

Clinical Research

A Single Bout of High-Intensity Interval Exercise Does Not Increase Endothelial or Platelet Microparticles in Stable, Physically Fit Men With Coronary Heart Disease

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ABSTRACT

Background: High-intensity interval exercise (HIIE) is gaining in popularity in fitness centres, even among coronary heart disease (CHD) patients. However, whether HIIE can have deleterious acute effects on the vasculature in CHD has not been studied. We hypothesized that when compared with moderate-intensity continuous exercise (MICE), a single bout of HIIE could lead to vascular damage and increasing numbers of circulating endothelial and platelet microparticles (EMPs, PMPs) in stable, physically fit CHD patients.

Methods: Nineteen male CHD patients (aged 62 ± 11 years) underwent, in random order, a single session of HIIE corresponding to 15-second intervals at 100% of peak power output and 15-second passive recovery intervals, and an isocaloric MICE session. EMPs (CD31⁺ and/or CD62E⁺ and CD42b⁻); PMPs (CD42b⁺); nitrates and

RÉSUMÉ

Introduction : L'exercice intermittent à haute intensité (EIHI) gagne en popularité dans les centres de conditionnement physique, même chez les patients ayant une coronaropathie. Cependant, la question de savoir s'il existe des effets délétères à court terme de ce type d'exercice sur la paroi vasculaire chez des patients coronariens n'a jamais été étudiée. Nous posons l'hypothèse que lorsque l'EIHI est comparé à l'exercice continu d'intensité modérée (ECIM), une seule séance pourrait mener à des lésions vasculaires et à l'augmentation du nombre de microparticules circulantes endothéliales et plaquettaires (MPE et MPP) chez les patients ayant une coronaropathie qui sont stables et en bonne forme physique.

Méthodes : Dix-neuf (19) patients de sexe masculin ayant une coronaropathie (âgés de 62 ± 11 ans) ont subi, selon un ordre au hasard,

Regular exercise is widely promoted to improve functional capacity and prognosis in patients with coronary heart disease (CHD). Exercise may potentially improve prognosis through multiple mechanisms, including improved endothelial function.¹ Moderate-intensity continuous exercise (MICE) training has been shown to improve endothelial function in

patients with CHD, chronic heart failure, and cardiovascular (CV) risk factors.^{2,3} However, there is growing interest in the use of high-intensity interval exercise (HIIE) training in subjects with CV disease.⁴⁻⁶ Two recent studies highlighted the superior CV effects of HIIE training compared with MICE training in patients with CHD.^{7,8} However, no data are available regarding the acute effects of HIIE on endothelial function in individuals with CHD. Acute exercise has been shown to improve endothelial function in the immediate postexercise period⁹ and on the morning after HIIE, but not on the morning after moderate-intensity exercise¹⁰ in healthy men. We recently reported that an “optimized” HIIE protocol

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See page 1290 for disclosure information.

nitrites; prostacycline; and troponin T, cardiac form (cTnT), were measured 10 minutes before exercise and 20 minutes, 24 hours, and 72 hours after both exercise sessions.

Results: EMPs, PMPs, nitrates and nitrites, prostacycline, and cTnT remained unchanged after both HIIE and MICE exercise sessions. Initial EMP concentration correlated inversely with EMP concentration 20 minutes post exercise, irrespective of exercise modality ($r = 0.78$, $P < 0.0001$).

Conclusions: A single HIIE session with very short exercise and passive recovery periods appears safe and does not induce changes to markers of endothelial function. Future studies are required to determine the safety of a long-term HIIE training program.

in CHD consisting of repeated 15-second bouts of exercise at 100% of peak power output interspersed with recovery intervals of equal duration is well tolerated and more efficient and allows subjects to exercise longer relative to an isocaloric single bout of MICE.^{11,12}

Endothelial microparticles (EMPs) are membrane-shed vesicles $< 1.0 \mu\text{m}$ in size that are released from endothelial cells in response to either cellular injury or dysfunction or apoptosis and possess procoagulant and inflammatory properties.¹³⁻¹⁶ Circulating EMP levels are increased in patients with CV disease or its risk factors¹⁴ and have been shown to correlate negatively with endothelial function measured *in vivo*.^{16,17} In addition to EMP levels, platelet microparticle (PMP) levels are increased in acute coronary syndromes¹⁸ and may be associated with high shear stress.¹⁴ It is believed that PMPs may interact with endothelial cells and result in endothelial activation.¹⁴

Currently, there are no data on the effects of acute exercise on circulating EMP or PMP levels in patients with CHD or on whether certain forms of aerobic exercise may be harmful to the vasculature, in particular through excessive shear stress. The subject is of great relevance given the increasing popularity of spinning and other types of interval training that are being routinely incorporated into fitness centre training programs. Therefore, the main objective of the present study was to measure the effects of single bouts of HIIE and MICE on circulating EMP levels in patients with stable documented CHD. We hypothesized that a single bout of HIIE could lead to vascular damage and increasing numbers of circulating EMPs.

Methods

Participants

Nineteen fit men with stable CHD and providing written informed consent were recruited from the Montreal Heart Institute cardiovascular prevention centre. See the *Inclusion and Exclusion Criteria* section of the [Supplementary Material](#) for details. All subjects provided written informed consent. The research protocol was approved by the Montreal Heart Institute Ethics Committee.

une simple séance d'EIHI correspondant à des intervalles de 15 secondes à 100 % du pic de puissance maximale et des intervalles de récupération passive de 15 secondes, et une séance isoénergétique d'ECIM. Les MPE (CD31⁺ et/ou CD62E⁺ et CD42b⁻), les MPP (CD42b⁺), les nitrates et les nitrites; la prostacycline, la troponine T et la troponine T cardiaque (TnTc) ont été mesurés 10 minutes avant l'exercice, et 20 minutes, 24 heures et 72 heures après les deux séances d'exercice.

Résultats : Les MPE, les MPP, les nitrates et les nitrites, la prostacycline et la TnTc sont demeurés inchangés après les séances d'EIHI et d'ECIM. La concentration initiale de MPE a été inversement corrélée à la concentration de MPE 20 minutes après l'exercice, sans distinction du type d'exercice ($r = 0,78$, $P < 0,0001$).

Conclusions : Une seule séance d'EIHI au moyen d'exercices très courts et de périodes de récupération passive semble sécuritaire et n'induit pas de changements dans les marqueurs du fonctionnement endothélial. D'autres études sont nécessaires pour déterminer l'innocuité à long terme du programme d'entraînement de type EIHI.

Experimental design

On the first visit, patients underwent a complete medical evaluation, including measurement of height, weight, body composition (bioimpedance analysis; Tanita, model BC 418, Japan), and resting ECG and completion of a maximal continuous graded exercise test. During 2 subsequent weeks, participants, in random order, performed the 2 exercise sessions (interval and continuous) under the supervision of an exercise physiologist, nurse, and cardiologist. Testing was conducted on an electromechanically braked bicycle ergometer (Ergoline 800S, Bitz, Germany). Cycling position, known to affect energy expenditure, was standardized by adoption of a top bar position. Saddle height was adjusted according to inseam leg length. Each participant used toe clips and was instructed to stay seated during exercise sessions. Participants were instructed to take their habitual medications on the mornings of the exercise test and exercise sessions.

Exercise testing and single exercise sessions

Exercise testing was performed according to previously described methodology.¹² For details, see the *Exercise Testing* and *Exercise Sessions* sections of the [Supplementary Material](#). MICE and HIIE sessions were also performed according to previously published methodology¹² and are detailed in the supplementary materials section.

Laboratory analyses

Citrated venous blood samples were collected at 4 times for each exercise session (10 minutes before exercise and 20 minutes, 24 hours, and 72 hours after exercise) and centrifuged immediately after collection with separated serum stored at -80°C for subsequent analysis. Patients were asked to refrain from exercise between the third and fourth blood samples. See [Supplemental Figure S1](#) for details.

EMPs were defined as particles with a diameter $< 0.9 \mu\text{m}$ that are negative for CD42b and positive for CD31 (EMP31), CD62E (EMP62E), or both. PMPs were defined as particles with a diameter $< 0.9 \mu\text{m}$ that are positive for CD42b. See

the *Microparticle Measurement* section of the [Supplementary Material](#) for details regarding methodology.

Nitric oxide metabolites (NOx) were measured with the Greiss reagent, prostacycline (6-keto-PGF) was measured with a commercially available enzyme-linked immunosorbent assay kit (Cayman Chemical, Ann Arbor, MI), and troponin T, cardiac form (cTnT), was measured with a single commercial assay (Roche Diagnostics, Mannheim, Germany). The decision limit for myocardial injury was set at 0.04 mg/L.¹⁹

Statistical analysis

Baseline characteristics and exercise parameters are reported as percentages for categorical variables and mean values ± SD for continuous variables. A 1-way analysis of variance with repeated measures including visit as the sole factor was done to assess differences in endothelial markers over time. To compare the response profiles of both types of exercise, a 2-way analysis of variance (time × mode) with repeated measures on the time factor was performed for EMPs, NOx, and prostacycline. Because of the lack of normality for PMP, *P* values were calculated from log-transformed data. As such, the median (Q1,Q3) is reported as summary statistics at visits 1, 2, 3, and 4 (Table 1). Spearman correlations were also generated to evaluate the relationship between EMP parameters and clinical data and exercise parameters. Finally, we assessed the concordance of pre-exercise and 20-minute postexercise EMP levels for the 2 exercise sessions (Fig. 1, A and B). All analyses were performed with SAS version 9.1 (SAS Institute Inc, Cary, NC) and conducted at the 0.05 significance level.

Results

Baseline characteristics and exercise stress testing data

Baseline characteristics are presented in [Supplemental Table S1](#). Subjects were generally overweight, with a significant proportion having a history of angina. Results from the maximal graded exercise test are presented in [Supplemental Table S2](#). Mean peak oxygen consumption (VO_{2peak}) indicates that exercise tolerance was comparable to age-predicted values.²⁰

Exercise sessions and electrocardiogram safety parameters

No significant ventricular arrhythmias or blood pressure reduction occurred during either exercise session. Only 3 subjects had demonstrable myocardial ischemia during the HIIE session, with ST-segment depression never surpassing 2 mm and always normalizing during the 15-second passive recovery periods. Although 40% of our sample had a history of exertional angina, none developed chest pain during the exercise sessions.

Microparticles, vascular biomarkers, and troponin T, cardiac form

Biomarkers are presented in Table 1. EMP, PMP, nitrates and nitrites, and prostacycline remained unchanged after exercise, irrespective of mode of training. However, we found an inverse relationship between initial EMP concentration and change in EMP concentration immediately following exercise. Individuals with the highest EMP levels pre-exercise showed

Table 1. Endothelial and platelet microparticles, nitric oxide metabolites, and prostacyclin response to a single bout of MICE or HIIE

	MICE				HIIE				<i>P</i> value for interaction*
	T0	T1	T2	T3	T0	T1	T2	T3	
EMP	27.0 ± 23.1	24.9 ± 17.9	25.5 ± 17.7	24.4 ± 18.0	30.4 ± 26.0	23.7 ± 13.0	25.3 ± 13.0	29.6 ± 17.7	0.46
EMP31	17.3 ± 13.2	17.0 ± 9.6	17.0 ± 11.8	16.5 ± 11.7	18.8 ± 12.7	16.4 ± 7.4	18.7 ± 11.1	22.5 ± 14.6	0.44
EMP62E	10.8 ± 10.8	9.1 ± 9.8	9.8 ± 8.3	9.1 ± 8.6	12.8 ± 15.6	8.5 ± 7.7	7.7 ± 5.2	8.3 ± 4.5	0.66
PMP42b	912 (447, 2057)	1311 (404, 2941)	958 (584, 2626)	836 (595, 1104)	812 (431, 2400)	1034 (715, 2342)	892 (347, 1989)	1117 (480, 2761)	0.10
EMP62E/EMP31 ratio	0.5 ± 0.3	0.5 ± 0.2	0.6 ± 0.4	0.6 ± 0.3	0.6 ± 0.4	0.5 ± 0.3	0.5 ± 0.3	0.4 ± 0.2	0.09
Nitrites	1.0 ± 0.4	0.8 ± 0.4	1 ± 0.5	1.0 ± 0.5	1.0 ± 0.7	0.8 ± 0.5	1.0 ± 0.3	1.0 ± 0.2	0.088
Nitrates	37.5 ± 42.9	26.9 ± 9.9	32.4 ± 16.1	21.4 ± 9.1	27.2 ± 16.9	29.0 ± 18.2	27.8 ± 13.1	23.0 ± 18.4	0.33
6-keto-PGF	98.0 ± 45.6	111.3 ± 36.5	99.9 ± 44.1	97.1 ± 37.3	113.7 ± 50.1	114.0 ± 45.0	107.2 ± 53.5	104.2 ± 47.4	0.71

EMP, endothelial microparticles positive for CD31 and/or CD62E; EMP31, all microparticles presenting CD31; EMP62E, all microparticles showing CD62E; HIIE, high-intensity interval exercise; MICE, moderate-intensity continuous exercise; PMP, platelet microparticles; T0, baseline; T1, 20 minutes post exercise; T2, 24 hours post exercise; T3, 72 hours post exercise.

* Group × time interaction. No significant group or time effects were observed.

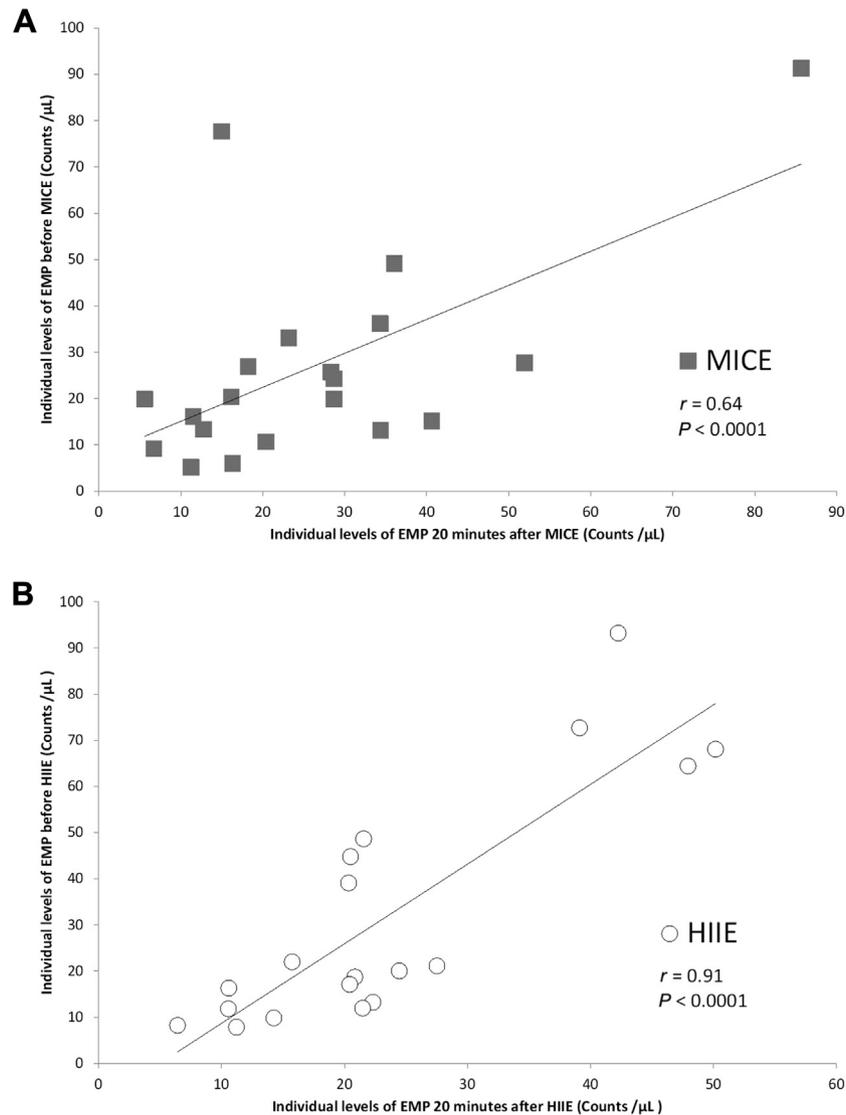


Figure 1. (A) Relation between levels of endothelial microparticles (EMPs) at baseline and change at 20 minutes after moderate-intensity continuous exercise (MICE). (B) Relation between levels of EMP at baseline and change at 20 minutes after high-intensity interval exercise (HIIE).

the greatest reduction in EMP levels at 20 minutes following exercise (Fig. 1, A and B). A statistically significant group \times time interaction was observed for EMP62E/EMP31 ratio. Serum cTnT levels were normal in all participants at baseline and did not significantly increase up to 72 hours following exercise, thus excluding the presence of exercise-induced myocardial injury.

Associations between clinical data, exercise parameters, and EMP levels

Statistically significant positive correlations between diabetes and both EMP31 and EMP62E were observed, while family history of premature CHD correlated positively with EMP31, EMP62E, and EMP42b (data not shown). No correlations were identified between EMP levels at baseline or exercise-induced EMP changes and medication usage (data not shown). Finally, no relationship was observed between baseline

EMP levels or EMP response to exercise and the presence or absence of myocardial ischemia during exercise sessions.

Discussion

Chronic exercise has consistently been shown to improve markers of endothelial function³; however, the effects of acute exercise on the vasculature are less well known. To our knowledge, this is the first study to examine the influence of acute exercise on circulating microparticle (MP) levels and vascular parameters in patients with stable CHD with and without exercise-induced ischemia. We hypothesized that a single bout of HIIE could lead to vascular damage and increasing numbers of circulating EMPs.

We found that irrespective of exercise intensity or mode (MICE or HIIE), neither levels of circulating EMPs nor vascular biomarkers increased after a single bout of exercise, and biomarkers remained unchanged during the subsequent 72 hours. The response of circulating EMPs to exercise was

different in different individuals, with some showing little change in EMP concentration, while others exhibited more marked changes (Fig. 1, A and B). Furthermore, the initial EMP concentration was inversely correlated with the change in EMP concentration at 20 minutes post exercise. Overall, in other words despite no significant decrease following exercise, individuals with the highest EMP levels pre-exercise showed the greatest reduction in EMP concentration immediately after exercise, irrespective of the exercise mode (HIIE or MICE, $r = 0.78$, $P < 0.0001$). The latter observation is probably a result, at least in part, of the phenomenon of regression to the mean. While no significant group or time effects were noted for EMPs or EMP subtypes, a statistically significant group- \times -time interaction was noted for EMP62E/EMP31 ratio. This interaction appears to be a result of the slight decrease in EMP62E/EMP31 ratio from baseline to 72 hours post exercise in the HIIE group. The significance of this result is unclear, given its small magnitude, and may also reflect a regression-to-the-mean phenomenon.

Conflicting data exist with respect to the impact of acute exercise on MP levels in healthy individuals.^{21,22} One recent study demonstrated an increase in EMP62E levels following a 90-minute endurance exercise,²¹ while another study observed an increase in monocyte-derived and platelet MPs following a maximal exercise test, with no increase in EMP levels.²² Our results are more consistent with the latter study. Explanations for this phenomenon include the intensity and duration of the exercise sessions (no endurance exercise performed in our study), as well as the medications used by most patients, including statins and antiplatelet agents. These agents have been shown to lower EMP and PMP levels²³⁻²⁵ and may have somehow influenced the MP response to exercise. Finally, our study participants were almost all habitual exercisers, performing aerobic exercise 2 to 3 times per week, such that EMP levels could have in theory reached a plateau or steady state. It is important to note that we did not restrict exercise training prior to study enrollment. Whether similar results would be obtained in untrained individuals is unknown, although no differential EMP response to exercise was observed in trained and untrained healthy subjects.²¹

As described in 2 recent studies from our group, the HIIE protocol we employed allows participants to sustain a high percentage of $\dot{V}O_{2max}$ by alternating high-intensity exercise and passive recovery phases,¹¹ while resulting in a lower ventilatory demand than an isocaloric MICE session does.¹² As mentioned above, this protocol did not appear to induce vascular damage in the acute setting. Furthermore, the repetitive cycle of low and high vascular laminar shear stress imposed by repeated bouts of HIIE could provide an important stimulus to the endothelium. High shear stress is a potent physiological stimulus for NO release and generally leads to improved endothelial function.²⁶ In highly-endurance-trained men and sedentary controls, a single bout of HIIE was associated with a reduction in flow-mediated dilatation (FMD) of the brachial artery 1 hour after exercise, despite increased NO bioavailability and improved antioxidant status.²⁷ The authors suggested that oxidative stress and other mechanisms independent of NO and antioxidant levels contribute to impaired FMD in the immediate postexercise period. Padilla et al. showed that in healthy

adults, a single bout of high-intensity walking exercise (75% of $\dot{V}O_{2peak}$) elicited the greatest improvement in FMD during a 3-hour period, relative to single bouts of low- (25%), and moderate-intensity (50%) exercise.²⁸ Our study was different in that it assessed patients with stable CHD, not healthy individuals. Previous data have shown that acute and long-term high-intensity exercise provoke oxidative stress,²⁹ but not moderate- or low-intensity exercise.³⁰ In view of these findings and our results, it is plausible to hypothesize that repeated bouts of high-intensity exercise interspersed with passive recovery periods could potentially elicit a positive effect on endothelial function related to high shear stress while also reducing exercise-related oxidative stress. It is important to note that endothelial function as reflected by brachial artery FMD was not measured in our study.

Our results regarding the MICE session are consistent with those of Mobius-Winkler et al., who studied the time-dependent release of endothelial progenitor cells and EMPs during MICE in 18 healthy subjects.³¹ No change was observed for EMPs, while endothelial progenitor cells, defined as CD34/KDR or CD133/KDR, showed a significant time-dependent rise. These data suggest that 240 minutes of cycling at 70% of the individual anaerobic threshold is not accompanied by significant irreversible endothelial damage. Harrison et al. examined changes in EMP levels in healthy, active young men following a high-fat meal, consumed with and without prior exercise consisting of 100 minutes at 70% of $\dot{V}O_{2peak}$.³² Levels of EMPs (CD31+/42b^{neg}) increased postprandially but were not influenced by exercise.

Limitations

Limitations of the current study include the small, exclusively male sample. Whether results would have differed in women is unclear. FMD of the brachial artery has been shown to increase in both healthy women^{33,34} and healthy men³⁵⁻³⁷ in response to acute exercise. Whether women and men exhibit a differential EMP response to acute exercise is not known. In a recent study, healthy premenopausal women were noted to have higher EMP levels than did age-matched men, with differences being attributed to hormonal variation within the menstrual cycle.³⁸ Even if the number of participants in our study is larger than that in previously published papers, the high interindividual variation limits our results. In addition, the sample was selected among a cohort of relatively young CHD patients who were habitual exercisers. Finally, the reproducibility of MP measurements is a recognized problem resulting from the lack of a standardized protocol for EMP and PMP measurement, an issue that goes beyond the scope of the present study.

Conclusion

Our findings suggest that a single bout of HIIE employing very short periods of exercise interspersed with short periods of passive recovery does not induce deleterious effects on the vascular wall. Given that our HIIE protocol appears safe and well tolerated and does not induce myocardial injury in the short term, future studies are required to evaluate the effect of a long-term HIIE training program on markers of endothelial function in the hopes of potentially implementing this type of

training into phase III cardiac rehabilitation for selected patients.

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Disclosures

The authors have no conflicts of interest to declare.

References

- Taylor RS, Brown A, Ebrahim S, et al. Exercise-based rehabilitation for patients with coronary heart disease: systematic review and meta-analysis of randomized controlled trials. *Am J Med* 2004;116:682-92.
- Hambrecht R, Adams V, Erbs S, et al. Regular physical activity improves endothelial function in patients with coronary artery disease by increasing phosphorylation of endothelial nitric oxide synthase. *Circulation* 2003;107:3152-8.
- Walther C, Gielen S, Hambrecht R. The effect of exercise training on endothelial function in cardiovascular disease in humans. *Exerc Sport Sci Rev* 2004;32:129-34.
- Tjonna AE, Lee SJ, Rognmo O, et al. Aerobic interval training versus continuous moderate exercise as a treatment for the metabolic syndrome: a pilot study. *Circulation* 2008;118:346-54.
- Wisloff U, Ellingsen O, Kemi OJ. High-intensity interval training to maximize cardiac benefits of exercise training? *Exerc Sport Sci Rev* 2009;37:139-46.
- Guiraud T, Nigam A, Gremaux V, Meyer P, Juneau M, Bosquet L. High-intensity interval training in cardiac rehabilitation. *Sports Medicine* 2012;42:587-605.
- Rognmo O, Hetland E, Helgerud J, Hoff J, Slordahl SA. High intensity aerobic interval exercise is superior to moderate intensity exercise for increasing aerobic capacity in patients with coronary artery disease. *Eur J Cardiovasc Prev Rehabil* 2004;11:216-22.
- Warburton DE, McKenzie DC, Haykowsky MJ, et al. Effectiveness of high-intensity interval training for the rehabilitation of patients with coronary artery disease. *Am J Cardiol* 2005;95:1080-4.
- Padilla J, Harris RA, Fly AD, Rink LD, Wallace JP. The effect of acute exercise on endothelial function following a high-fat meal. *Eur J Appl Physiol* 2006;98:256-62.
- Tyldum GA, Schjerve IE, Tjonna AE, et al. Endothelial dysfunction induced by post-prandial lipemia: complete protection afforded by high-intensity aerobic interval exercise. *J Am Coll Cardiol* 2009;53:200-6.
- Guiraud T, Juneau M, Nigam A, et al. Optimization of high intensity interval exercise in coronary heart disease. *Eur J Appl Physiol* 2010;108:733-40.
- Guiraud T, Nigam A, Juneau M, Meyer P, Gayda M, Bosquet L. Acute responses to high-intensity intermittent exercise in CHD patients. *Med Sci Sports Exerc* 2011;43:211-7.
- Boulanger CM, Scoazec A, Ebrahimi T, et al. Circulating microparticles from patients with myocardial infarction cause endothelial dysfunction. *Circulation* 2001;104:2649-52.
- Nomura S, Tandon NN, Nakamura T, Cone J, Fukuhara S, Kambayashi J. High-shear-stress-induced activation of platelets and microparticles enhances expression of cell adhesion molecules in THP-1 and endothelial cells. *Atherosclerosis* 2001;158:277-87.
- Esposito K, Ciotola M, Schisano B, et al. Endothelial microparticles correlate with endothelial dysfunction in obese women. *J Clin Endocrinol Metab* 2006;91:3676-9.
- Koga H, Sugiyama S, Kugiyama K, et al. Elevated levels of VE-cadherin-positive endothelial microparticles in patients with type 2 diabetes mellitus and coronary artery disease. *J Am Coll Cardiol* 2005;45:1622-30.
- Werner N, Wassmann S, Ahlers P, Kosiol S, Nickenig G. Circulating CD31+/annexin V+ apoptotic microparticles correlate with coronary endothelial function in patients with coronary artery disease. *Arterioscler Thromb Vasc Biol* 2006;26:112-6.
- Blann A, Shantsila E, Shantsila A. Microparticles and arterial disease. *Semin Thromb Hemost* 2009;35:488-96.
- Alpert JS, Thygesen K, Antman E, Bassand JP. Myocardial infarction redefined—a consensus document of the Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. *J Am Coll Cardiol* 2000;36:959-69.
- Shvartz E, Reibold RC. Aerobic fitness norms for males and females aged 6 to 75 years: a review. *Aviat Space Environ Med* 1990;61:3-11.
- Sosdorf M, Otto GP, Claus RA, Gabriel HH, Losche W. Cell-derived microparticles promote coagulation after moderate exercise. *Med Sci Sports Exerc* 2011;43:1169-76.
- Chaar V, Romana M, Tripette J, et al. Effect of strenuous physical exercise on circulating cell-derived microparticles. *Clin Hemorheol Microcirc* 2011;47:15-25.
- Bulut D, Becker V, Mugge A. Acetylsalicylate reduces endothelial and platelet-derived microparticles in patients with coronary artery disease. *Can J Physiol Pharmacol* 2011;89:239-44.
- Nomura S, Shouzu A, Omoto S, Nishikawa M, Iwasaka T. Effects of losartan and simvastatin on monocyte-derived microparticles in hypertensive patients with and without type 2 diabetes mellitus. *Clin Appl Thromb Hemost* 2004;10:133-41.
- Tramontano AF, O'Leary J, Black AD, Muniyappa R, Cutaia MV, El-Sherif N. Statin decreases endothelial microparticle release from human coronary artery endothelial cells: implication for the Rho-kinase pathway. *Biochem Biophys Res Commun* 2004;320:34-8.
- Miller VM, Vanhoutte PM. Enhanced release of endothelium-derived factor(s) by chronic increases in blood flow. *Am J Physiol* 1988;255:H446-51.
- Rognmo O, Bjornstad TH, Kahrs C, et al. Endothelial function in highly endurance-trained men: effects of acute exercise. *J Strength Cond Res* 2008;22:535-42.
- Padilla J, Harris RA, Rink LD, Wallace JP. Characterization of the brachial artery shear stress following walking exercise. *Vasc Med* 2008;13:105-11.
- Ji LL. Antioxidants and oxidative stress in exercise. *Proc Soc Exp Biol Med* 1999;222:283-92.
- Goto C, Higashi Y, Kimura M, et al. Effect of different intensities of exercise on endothelium-dependent vasodilation in humans: role of endothelium-dependent nitric oxide and oxidative stress. *Circulation* 2003;108:530-5.

31. Mobius-Winkler S, Hilberg T, Menzel K, et al. Time dependent mobilization of circulating progenitor cells during strenuous exercise in healthy individuals. *J Appl Physiol* 2009;6:1943-50.
32. Harrison M, Murphy RP, O'Connor PL, et al. The endothelial micro-particle response to a high fat meal is not attenuated by prior exercise. *Eur J Appl Physiol* 2009;106:555-62.
33. Harvey PJ, Morris BL, Kubo T, et al. Hemodynamic after-effects of acute dynamic exercise in sedentary normotensive postmenopausal women. *J Hypertens* 2005;23:285-92.
34. Harvey PJ, Picton PE, Su WS, Morris BL, Notarius CF, Floras JS. Exercise as an alternative to oral estrogen for amelioration of endothelial dysfunction in postmenopausal women. *Am Heart J* 2005;149:291-7.
35. Cosio-Lima LM, Thompson PD, Reynolds KL, et al. The acute effect of aerobic exercise on brachial artery endothelial function in renal transplant recipients. *Prev Cardiol* 2006;9:211-4.
36. Harris RA, Padilla J, Hanlon KP, Rink LD, Wallace JP. The flow-mediated dilation response to acute exercise in overweight active and inactive men. *Obesity (Silver Spring)* 2008;16:578-84.
37. Pullin C, Bellamy M, Bailey D, et al. Time course of changes in endothelial function following exercise in habitually sedentary men. *J Exercise Physiol* 2004;7:14-22.
38. Toth B, Nikolajek K, Rank A, et al. Gender-specific and menstrual cycle dependent differences in circulating microparticles. *Platelets* 2007;18:515-21.

Supplementary Material

To access the supplementary material accompanying this article, visit the online version of the *Canadian Journal of Cardiology* at www.onlinecjc.ca and at <http://10.1016/j.cjca.2013.03.024>.

SUPPLEMENTARY MATERIAL

Inclusion and exclusion criteria

Inclusion criteria consisted of documented CHD (history of $\geq 70\%$ arterial diameter narrowing of at least one major coronary artery, prior coronary revascularization, documented prior myocardial infarction and/or perfusion defect on myocardial scintigraphy) and performing regular exercise ≥ 2 times per week. Exclusion criteria were: 1) recent acute coronary syndrome (≤ 3 months), 2) recent percutaneous or surgical coronary revascularization (≤ 3 months), 3) significant resting ECG abnormality (major ST-T wave change, complete bundle branch block or intraventricular conduction delay, atrial fibrillation or flutter), 4) history of ventricular tachycardia or ventricular fibrillation, 5) automated implantable cardioverter defibrillator or pacemaker, 6) left ventricular ejection fraction $< 45\%$, 7) chronic congestive heart failure, 8) uncontrolled hypertension (systolic blood pressure ≥ 180 mmHg or diastolic pressure ≥ 110 mmHg), 9) recent modification of cardiovascular medications (≤ 6 weeks), and 10) musculoskeletal conditions making exercise difficult or contraindicated.

Exercise testing

After a 3-min warm-up at 20 W, a maximal continuous graded exercise test was performed. Initial power was set at 60 W and increased by 15 W every minute. Verbal encouragement was given throughout the test. Criteria for exercise test cessation were volitional exhaustion, significant ECG abnormalities (ST-depression > 2 mm or significant ventricular arrhythmias), or abnormal blood pressure response. Oxygen uptake (VO_2) was determined continuously on a 15-sec basis using an automated

cardiopulmonary exercise system (Oxycon Alpha, Jaegger, Germany). Gas analyzers were calibrated before each test, using a gas mixture of known concentration (5% CO₂) and ambient air. Participants breathed through a facemask connected with the turbine. The turbine was calibrated before each test using a 3-liter syringe at several flow rates. Heart rate/rhythm monitoring was performed continuously using 12-lead ECG (Marquette, Missouri) and blood pressure was measured manually every 2 minutes. The highest VO₂ over a 15-sec period and the highest heart rate over a 5-sec period during the test were considered as peak oxygen consumption (VO_{2peak}, in ml/kg/min) and peak heart rate (HR peak, in bpm). Power of the last completed stage was considered as the peak power output (PPO, in W).

Exercise sessions

Participants were asked to arrive fully hydrated to the laboratory, at least 3 h after their last meal. No attempt was made to control meal size or content. The MICE protocol consisted of cycling during 28.7 minutes at 70% of PPO. The HIIE session started by a 10-minute warm-up at 50% of PPO, followed by two 10-minute sets composed of repeated bouts of 15 seconds at 100% of PPO interspersed by 15 seconds of passive recovery. Four minutes of passive recovery were allowed between the two sets, followed by a 5-minute cool-down at the end of the exercise sessions. Both exercise sessions were isocaloric, as detailed previously.¹² Oxygen consumption measurement, ECG and blood pressure monitoring were performed as described above for the maximal graded exercise test. Perceived exertion was measured every 3 min with the 20-point Borg scale. Feedback on elapsed time and verbal encouragement were given throughout the sessions.

Exercise cessation criteria were the same as those used during the maximal stress test. Participants were monitored for at least 5 min after exercise cessation in a sitting position.

Microparticle measurement

Platelet-free plasma (PFP) was obtained by centrifugation at 1500 g for 15 min followed by a single centrifugation at 13 000 g for 2 min to avoid platelet contamination. The PFP was then aliquoted and stored at -80 °C until analysis by flow cytometry.¹³ For flow cytometry analysis, 100 µl of PFP in a TruCOUNT tube (BD Biosciences) was incubated with anti-CD31 PE, anti-CD62E APC and anti-CD42 FITC (BD Biosciences) antibodies at room temperature for 30 min. IgG1-PE, IgG1-APC and IgG1-FITC (BD Biosciences) were used for fluorescence minus one (FMO) negative controls. Acquisition of flow cytometry data was obtained using a LSRII flow cytometer (BD Biosciences). Events with a diameter ≤ 0.9 µm, as estimated using Megamix size beads (Biocytex), were identified in forward scatter and side scatter intensity dot plot representation, gated as MP and then plotted on one- or two-color histograms. Endothelial microparticles were defined as particles with a diameter < 0.9 µm, negative for CD42b and positive for CD31 (EMP31) or CD62E (EMP62E). Importantly, we refer to EMPs as microparticles that are either positive for CD31 or CD62E or both in contrast to the single positive CD31 (EMP31) or CD62E (EMP62E) microparticles. Platelet microparticles (PMP) were defined as particles with a diameter < 0.9 µm, positive for CD42b. For each PFP sample, microparticle quantification was performed in triplicates and the mean value was determined after flow cytometry analysis using the FACSDiva software version 5.0.3.

The coefficients of variation of the triplicates for the different microparticles typically varied from 6.3-11.3% for the more abundant PMP, 10.2-11.4% for EMP, 11.1-12.2% for the EMP31, and from 17-19.8% for EMP62E.

Supplemental Table S1. Patient characteristics and medication use

Anthropometrics	
Age (years)	62 ± 12
Body mass index (kg/m ²)	27 ± 4
Waist circumference (cm)	97 ± 10
Body fat (%)	27 ± 5
Left ventricular ejection fraction (%)	59 ± 5
Angina pectoris	8 (42%)
Prior myocardial infarction	10 (53%)
Prior percutaneous coronary intervention	7 (37%)
Prior coronary bypass surgery	6 (32%)
Cardiovascular risk factors	n (%)
Diabetes mellitus	2 (10%)
Hypertension	5 (26%)
Dyslipidemia	17 (90%)
Smoking	0 (0%)
Medications	n (%)
Anti-platelet agents	19 (100%)
Beta-blockers	10 (53%)
Calcium channel blockers	0 (0%)
Angiotensin-converting enzyme inhibitors	3 (16%)

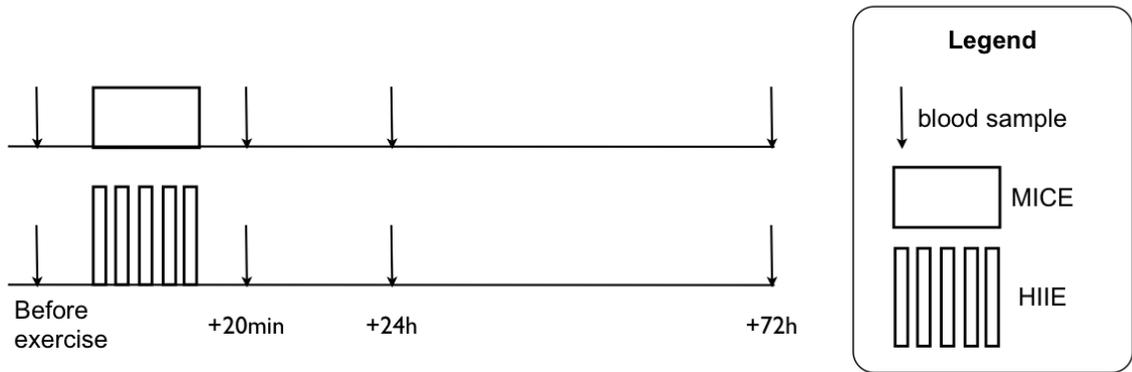
Angiotensin receptor antagonists	6 (32%)
Statins	17 (90%)
Nitrates	0 (0%)

Supplemental Table S2. Results from maximal continuous graded exercise test

VO _{2peak} (ml/kg/min)	29.1 ± 9.1
VO _{2peak} (ml/min)	2369 ± 800
Exercise tolerance (METs)	8.3 ± 2
Peak power output (Watt)	181 ± 62
Resting heart rate (bpm)	62 ± 10
Peak heart rate (bpm)	144 ± 20
Resting systolic blood pressure (mmHg)	129 ± 13
Maximal systolic blood pressure (mmHg)	176 ± 25

MET, metabolic equivalent (multiple of 3.5 ml/kg/min); VO_{2peak}, peak oxygen consumption.

Supplemental Figure



Experimental design. MICE duration was 28.7 minutes.