest rise in ANGPTL2 levels 24 hours after MICE was noted in the patient with the most severe condition (angina, myocardial infarction, and previous angioplasty) in whom ST-segment depression (−1 mm; ischemia maintained for 7 minutes) was observed during MICE (data not shown). In contrast, a sustained reduction in ANGPTL2 levels was observed 24 hours after intermittent MICE (Fig. 4B), including in the patient with CAD who displayed the highest rise in ANGPTL2 levels 24 hours after MICE and in whom complete disappearance of symptoms and signs of myocardial ischemia were reported 20 minutes after HIIE.35 In contrast to MICE, it is possible that HIIE leads to short intermittent periods of subclinical ischemia that might potentially induce an ischemic preconditioning-like response,28 preventing the need for an adaptive rise in ANGPTL2 levels.

Conclusions

In summary, our study demonstrates that high circulating levels of proinflammatory ANGPTL2 in patients with CAD are sensitive to a single bout of aerobic exercise. In addition, circulating ANGPTL2 is negatively related to cardiopulmonary fitness (VO2max). Both acute MICE and HIIE lead to a reduction in circulating ANGPTL2 after 20 minutes of exercise. This reduction in ANGPTL2 levels could be the result of an exercise-induced acute rise in epinephrine or IL-6, or both, reducing ANGPTL2 release. Although acute HIIE leads to a 3-day reduction in ANGPTL2 levels, 1 MICE session induces an unexpected transient rise in ANGPTL2 levels after 24 hours, characterizing patients with CAD sensitive to ischemic episodes. Because ANGPTL2 is proinflammatory and accelerates the atherosclerotic process,15,17,18 we propose that any intervention capable of reducing abnormally high levels of ANGPTL2 would be beneficial. Although chronic MICE and HIIE are both safe and beneficial in patients with CAD,24-26 their long-term impact on circulating ANGPTL2 levels remains unknown.

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Disclosures

The authors have no conflicts of interest to disclose.

References


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