Exercise capacity is associated with endothelin-1 release during emotional excitement in coronary artery disease patients

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Submitted 15 November 2013; accepted in final form 23 May 2014

Tulppo MP, Piira OP, Hautala AJ, Kiviniemi AM, Miettinen JA, Huikuri HV. Exercise capacity is associated with endothelin-1 release during emotional excitement in coronary artery disease patients. Am J Physiol Heart Circ Physiol 307: H391–H396, 2014. First published May 30, 2014; doi:10.1152/ajpheart.00902.2013.—Endothelin-1 (ET-1), a potent vasoconstrictor, IL-6, and catecholamines are increased and heart rate variability [SD of normal to normal R-R intervals (SDNN)] decreased during emotional excitement, but individual responses vary. We tested the hypothesis that exercise capacity is associated with physiological responses caused by real-life emotional excitement. We measured the plasma levels of ET-1, IL-6, catecholamines, heart rate, and SDNN in enthusiastic male ice hockey spectators (n = 51; age, 59 ± 9 years) with stable coronary artery disease (CAD) at baseline and during the Finnish National Ice Hockey League’s final play-off matches. Maximal exercise capacity (METs) by bicycle exercise test and left ventricular ejection fraction (LVEF) were measured on a separate day. ET-1 response from baseline to emotional excitement correlated with maximal METs (r = −0.30; P = 0.040). In a linear stepwise regression analysis age, body mass index (BMI), METs, LVEF, basal ET-1, and subjective experience of excitement were entered as independent variables to explain ET-1 response. This model explained 27% of ET-1 response (P = 0.003). Maximal METs were most strongly correlated with ET-1 response (β = −0.45; partial correlation r = −0.43; P = 0.002), followed by BMI (β = −0.31; partial correlation r = −0.31; P = 0.033) and LVEF (β = −0.30; partial correlation r = −0.33; P = 0.023). Exercise capacity may protect against further cardiovascular events in CAD patients, because it is associated with reduced ET-1 release during emotional excitement.

EMOTIONAL EXCITEMENT HAS PROVED to be an important trigger of cardiovascular events. For example, earthquakes, wars, and sports events are associated with peaks in the incidence of sudden cardiac death, myocardial infarction, and acute coronary syndrome (18, 22, 35). Circulating endothelin-1 (ET-1) concentration and inflammatory factors has been shown to be higher in patients with emotional stress-induced acute coronary syndrome than in patients with acute coronary syndrome not associated with emotional stress (44). Elevated ET-1 has also been linked to plaque rupture and subsequent acute coronary syndrome (44). We have recently shown that circulating ET-1 and IL-6 are increased in coronary artery disease patients (CAD) and healthy subjects during emotional excitement caused by watching a thrilling ice hockey match (31, 32). The changes in ET-1 and IL-6 from baseline to the ice hockey match were more marked in CAD patients than in age-matched healthy subjects despite optimal medication, including β-blocking medication (31). Also, these differences were obvious despite equal emotional excitement in the patients and the healthy subjects, documented by a questionnaire on subjective experience of excitement answered during the match. Second, circulating catecholamines and heart rate are increased and heart rate variability decreased as evidence of autonomic regulation toward sympathetic dominance during emotional excitement (13, 24 –27, 30 –32). However, marked individual differences have been observed in physiological responses to emotional excitement, which were not explained by differences, for example, in subjective experience of excitement (30–32).

It has been suggested that good exercise capacity or cardiovascular conditioning by exercise training is associated with attenuated physiological responses to mental stress measured, for example, by heart rate, heart rate variability, blood pressure, and catecholamines (4, 5a, 10, 12, 34, 41). The association between good exercise capacity and mental stress responses is called the “cross-stressor adaptation” hypothesis, meaning that adaptation to exercise training leads to adaptation of the responses to other challenges like mental stress (38). Some controversial results regarding this association have also been reported, and prior studies have mainly included subjects without a known cardiac disease. Therefore, we set out to test the hypothesis that physical fitness is associated with physiological response among patients with documented CAD by studying the possible association between maximal exercise capacity and individual responses in plasma ET-1, IL-6, catecholamines, heart rate, and heart rate variability caused by leisure-time emotional excitement in patients with stable CAD.

METHODS

Subject selection. The patients were selected from the ARTEMIS (Innovation to Reduce Cardiovascular Complications of Diabetes at the Intersection; ClinicalTrials.gov, Record 1539/31/06) study database comprising 665 patients with CAD who underwent a thorough evaluation of their cardiovascular status, including a baseline examination performed by a cardiologist, echocardiography, an exercise stress test, and 24-h Holter recordings. CAD and its severity were assessed by coronary angiography at Oulu University Hospital within 6 mo of inclusion. All the subjects gave informed consent before participating. This sub-study of the ARTEMIS project was approved by the Ethics Committee of Oulu University Hospital and was deemed to comply with the Declaration of Helsinki. To maximize the likelihood of emotional arousal, 162 candidates were interviewed by telephone about their devotion to spectator sports and the most preferred sports event. Inclusion criteria were a self-reported high level of excitement during major spectator sports events in CAD patients, because it is associated with reduced ET-1 release during emotional excitement in coronary artery disease patients.
enthusiastic ice hockey fans were chosen for the present investigation (55 males) based on their interest in ice hockey during the season (Fig. 1). Ice hockey is the most important and most passionately followed team sport in Finland. Two patients were excluded from the final analyses because of a change in cardiac medication between the match and the exercise test, and two patients did not participate in the exercise test (n = 51, all males).

Protocol. The subjects were spectators at Finnish National Ice Hockey League play-off matches held in the city of Oulu, Finland, in 2008, 2009, and 2011. The characteristics and clinical data of the patients are shown in Table 1. The subjects watched the match inside a private balcony of the ice hockey arena with an excellent view of the rink and a constant temperature of 20°C. A baseline blood sample was collected before the match with the patient in a sitting position, and a second sample was collected in the private balcony of the ice hockey arena 1.5 h after the beginning of the match, close to the second break. Subjective experience of excitement was assessed with a questionnaire (1 value) during the match at the same time with blood sample (0 = none, 1 = very low, 2 = low, 3 = medium, 4 = high, 5 = very high excitement).

Maximal exercise test and echocardiography. After the match days, all the subjects performed a maximal bicycle exercise stress test, which started at 30 W, followed by an incremental protocol with the work rate increasing at a rate of 15 W every minute. The CAD patients were encouraged to reach a symptom-limited maximal workload (METs). Maximal ST-segment depression from 12-lead ECG was used as a measure of ischemic changes at the time of exercise. Myocardial ischemia certainly limits the exercise capacity and may play a major role in the results. This was a stable coronary artery disease patient’s population on optimal medication, and there were very few significant ST-segment depressions (none over 0.2 mV) as a sign of myocardial ischemia, which could potentially limit the exercise capacity. An experienced cardiologist performed two-dimensional echocardiography (Vivid 7; GE Healthcare) where the left ventricular ejection fraction (LVEF) was measured from a 4-chamber view according to the American Society of Echocardiography’s recommendations. There was always a time delay, which was less than 6 mo (median, 2.5 mo) between the measurement of LVEF and the ice-hockey match and less than a week between the match and the exercise test (median, 2.3 days).

Heart rate variability. A 24-h ECG recording was performed on all attending subjects on the match day and during a reference day within 1 wk after the match. Heart rate variability was analyzed during the match hours (3 h from the beginning of the match) and at the corresponding time of the day of the reference measurement. Average heart rate and standard deviation of normal to normal R-R intervals (SDNN) were analyzed from R-R interval data using standard methods (2).

Blood collection, sample preparation, and laboratory analysis. Blood samples for determination of plasma ET-1 and catecholamines were collected by venipuncture from the cubital vein via a 20-gauge needle (Venoeject; Terumo Medical, Somerset, NJ) connected to evacuated tubes. The first 5 ml of blood were discarded. Samples were collected in a sitting position. The baseline samples were collected after a quiet steady period of 3–5 min. To avoid any confounding effects, considerable attention was paid to proper blood collection, handling, and storage. All the laboratory analyses were performed in high-quality national reference laboratories.

Analysis of ET-1, IL-6, and catecholamines. The concentrations of ET-1 and IL-6 were determined from serum samples. Serum was prepared by allowing the blood to clot for 30 min, followed by centrifugation at 2,000 g for 10 min. The serum was stored at −20°C until analyzed. ET-1 levels were analyzed using a sandwich ELISA (QuantiGlo Chemiluminescent Immunoassay; R&D Systems, Minneapolis, MN) with a sensitivity of 0.064 pg/ml. Serum IL-6 levels were also analyzed using a sandwich ELISA (Quantikine High Sensitivity Immunoassay; R&D Systems) with a sensitivity of 0.039 pg/ml.

Catecholamines (norepinephrine and epinephrine) were determined from blood samples collected in 10-ml plastic tubes containing EGTA and reduced glutathione as a preservative and placed on ice. The samples were centrifuged immediately, and the plasma was stored frozen at −70°C until analyzed. The epinephrine and norepinephrine were analyzed by high-performance liquid chromatography with electrochemical detection (plasma catecholamine reagent kit and column from Chromsystems GmbH, Munich, Germany). Absolute recovery of catecholamines was 70–72% and analytical recovery was 96–99%, the linear range of the method was 0.06–40.0 nmol/l, intra-assay variation was 4.1–6.7% for norepinephrine and 3.5–8.5% for epinephrine, and inter-assay variation was 7.1–7.2% for norepinephrine and 7.6–10.1% for epinephrine.

Statistical analysis. Statistical methods were used to calculate means and standard deviations. Normal Gaussian distribution in the data was verified with the Kolmogorov-Smirnov goodness-of-fit test (z value < 1.0). A paired t-test or Wilcoxon test was used when appropriate to study the change of different parameters from baseline to the match. Because some of the data were skewed (ET-1 and IL-6), Spearman’s correlation analysis was used to study associations between the maximal METs and the different parameters at baseline and during the match and responses from baseline to the match. A linear stepwise regression analyses were used to explain ET-1, IL-6, catecholamines, heart rate, and SDNN at rest and their changes during emotional excitement by adjusting for relevant covariates [maximal exercise capacity, age, body mass index (BMI), and LVEF] and also for the resting value of dependent variable and subjective experience of excitement when the stress responses were examined. The data were analyzed using IBM SPSS Statistics 21.0 (IBM, Somers, NY). A P value < 0.05 was considered statistically significant.
RESULTS

Demographics and maximal exercise test. The age of the subjects was 59 ± 9 years; BMI, 29 ± 4 kg/m²; maximal load, 170 ± 39 W; and maximal METs, 6.9 ± 1.7. Maximal ST-depression during the exercise test was 0.082 ± 0.089 mV, and ST-depression was more than 0.1 mV in 19 patients. Ten CAD patients reported low, 20 medium, 19 high, and two very high subjective experience of excitement during the match. The clinical data of the subjects are presented in Table 1.

Heart rate dynamics, catecholamines, IL-6, and ET-1. Heart rate increased (68 ± 11 vs. 76 ± 13 beats/min; \( P < 0.001 \)) and SDNN decreased (77 ± 25 vs. 65 ± 22 ms; \( P = 0.001 \)) from baseline to the match. ET-1 (2.81 ± 0.18 vs. 2.93 ± 0.24 pg/ml; \( P < 0.001 \)), IL-6 (1.94 ± 1.94 vs. 2.77 ± 2.33 pg/ml; \( P < 0.001 \)), and norepinephrine increased (4.38 ± 1.79 vs. 4.77 ± 1.75 nmol/l; \( P = 0.015 \)), but epinephrine did not change (0.33 ± 0.19 vs. 0.39 ± 0.25 nmol/l; \( P = 0.176 \)) from baseline to the match.

Association between exercise capacity and ET-1. Maximal METs correlated with circulating ET-1 at rest (\( r = -0.43; P = 0.002 \)) and during emotional excitement (\( r = -0.52; P < 0.001 \)). The change in ET-1 from baseline to emotional excitement was used as a dependent variable and age, BMI, METs, LVEF, basal ET-1, and subjective experience of excitement were used as independent variables. METs, BMI, and LVEF correlated with ET-1 response (\( R = 0.52; P = 0.002 \) for the model). The strongest association was observed between ET-1 response and METs (\( \beta = -0.45 \); partial correlation \( r = -0.43; P = 0.002 \)), followed by BMI (\( \beta = -0.31; \) partial correlation \( r = -0.31; P = 0.033 \)) and LVEF (\( \beta = -0.30; \) partial correlation \( r = -0.33; P = 0.023 \)).

Association between exercise capacity and IL-6. Maximal METs correlated with circulating IL-6 at rest (\( r = -0.55; P < 0.001 \)) and during emotional excitement (\( r = -0.42; P < 0.002 \)) but not with the change from baseline to the match (\( r = 0.13; P = 0.138 \)). In a linear regression analysis with basal LVEF as independent variables, only BMI correlated with basal ET-1 (\( r = 0.48; P < 0.001 \)). When the change in ET-1 from baseline to emotional excitement was used as a dependent variable, and age, BMI, METs, LVEF, basal ET-1, and subjective experience of excitement were used as independent variables, METs, BMI, and LVEF correlated with ET-1 response (\( R = 0.52; P = 0.002 \) for the model). The strongest association was observed between ET-1 response and METs (\( \beta = -0.45 \); partial correlation \( r = -0.43; P = 0.002 \)), followed by BMI (\( \beta = -0.31; \) partial correlation \( r = -0.31; P = 0.033 \)) and LVEF (\( \beta = -0.30; \) partial correlation \( r = -0.33; P = 0.023 \)).
IL-6 as a dependent variable and age, BMI, METs, and LVEF as independent variables, only maximal METs correlated with basal IL-6 (β = −0.37; partial correlation r = −0.30; P = 0.038). There was no correlation when the change in IL-6 from baseline to emotional excitement was used as a dependent variable and age, BMI, METs, LVEF, basal IL-6, and subjective experience of excitement were used as independent variables (R = 0.47; P = 0.098). Maximal METs did not correlate with the responses in any other variables (Table 2).

**DISCUSSION**

The main finding of the present study is that maximal exercise capacity is moderately (4) associated with ET-1 response to emotional excitement in CAD patients. Good exercise capacity attenuated ET-1 response to emotional excitement in a real-life study design. The association was more obvious after adjustment with appropriate covariates including age, BMI, LVEF, basal level of ET-1, and subjective experience of excitement. The association between exercise capacity and circulating ET-1 responses to emotional excitement may partly explain the well-known cardioprotective evidence of exercise capacity in large epidemiological studies (16, 17). The present findings also support the concept of the “cross-stressor adaptation” hypothesis, particularly concerning the association between exercise capacity and vasoconstriction during emotional excitement in patients with CAD. Second, acute inflammatory, catecholamine, and autonomic responses to emotional excitement were not associated with maximal exercise capacity.

**Methodological considerations.** Previous reports have concentrated on investigating the association between exercise capacity and physiological responses to mental stress, such as a frustrating computer task, a public speech, or arithmetic tests in a laboratory environment. The most commonly used markers of stress responses to a mental challenge have been heart rate (5a–7, 10–12, 33, 34, 36), heart rate variability (7, 9, 11, 36), blood pressure (6, 7, 10, 11, 33, 34, 36), catecholamines (6), and more recently also inflammation markers like IL-6 (11). The subjects in previous studies have been children (5a, 34), healthy young men and women (6, 7, 12, 33, 36), healthy middle-age and elderly subjects (9, 11), and subjects with hypertension without any medication (10). Finally, previous study designs have mostly focused on stressful tasks that are relatively short (5–10 min). Therefore, there are several fundamental methodological differences between previous studies and the present study design. First, our study design represents real-life and long-lasting (3 h) emotional excitement during a thrilling sports event among enthusiastic sports fans. Most important, our subjects were angiographically documented CAD patients whose stress reactions to emotional excitement, particularly ET-1 and IL-6, are known to be more marked than in age-matched healthy subjects despite the patients’ optimal medication (31). Finally, the present study is the first one where the association between exercise capacity and ET-1 response to emotional excitement was studied.

**Exercise capacity and ET-1.** ET-1 shows a terminal elimination half-life of several hours (28), making it an ideal biomarker for studying stress reactions during long-lasting real-life conditions like in the present study design. Circulating ET-1 is the most potent endogenous vasoconstrictor in the human body, produced by vascular endothelial cells (45). ET-1 levels increase during mental stress in healthy subjects (31) and in various clinical populations with cardiac (8, 32) and peripheral atherosclerotic disease patients (21). In our recent study ET-1 increased during emotional excitement even more markedly in CAD patients than in healthy age-matched controls (31). ET-1 is a very powerful vasoconstrictor affecting both the peripheral and coronary circulations, and there is evidence that it induces a long-lasting constriction of coronary arteries, making it an ideal candidate for initiation and maintenance of a coronary artery spasm (23). ET-1 levels have also been shown to be higher in patients who suffer from acute coronary syndrome during emotional stress than in acute coronary syndrome patients without emotional triggers (44). Elevated ET-1 has also been linked to plaque rupture and subsequent acute coronary syndromes (14, 40).

The novel finding of the present study is that ET-1 release during emotional excitement is moderately associated with exercise capacity in CAD patients. ET-1 is released in response to various stimuli, including increases in pulsatile stretch (19), shear stress (20), and hypoxia (15). In our previous study, beat-to-beat blood pressure measurements showed that blood pressure is rapidly and markedly increased and decreased within tens of seconds during emotional excitement in CAD patients (30). The changes in blood pressure occurred rapidly during exciting periods of the match without any body movement or other confounding factors (30). These rapid changes in excitement are typical in team sports like ice hockey and soccer and could be difficult to mimic in laboratory conditions, underlining the unique study design of the present study. The rapid changes in blood pressure are most probably due to neural sympathetic activation and/or norepinephrine pulsation.

### Table 2. Correlation between maximal exercise capacity and stress markers at rest (adjusted with age, body mass index, and LVEF) and their responses (change from baseline to the match) to the emotional experience (adjusted with age, body mass index, LVEF, basal value of each marker, and subjective experience of excitement)

<table>
<thead>
<tr>
<th>Dependent Variables</th>
<th>Endothelin-1</th>
<th>IL-6</th>
<th>Norepinephrine</th>
<th>Epinephrine</th>
<th>Heart Rate</th>
<th>SD of Normal to Normal R-R Intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Exercise capacity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>−0.43†</td>
<td>−0.55‖</td>
<td>−0.03</td>
<td>0.01</td>
<td>0.06</td>
<td>0.38*</td>
</tr>
<tr>
<td>Response</td>
<td>−0.30*</td>
<td>0.19</td>
<td>0.03</td>
<td>−0.04</td>
<td>−0.13</td>
<td>−0.24</td>
</tr>
<tr>
<td><strong>Exercise capacity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest, adjusted</td>
<td>−0.04</td>
<td>−0.30*</td>
<td>−0.16</td>
<td>0.05</td>
<td>−0.36*</td>
<td>0.19</td>
</tr>
<tr>
<td>Response, adjusted</td>
<td>−0.52‡</td>
<td>−0.26</td>
<td>0.06</td>
<td>0.12</td>
<td>−0.01</td>
<td>−0.15</td>
</tr>
</tbody>
</table>

*P < 0.05; †P < 0.01; ‡P < 0.001.
(half-life, $\sim 1$ min) rather than via the ET-1 pathway, since an elevated ET-1 level is the result of long-term vasoconstriction ($>30$ min) (29). The rapid and marked changes in blood pressure increases pulsatile stretch and shear stress, and this could result in the elevated ET-1 release observed in the present study.

Exercise capacity is limited by various factors, including vascular stiffness in cardiovascular diseases in particular (1, 3, 37). It could be speculated that vascular stiffness is the key component explaining the association between low exercise capacity and increased ET-1 release during emotional excitement in the present study. High vascular stiffness limits exercise capacity and, on the other hand, results in high pulsatile stretch/shear stress and leads to elevated ET-1 release during emotional excitement. Furthermore, BMI and LVEF were associated with ET-1 response to emotional excitement. Both obesity (42, 43) and LVEF (43) are associated with vascular stiffness, further supporting the present hypothesis that vascular stiffness is the key mechanism explaining the association between low exercise capacity and high ET-1 release during emotional excitement.

Exercise capacity and other stress markers. A majority of studies have shown that exercise capacity or exercise conditioning is associated with mental stress responses including heart rate (7), heart rate variability (5a, 7, 10–12), blood pressure (10, 34), and inflammatory markers (11). There are also studies where the association between exercise capacity and mental stress responses is lacking (6, 9, 33, 36). There is no a common denominator that could explain the differences between study findings; for example, the age of the study subjects has varied from children to elderly and the mental challenges are different in almost every laboratory. Hamer et al. (11) showed that physical fitness is associated with inflammatory responses to short-term mental stress, documented by circulating IL-6 and TNF-α. The subjects were healthy middle-age men and women from the Whitehall II epidemiological cohort, and the mental challenge lasted 10 min in laboratory condition. In the present study, exercise capacity was associated with circulating IL-6 at baseline but not with IL-6 response. It is highly possible that cardiac medication is masking the role of exercise capacity in the IL-6 response. Furthermore, the responses in catecholamines or the global heart rate variability index (SDNN) were not associated with exercise capacity. This is not surprising in the present study population with various cardiac medications targeting reduced sympathetic outflow, including β-blockades, in all the subjects.

Limitations. There are certain limitations in the present study design. For practical reasons, sample collection during the match was scheduled for the second break, which by no means represents a moment of maximal emotion. However, because the half-life of ET-1 and IL-6 is several hours, the values represent the total stress burden. There are also many other pro-inflammatory and vasoconstrictive markers in addition to ET-1 and IL-6, which may play a role in stress-mediated coronary events. We nevertheless decided here to analyze only these two markers because of the widespread evidence of their role as triggers of adverse cardiovascular events. Subjective experience of excitement was assessed with a questionnaire. We are aware that this method of assessing emotional excitement is subjective and more inaccurate than, for example, a continuous measure on skin conductance. However, this was quite a heavy protocol for the patients in the ice-hockey hall, and it was not practically possible to include any other devices or measurements during the ice-hockey match. Finally, we had only male subjects in the present study and the results may be different in females, since we know that there are differences in physiological responses to mental stress between men and women.

Conclusion. Exercise capacity may protect against further cardiovascular events in CAD patients, because it is associated with reduced ET-1 release during emotional excitement.

ACKNOWLEDGMENTS
Pirkko Huikuri, Päivi Koski, Sari Kaarlenkaski, and Päivi Kastell are acknowledged for excellent technical assistance.

GRANTS
This study was supported financially by the Finnish Red Cross Blood Service Research Fund; the Finnish Funding Agency for Technology and Innovation, Helsinki, Finland; and the Oulun Kärpät team, which provided the facilities.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

REFERENCES
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PHYSICAL FITNESS AND MENTAL STRESS


