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Aerobic exercise modulation of mental stress-induced responses in cultured endothelial progenitor cells from healthy and metabolic syndrome subjects

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ABSTRACT

Aim: Numerous studies have demonstrated that exercise acutely prevents the reduction in flow-mediated dilation induced by mental stress in subjects with metabolic syndrome (MetS). However, it is unknown whether a similar effect occurs in endothelial progenitors cells (EPCs). This study investigated whether exercise protects from the deleterious effect of mental stress on cultured EPCs in healthy subjects and those with MetS.
Main methods: Ten healthy subjects (aged 31 ± 2) and ten subjects with MetS (aged 36 ± 2) were enrolled. Subjects underwent a mental stress test, followed immediately by either 40 min of leg cycling or rest across two randomized sessions: mental stress + non-exercise control (MS) and mental stress + exercise (MS + EXE). The Stroop Color-Word Test was used to elicit mental stress. Blood samples were drawn at baseline and following sessions to isolate mononuclear cells. These cells were cultured in fibronectin-coated plates for seven days, and EPCs were identified by immunofluorescence ($acLDL^+$/ UEA-I $Lectin^+$).
Key findings: All subjects presented similar increases in mean blood pressure and heart rate during the mental stress test ($P < 0.01$) in both the MS and MS + EXE sessions. Number of EPCs was not different between groups at baseline in both sessions ($P > 0.05$). The EPC response to MS and MS + EXE was increased in healthy subjects, whereas it was decreased in subjects with MetS ($P < 0.04$). In healthy subjects, the EPC response to MS + EXE was greater than the response to MS alone ($P = 0.03$).
Significance: An exercise session increased EPCs in healthy subjects but did not prevent the EPC reduction induced by mental stress among subjects with MetS.

Introduction

Metabolic syndrome (MetS) is a constellation of metabolic risk factors, including high blood pressure, hyperglycemia, dyslipidemia and abdominal obesity. When these risk factors present together, the probability for future cardiovascular problems becomes greater than that of any one factor alone [1]. Subjects with MetS have a two-fold increased risk for heart attack or stroke, and a five-fold increased risk for developing diabetes when compared with individuals who do not have metabolic syndrome [1].

MetS is also associated with endothelial dysfunction, as evidenced by reduced flow-mediated dilation [2,3]. Endothelial dysfunction is a systemic pathological state characterized by an imbalance between mediators of vasodilation and vasoconstriction produced by (or acting on) the endothelium [4]. These alterations can increase both inflammatory markers and the production of reactive oxygen species from the endothelium, contributing to atherogenesis [5].

Endothelial repair can occur by the proliferation of surrounding mature endothelial cells [6]. However, mature endothelial cells have a low proliferative potential, and their capacity to replace damaged endothelium is limited [6]. Therefore, endothelial repair depends on other cell types. Accumulating evidence indicates that the peripheral blood of adults contains a unique subtype of circulating endothelial progenitor cells (EPCs) with similar properties to embryonic angioblasts [7,8]. Recruitment of EPCs requires a coordinated sequence of signaling events, including chemoattraction, migration and adhesion. Jialal et al. [9] demonstrated that the mobilization and migration of EPCs is usually impaired in subjects with MetS. However, it is unclear whether the adhesion capacity of EPCs is preserved in MetS.

In addition to MetS, psychosocial disorders such as mental stress represent important risk factors for cardiovascular morbidity and mortality [10]. Chronic exposure to stress is usually associated with endothelial dysfunction [11–13] and the depletion of circulating EPCs

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in healthy subjects [14]. This deleterious process on endothelial function begins during mental stress, can last up to 90 min [11], and its magnitude is considered clinically meaningful [15]. Consequently, preventing this process might be crucial for cardiovascular health protection, particularly among subjects with a cluster of risk factors, such as those with MetS [16,17].

Lower-limb aerobic exercise acutely improves endothelial function [18,19] and prevents a reduction in the brachial artery flow-mediated dilation induced by mental stress among subjects with cardiometabolic risk factors [3]. This type of exercise is also capable of modulating hemodynamic responses (i.e., blood pressure, stroke volume and cardiac output) and mental stress in healthy and pre-hypertensive subjects [20,21]. As such, it is expected that a single bout of exercise would protect the number and function of EPCs from the deleterious effects of mental stress; however, this hypothesis currently remains untested.

This study aimed to investigate the effect of mental stress followed by a bout of exercise on cultured EPCs in both healthy subjects and those with MetS. We hypothesized that exercise prevents a reduction in the EPC response to mental stress in subjects with MetS.

Materials and methods

Ethics statement

All experimental procedures and protocols were consistent with the principles of the Declaration of Helsinki, and were approved by the Institutional Review Boards of the Fluminense Federal University (CAAE 006.0.258.000-10). All subjects provided written informed consent before the study and their rights were protected.

Subjects

Subjects were recruited through advertisements at the University and in local newspapers. Twenty subjects were enrolled, ten subjects with early MetS (MetS group, age: 36 ± 2) and ten healthy subjects (controls) with none of the five criteria for MetS (Healthy group, age: 31 ± 2). The MetS group presented at least three of the following five criteria defined by the American Heart Association [17]: waist circumference >90 cm (men) or >80 cm (women); systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥85 mmHg; fasting blood glucose ≥100 mg.dL^{-1}; plasma triglycerides ≥150 mg.dL^{-1}; high-density lipoprotein cholesterol (HDL-c) <40 mg.dL^{-1} (men) or <50 mg.dL^{-1} (women). Other inclusion criteria included the absence of any diagnosed disease, no recent infection, no medication, non-smoker, women with regular menstrual cycles, and maintaining a sedentary lifestyle (not engaged in exercise activities lasting ≥30 min for at least three times weekly during the last three months). Women who had regular menstrual cycles were evaluated during the early follicular phase (up to 5 days after the onset of menstruation). The eligibility requirements were verified through a clinical history assessment, physical examination, blood pressure measurement, biochemical blood analyses, resting electrocardiogram, and peak cardiopulmonary exercise testing.

Biochemical blood analyses

Blood was drawn in the morning from an anterior cubital vein following a 12 h fast. Cholesterol and its subfractions [HDL-c and low-density lipoprotein (LDL-c)], as well as circulating triglycerides and glucose were determined using enzymatic colorimetric methods. Total leukocyte count was measured by an electronic counter, the HST-302 N system (Sysmex Corporation, Kobe, Hyōgo Prefecture, Japan).

Clinical evaluation

A physician conducted the evaluation, which included a clinical history assessment and a resting electrocardiogram (CardioCare 2000, Bionet, Tustin, CA, USA). With the participant in an upright sitting position, resting blood pressure measurements were performed twice, one on each arm, on two separate days. Recordings were made under quiet and comfortable (approximately 24 °C) laboratory conditions. An appropriately sized cuff (cuff bladder encircling at least 80% of the arm) was used.

Physical examination

Weight and height were measured using a medical beam balance (Welmy, Santa Bárbara d'Oeste, SP, Brazil). Body mass index (BMI) was calculated as weight (kg) divided by the square of the height (m). Waist circumference was considered as the midpoint between the iliac crest and the last floating rib (XII rib).

Cardiopulmonary exercise testing

Subjects underwent a standard cardiopulmonary exercise test, performed until exhaustion on a cycle ergometer (CG400 model, Inbrasport, Porto Alegre, RS, Brazil) [3]. The protocol was individualized according to predicted maximal exercise capacity. Subjects were verbally encouraged to exercise until exhaustion to reach volitional fatigue at approximately 10 min of testing. Ventilation, oxygen uptake, and carbon dioxide output were measured with each breath (CPX Ultima Gas Exchange System, Medgraphics Corp, St Paul, MN, USA). An electrocardiogram was monitored through 12 leads (Welch Allyn CardioPerfect Workstation, Welch Allyn, Skaneateles Falls, NY, USA), and perceived exertion was assessed every minute using the 0–10 Borg scale. Breath-by-breath ventilation and expired gas data were averaged every 20 s to identify the peak oxygen consumption (VO_2peak), which was considered the highest value of oxygen uptake recorded during exercise. The ventilatory threshold was identified by a combination of the following methods: 1) Inflection of ventilation vs. time curve and 2) A consistent increase in the ventilatory equivalent of oxygen (VE/VO_2) without a concomitant increase in the ventilatory equivalent of carbon dioxide (VE/VCO_2).

Experimental protocol

On two separate days, at least two days apart, subjects from both the healthy and MetS groups underwent a mental stress + non-exercise control session (MS) and a mental stress + exercise session (MS + EXE) in random order (Fig. 1). Both sessions began with a blood draw for EPC isolation. Then, a mental stress test was induced, and the subjects underwent either a non-exercise control period or a bout of exercise. A blood draw was repeated after the control period or bout of exercise. During the MS session, subjects sat still on the cycler ergometer for the same period of time as the MS + EXE session. These experimental sessions were always conducted at the same time of day after a 1-h fast. Participants were also given standard feeding orientations for the previous day and abstained from caffeine and alcohol consumption, as well as physical exercise for at least 48 h.

Mental stress test

Mental stress was elicited over a 3-min period using an adapted, computerized version of the Stroop Color-Word Test [22]. The test consisted of a slideshow projected on the ceiling in front of the subjects. The slides changed every 2 s. Auditory conflicts were continuously delivered via earphones using a standardized audio clip integrated into the slideshow. Perceived stress level was recorded after each test