Association of Lower Habitual Physical Activity Level With Mitochondrial and Endothelial Dysfunction in Patients With Stable Coronary Artery Disease

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Background: Exercise training improves endothelial function in patients with coronary artery disease (CAD) through unclear mechanisms. We hypothesized that mitochondrial dysfunction related to a lower habitual physical activity level (PAL) is associated with endothelial dysfunction.

Methods and Results: We assessed habitual PAL by a validated International Physical Activity Questionnaire, brachial flow-mediated dilatation (FMD) and serum lactate, pyruvate, fasting glucose and lipid profiles in 105 CAD patients (age 68±10; 87% men). As defined by the lactate/pyruvate ratio (LP ratio) ≥75th percentile of the age-and sex-matched controls (ie, ≥18), mitochondrial dysfunction was observed in 33/105 (31%) patients. With decreasing PAL tertiles, there were significant linear trends of lower FMD (P=0.004) and higher LP ratio (P=0.009). Multivariate logistic regression found that the lowest compared with the highest PAL tertile (adjusted odds ratio=3.78, P=0.02) had more patients with high LP ratio. After adjustment for cardiovascular risk factors and medications, the lowest compared to the highest PAL tertile had significantly lower FMD (absolute decrease 1.25%, P=0.03) and high LP ratio was associated with impaired FMD (absolute reduction 1.09%, P=0.03).

Conclusions: In CAD patients, a lower level of habitual PAL is associated with impaired FMD and increased prevalence of mitochondrial dysfunction as defined by high LP ratio. Moreover, high LP ratio predicts a lower FMD, suggesting that the occurrence of mitochondrial dysfunction with lower habitual PAL is associated with endothelial dysfunction in CAD patients. (Circ J 2012; 76: 2572–2578)

Key Words: Coronary artery disease; Endothelial function; Lactate/pyruvate ratio; Physical activity

Exercise training has been shown to reduce total and cardiovascular mortality in patients with coronary artery disease (CAD), but the mechanisms remain unclear. Endothelial dysfunction precedes atherosclerosis and is an independent predictor of cardiovascular events. Emerging evidence demonstrates that exercise improves both the cardiometabolic risk profile and endothelial function in high-risk patients for CAD. Furthermore, it has been reported that a higher level of habitual physical activity level (PAL) is associated with improved endothelial function in patients with CAD.

Recent studies have highlighted the potential link between mitochondrial dysfunction and the pathogenesis of atherosclerosis. Cardiovascular risk factors such as hypercholesterolemia and diabetes mellitus induce mitochondrial dysfunction, leading to an overproduction of reactive oxygen species (ROS). The resulting increase in oxidative stress impairs endothelial function by lowering the bioavailability of nitric oxide. Oxidative mitochondrial damage has been shown to induce mitochondrial dysfunction in experimental studies. Clinical studies have also demonstrated an association between mitochondrial dysfunction and impaired endothelial function in patients with CAD. The lactate/pyruvate (LP) ratio is an established indirect serum marker of mitochondrial dysfunction as the impaired oxidative phosphorylation in the mitochondria is associated with excessive hydrolysis of ATP and production of more protons, which drives the equilibrium reaction from pyruvate to lactate, leading to an increased LP.
ratio. Although exercise training has been reported to augment mitochondrial content and function in human skeletal muscle, there is limited evidence on whether physical activity modulates mitochondrial function in high-risk CAD patients. Therefore, we hypothesized that the endothelial dysfunction associated with lower PAL is related to the occurrence of mitochondrial dysfunction.

Methods

Study Population
Consecutive patients with stable CAD and >50% stenosis in at least 1 of the major coronary arteries as documented by coronary angiography were recruited between July 2007 and April 2008. All patients had received stable medical therapy for 26 months before enrollment. Patients with recent acute coronary syndrome or percutaneous coronary intervention in the past 3 months, significant valvular heart disease, left ventricular ejection fraction <45%, exacerbated chronic obstructive pulmonary disease, significant renal or hepatic dysfunction, and severe orthopedic conditions that prohibited exercise were excluded. As a result, a total of 105 patients were eligible for this study.

To determine the range of LP ratio in normal individuals, 30 age- and sex-matched subjects who had no history of cardiovascular diseases, a coronary artery calcium score <10 and were not on any cardiovascular medications were enrolled as controls. The study was approved by the local institutional review board, and all subjects provided informed consent.

Demographic and Laboratory Evaluations
Baseline demographic data and cardiovascular medications were documented. Cardiovascular risk factors, including tobacco smoking, diabetes mellitus, hypercholesterolemia and hypertension, were assessed. The body height and weight, blood pressure and body mass index of all subjects were measured as previously described. Hypertension was defined as either systolic or diastolic blood pressure ≥140/90 mmHg at 2 different clinical visits or the use of medications. Diabetes mellitus was defined as fasting glucose ≥7.0 mmol/L or on medication. Hypercholesterolemia was defined as a fasting total serum cholesterol level ≥4.9 mmol/L or on medication. Smoking status was recorded as either current smoker or non-current smoker.

Fasting blood samples were obtained from all subjects to determine serum creatinine, glucose and lipid levels. The plasma level of high-sensitivity C-reactive protein was measured by a Hitachi 747 analyzer (Boehringer Mannheim, Mannheim, Germany), and a particle-enhanced immunoturbidimetric assay (Roche Diagnostics, Mannheim, Germany) as described previously. Fasting venous blood samples for lactate and pyruvate analysis were collected by non-tourniquet sampling and immediately added to fluoride oxalate medium and a titrated volume of chilled perchlorate, respectively; as previously described. After thorough mixing, the samples were then centrifuged at 3,000 rpm for 20 min and the supernatants snap-frozen at −80°C until assay. Lactate was measured by an enzyme-coupled colorimetric assay automated on a Hitachi-912 analyzer (Roche Diagnostics). Pyruvate was measured by reverse-phase high-performance liquid chromatography (Waters Corp, Milford, MA, USA) using a C18 column with isocratic elution.

Assessment of Habitual PAL
Each subject’s habitual PAL was assessed by a validated Inter-
national Physical Activity Questionnaire (IPAQ) administered by a single experienced interviewer. The questionnaire has been validated internationally and its Chinese version has also been validated in both Hong Kong Chinese and older Chinese subjects. The IPAQ was conducted as previously described. In brief, subjects were asked to report on the time they spent walking, in vigorous- and moderate-intensity activities lasting at least 10 min and in sedentary activity over the past 7 days. Habitual PAL was obtained by estimating the total weekly energy expenditure (MET·min/week), which was calculated by weighting the reported duration of each activity category performed per week by a MET energy expenditure estimate assigned to each category of activity. The weight for each category of activity was defined as follows: 3.3 METs for walking, 4 METs for moderate activity, and 8 METs for vigorous activity. The intraobserver variability testing found an intraclass correlation coefficient of 0.94 (P<0.001).

Assessment of Vascular Endothelial Function
Vascular ultrasound examination was performed by 2 experienced operators without knowledge of the subjects, using a high-resolution ultrasound system (Agilent Sonos 5500; Philips, Andover, MA, USA) with a 7.5-MHz linear array transducer. All the scanned images were stored digitally and analyzed offline by the same operators, who were blinded to the identity of subjects. All subjects were studied in the fasting state, and all vasoactive medications were withheld for ≥12 h before the study. As previously described, longitudinal scans of the brachial artery were obtained at rest, and then brachial flow-mediated dilation (FMD) was induced by inflation of a pneumatic tourniquet placed on the forearm to a pressure of 250 mmHg for 5 min. The diameter of the brachial artery was allowed to return to baseline. Finally, the diameter of the brachial artery was obtained 5 min after administration of 400 μg sublingual nitroglycerin spray. FMD was calculated as the percentage change in diameter from baseline to the diameter at 60 s of reactive hyperemia. The FMD measurements were reproducible, as evidenced by intraobserver variability study in 25 patients showing an intraclass correlation coefficient of 0.88 (P<0.001), whereas the intraobserver variability correlation coefficients for 2 different operators were 0.90 and 0.84, respectively (both P<0.001).

Statistical Analysis
Based on the data from our previous observational study, we assumed a standard deviation of 3% for FMD. In order to detect a difference of 2% in FMD from any of the 3 tertiles of PAL with 80% power and a 5% false positive error rate, we would require at least 102 subjects in a 1-way ANOVA design. Continuous variables are expressed as mean±standard deviation (SD) and categorical data are presented as frequencies and percentages. Statistical comparisons were performed with Student’s t-test or Pearson’s chi-square test as appropriate. Relationships between continuous variables were studied using Pearson’s correlation coefficient. Comparisons of variables between different tertiles of PAL were performed with 1-way analysis of variance (ANOVA) with post-hoc Bonferroni’s correction for multiple comparisons and test for linear trend. The effect of habitual PAL on mitochondrial dysfunction was analyzed using univariate and multivariate binary logistic regression analyses. Absolute changes and 95% confidence intervals (CI) of FMD were calculated by univariate and multivariate linear regression analyses, to determine the effect of higher PAL after adjustment for confounders. A value of P<0.05 was considered statistically significant.
Table 1. Study Population’s Clinical Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All (n=105)</th>
<th>High LP ratio (n=33)</th>
<th>Normal LP ratio (n=72)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>68±10</td>
<td>68±9</td>
<td>68±10</td>
<td>0.83</td>
</tr>
<tr>
<td>Males, n (%)</td>
<td>88 (84)</td>
<td>30 (91)</td>
<td>58 (81)</td>
<td>0.18</td>
</tr>
<tr>
<td>Previous MI, n (%)</td>
<td>57 (54)</td>
<td>17 (52)</td>
<td>40 (56)</td>
<td>0.70</td>
</tr>
<tr>
<td>Previous revascularization, n (%)</td>
<td>81 (77)</td>
<td>28 (85)</td>
<td>53 (74)</td>
<td>0.20</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>73 (70)</td>
<td>21 (64)</td>
<td>52 (72)</td>
<td>0.38</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>38 (36)</td>
<td>16 (49)</td>
<td>22 (31)</td>
<td>0.08</td>
</tr>
<tr>
<td>Hypercholesterolemia, n (%)</td>
<td>68 (65)</td>
<td>22 (67)</td>
<td>46 (64)</td>
<td>0.78</td>
</tr>
<tr>
<td>Current smoker, n (%)</td>
<td>6 (6)</td>
<td>2 (6)</td>
<td>4 (6)</td>
<td>0.92</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25±3</td>
<td>25±3</td>
<td>25±3</td>
<td>0.30</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>52.2±11.6</td>
<td>50.9±11.3</td>
<td>52.7±11.7</td>
<td>0.48</td>
</tr>
<tr>
<td>BP, mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>145±19</td>
<td>150±24</td>
<td>143±17</td>
<td>0.16</td>
</tr>
<tr>
<td>Diastolic</td>
<td>82±9</td>
<td>82±10</td>
<td>81±9</td>
<td>0.74</td>
</tr>
<tr>
<td>Biochemical analysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>6.10±2.14</td>
<td>6.54±2.12</td>
<td>5.91±2.13</td>
<td>0.16</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>6.86±1.70</td>
<td>7.23±1.83</td>
<td>6.69±1.63</td>
<td>0.14</td>
</tr>
<tr>
<td>Triglyceride, mmol/L</td>
<td>1.59±1.07</td>
<td>1.48±0.66</td>
<td>1.63±1.22</td>
<td>0.50</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.11±0.71</td>
<td>4.12±0.69</td>
<td>4.07±0.72</td>
<td>0.51</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.14±0.25</td>
<td>1.14±0.21</td>
<td>1.14±0.27</td>
<td>0.97</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>2.28±0.52</td>
<td>2.35±0.51</td>
<td>2.24±0.52</td>
<td>0.31</td>
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<tr>
<td>Creatinine, μmol/L</td>
<td>91±19</td>
<td>90±12</td>
<td>91±22</td>
<td>0.73</td>
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<tr>
<td>hsCRP, mg/L</td>
<td>1.84±2.81</td>
<td>1.33±1.85</td>
<td>2.06±3.12</td>
<td>0.24</td>
</tr>
<tr>
<td>Medications, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-blocker</td>
<td>84 (80)</td>
<td>27 (82)</td>
<td>57 (79)</td>
<td>0.75</td>
</tr>
<tr>
<td>Calcium-channel blocker</td>
<td>26 (25)</td>
<td>9 (27)</td>
<td>17 (24)</td>
<td>0.69</td>
</tr>
<tr>
<td>ACEI/ARB</td>
<td>78 (74)</td>
<td>25 (76)</td>
<td>53 (74)</td>
<td>0.82</td>
</tr>
<tr>
<td>Nitrate</td>
<td>58 (56)</td>
<td>19 (58)</td>
<td>39 (55)</td>
<td>0.80</td>
</tr>
<tr>
<td>Aspirin</td>
<td>100 (95)</td>
<td>32 (97)</td>
<td>68 (94)</td>
<td>0.57</td>
</tr>
<tr>
<td>Statin</td>
<td>99 (94)</td>
<td>30 (91)</td>
<td>69 (96)</td>
<td>0.31</td>
</tr>
<tr>
<td>Brachial FMD, %</td>
<td>3.28±2.33</td>
<td>2.22±2.53</td>
<td>3.76±2.07</td>
<td>0.001</td>
</tr>
<tr>
<td>LP ratio</td>
<td>17.19±3.61</td>
<td>22.71±2.31</td>
<td>15.13±1.73</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Habitual PAL (MET · min/week)</td>
<td>1,696±898</td>
<td>1,374±725</td>
<td>1,843±934</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*Comparison between patients with high or normal LP ratio.

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin-receptor blocker; BP, blood pressure; CAD, coronary artery disease; HbA1c, hemoglobin A1c; hsCRP, high-sensitivity C-reactive protein; FMD, flow-mediated dilation; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LP, lactate/pyruvate; LVEF, left ventricular ejection fraction; MI, myocardial infarction; PAL, physical activity level.

Results

Clinical Characteristics
A total of 105 subjects with stable CAD (mean age 68±10 years, 84% men) were recruited (Table 1). The compared with the age- and sex-matched healthy controls (n=30, mean age 68±8 years, 87% men), patients with CAD had a lower FMD (3.28±2.33% vs. 5.98±4.83%, P=0.006) and higher LP ratio (17.19±3.61 vs. 15.16±2.68, P=0.001). However, there was no significant difference in mean habitual PAL between CAD patients and controls (1,696±898 MET · min/week vs. 1,756±1,036 MET · min/week, P=0.76).

The 75th percentile of the LP ratio of the normal controls was 18, and thus was defined as the cutoff for mitochondrial dysfunction. As defined by a LP ratio ≥18, 33 (31%) patients had mitochondrial dysfunction. There were no significant differences in age, prevalence of males, hypertension, diabetes mellitus, hypercholesterolemia, or current smoking status in patients with or without mitochondrial dysfunction (all P>0.05, Table 1). The use of medications including β-blockers, angio-

tensin-converting enzyme inhibitors or angiotensin-receptor blockers, and statins also did not differ significantly between the 2 groups (P>0.05). Patients with mitochondrial dysfunction had lower FMD (2.22±2.53% vs. 3.76±2.07%, P=0.001) and lower habitual PAL (1,374±725 MET · min/week vs. 1,843±934 MET · min/week, P=0.01) as compared with patients without mitochondrial dysfunction. As expected, those with mitochondrial dysfunction had significantly higher LP ratio (22.71±2.31 vs. 15.13±1.73, P<0.001).

Relationships Between Habitual PAL, Mitochondrial Dysfunction and Endothelial Function
Among the control subjects, there were no significant correlations between habitual PAL, FMD and LP ratio (all P>0.05). To further investigate the relationship between the habitual PAL and mitochondrial dysfunction and endothelial function in patients with CAD, the group was divided into 3 tertiles of habitual PAL according to the patients’ total weekly energy expenditure (Table 2).

The effect of PAL tertiles on FMD and LP ratio were ana-
Physical Activity and Endothelial Function

lyzed with ANOVA with post-hoc test for linear trend and Bonferroni’s correction for multiple comparisons was performed (Figure, Table 3). There was a significant decrease in brachial FMD ($P=0.01$, $P$ for linear trend=$0.004$) and an increase in LP ratio ($P=0.03$, $P$ for linear trend=$0.009$) in patients with decreasing tertiles of habitual PAL. Patients in the lowest compared with the highest PAL tertile had significantly lower FMD (absolute difference 1.58%, $P=0.01$) and higher LP ratio (absolute difference 2.24, $P=0.03$). However, there were no significant differences in FMD and LP ratio between patients with 2nd vs. 1st or 3rd vs. 2nd tertiles of PAL. Also, there was no significant correlation between habitual PAL tertiles and conventional cardiovascular risk factors (Table 3).

Unadjusted and Adjusted Effects of Habitual PAL on Mitochondrial Dysfunction

The lowest compared with the highest PAL tertile had significantly more patients with a high LP ratio, an indirect indicator of mitochondrial dysfunction (unadjusted odds ratio [OR]=3.63, 95% CI 1.20–10.94, $P=0.02$, Table 4). Multivariate binary logistic regression analysis was then used to examine the adjusted effect of habitual PAL on the presence of high LP ratio. After correcting for age, sex, cardiovascular risk factors such as hypertension, hypercholesterolemia, diabetes mellitus and smoking status, as well as medications including β-blockers, angiotensin-converting enzyme inhibitors or angiotensin-receptor blockers and statins, the increased prevalence of mitochondrial dysfunction remained significant (adjusted OR=3.78, 95% CI 1.11–12.91, $P=0.03$, Hosmer and Lemeshow’s test of goodness-of-fit $P=0.91$) comparing the lowest with the highest PAL tertile. In both unadjusted and adjusted models, the 2nd compared with the 1st PAL tertile was not associated with a difference in risk of mitochondrial dysfunction ($P>0.05$). These findings suggest a potential threshold level at which the PAL can reduce the prevalence of mitochondrial dysfunction.

Effect of Habitual PAL and Mitochondrial Dysfunction on FMD

Multivariate linear regression was used to analyze the adjusted effect of habitual PAL on FMD, adjusting for age, sex, hypertension, diabetes mellitus, hypercholesterolemia, smoking status, and use of medications including β-blockers, angiotensin-converting enzyme inhibitors or angiotensin-receptor blockers and statins, as these factors are known to affect FMD (Table 5). After adjusting for these confounding factors, patients in the lowest PAL tertile had significantly lower FMD (absolute difference 1.51%, $P=0.009$) as compared with subjects in the highest PAL tertile, accounting for a relative difference of 31% in FMD.

In the univariate analysis, mitochondrial dysfunction ($r=−0.31$, $P=0.001$) and its blood marker, the LP ratio ($r=−0.26$, $P=0.008$),

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**Table 2. Habitual Physical Activity Level in Patients With CAD**

<table>
<thead>
<tr>
<th>Energy expenditure per week (MET · min/week)</th>
<th>Total</th>
<th>Vigorous activity</th>
<th>Moderate activity</th>
<th>Walking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall mean</td>
<td>1,696±898</td>
<td>140±465</td>
<td>579±662</td>
<td>1,005±742</td>
</tr>
<tr>
<td>1st tertile (n=35)</td>
<td>Mean</td>
<td>801±320</td>
<td>0±0</td>
<td>317±366</td>
</tr>
<tr>
<td>2nd tertile (n=35)</td>
<td>Mean</td>
<td>1,553±198</td>
<td>61±219</td>
<td>439±388</td>
</tr>
<tr>
<td>3rd tertile (n=35)</td>
<td>Mean</td>
<td>2,734±608</td>
<td>358±733</td>
<td>982±892</td>
</tr>
</tbody>
</table>

CAD, coronary artery disease.

**Figure.** (A) Brachial flow-mediated dilation (FMD) and (B) lactate/pyruvate ratio in patients with 3 different tertiles of physical activity level (PAL) in patients with coronary artery disease. *Significantly different from the 3rd tertile (1-way ANOVA with Bonferroni’s correction for multiple comparisons).
were both associated with an impaired FMD. The effects of higher habitual PAL together with mitochondrial dysfunction on FMD were further analyzed using the same multivariate linear regression model (Table 5). When entered into the same multivariate model, the presence of mitochondrial dysfunction independently accounted for an absolute 1.09% reduction in FMD (relative reduction 26%, 95% CI −2.09 to −0.09, P = 0.03), while the lowest compared with the highest PAL tertile was independently associated with an absolute decrease of 1.25% in FMD (relative decrease 28%, 95% CI 0.13–2.38, P=0.03; after adjusting for the aforementioned variables that are known to affect FMD.

Discussion
In this study, we demonstrated that lower PAL in CAD patients
was associated with impaired endothelial function and higher risk of mitochondrial dysfunction. Moreover, the presence of mitochondrial dysfunction predicted a lower FMD, suggesting that mitochondrial dysfunction together with lower habitual PAL may contribute to endothelial dysfunction in patients with CAD.

Endothelial dysfunction is an independent predictor of future cardiovascular events. Aging and cardiovascular diseases are associated with endothelial dysfunction through different mechanisms. Specifically, ROS induced by atherogenic factors exert significant detrimental effect on the vascular endothelium by decreasing NO bioavailability. Multiple studies have documented that physical activity reverses the age-dependent endothelial dysfunction in healthy subjects and exercise training could improve endothelial function in CAD patients. Our study showed that higher habitual PAL, apart from supervised exercise training programs, was associated with better endothelial function in high-risk CAD patients.

Aging and vascular risk factors such as hypercholesterolemia and type 2 diabetes mellitus have been implicated in oxidative damage leading to mitochondrial dysfunction. In humans, mitochondrial content and function are both increased after exercise training and exercise-induced benefits on the mitochondria have been observed in patient with diabetes, obesity and heart failure. Regular exercise is regarded as an effective antioxidant therapy and the decrease in ROS-induced mitochondrial damage may explain the cardioprotective effect of exercise. Our study demonstrated that, in patients with stable CAD, a low level of habitual PAL was associated with the occurrence of mitochondrial dysfunction, supporting the notion that physical inactivity may worsen the aging of the mitochondria.

The clinical implications of mitochondrial dysfunction in atherosclerosis are being increasingly recognized. Increased ROS generation in mitochondrial dysfunction may accelerate apoptosis of vascular smooth muscle cells and macrophages, contributing to the progression of atherosclerotic lesions and subsequently plaque rupture. Also, the reduction of NO bioavailability by oxidative stress may aggravate endothelial dysfunction. In patients with CAD, the presence of mitochondrial dysfunction has been associated with impaired endothelial function. Indeed, the findings from our study indicated that mitochondrial dysfunction and its blood marker, the LP ratio, both significantly correlated with impairment of FMD. Furthermore, mitochondrial dysfunction was associated with a significant relative impairment of 26% in endothelial function, suggesting that the endothelial dysfunction related to lower PAL was at least partly mediated through the occurrence of mitochondrial dysfunction.

It is well recognized that both the level (ie, total physical activity) and intensity of exercise are related to the risk of CAD and physical activity reduced the risk of fatal and non-fatal cardiovascular events among cardiovascular disease survivors. In our study, lower habitual PAL, measured as the total weekly amount of exercise, was associated with both endothelial dysfunction and mitochondrial dysfunction. In particular, significant differences were only observed in the highest compared with the lowest PAL tertile, indicating a possible threshold level of habitual physical activity for its vasoprotective effects. The participants who attained a significant better endothelial function and mitochondrial function were engaged in a mean habitual PAL of 2,734 METs · min/week. This threshold level of exercise could be translated into 2 h of daily walking exercise, or 1.5 h of moderate exercise such as bicycling or carrying light loads, or 50 min of vigorous activity per day.

Study Limitations
The present study was a cross-sectional observational study with a relatively small sample size. Therefore, a causal relationship between habitual exercise and the improvement in mitochondrial function and endothelial function could not be established. In particular, abnormal mitochondrial function has a profound negative effect on aerobic capacity leading to poor exercise tolerance and hence a reduced PAL. Whether increased PAL or exercise training in patients with CAD can improve endothelial function via attenuation of mitochondrial dysfunction requires further long-term prospective clinical studies.

Acknowledgments
This study was supported by the CRCG Small Project Funding of University of Hong Kong (Project No. 200907176063), and Sun Chaih Yeh Heart Foundation. We thank Dr Duncan Macfarlane, Institute of Human Performance, University of Hong Kong, for the use of the Chinese version of IPAQ.

References
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