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An active lifestyle induces positive antioxidant enzyme modulation in peripheral blood mononuclear cells of overweight/obese postmenopausal women

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ABSTRACT

Aims: The aim of this study was to investigate the effects of an active lifestyle on mitochondrial functioning, viability, bioenergetics, and redox status markers in peripheral blood mononuclear cells (PBMC) of overweight/obese postmenopausal women.
Materials and methods: We performed a cross-sectional study with postmenopausal women aged 45–64 years and body mass index > 25 kg/m^2, divided into physically active (n = 23) and sedentary (n = 12) groups. Mitochondria functioning and viability, bioenergetics and redox status parameters were assessed in PBMC with spectrophotometric and fluorometric assays.
Key findings: No differences were found in the enzyme activity of complexes I and II of the electron transport chain (ETC), mitochondrial superoxide dismutase (MnSOD) activity, methyl-tetrazolium reduction levels and reduced glutathione and oxidized glutathione levels between the groups. However, the physically active group presented higher levels of reactive oxygen species (ROS) (P = 0.04) and increased catalase (CAT) (P = 0.029), total (P = 0.011) and cytosolic SOD (CuZnSOD) (P = 0.009) activities.
Significance: An active lifestyle that includes aerobic exercise for at least 30 min, three times per week may improve antioxidant enzyme activities in PBMC in overweight/obese postmenopausal women, without changes in the activity of the ETC enzymes. However, this low intensity physical activity is not able to induce relevant mitochondrial adaptations.

Introduction

Ovarian function decline is associated with spontaneous increases in inflammation conditions, risk of atherosclerosis development, weight gain and bone loss in postmenopausal women [38]. On the same line, the combination of menopause and physical inactivity also increases cardiovascular risks factors, such as obesity, hypertension, dyslipidemia, oxidative stress and persistent inflammation [29,30]. Regarding these, recent data from the World Health Organization (WHO) demonstrated that at least 2.8 million people die yearly throughout the world as a result of being overweight or obese [3]. Similarly, a meta-analysis demonstrated that each 5 kg/m² increase in body mass index (BMI) is associated with 12% increased risk of postmenopausal breast cancer [40], representing a major challenge to health care systems. As such, obesity in menopause has become a public health concern in America considering its increased prevalence after 40 years of age, reaching a 65% prevalence between 40–59 years of age, and outstanding 73.8% in over 60 years old women [22].

The peripheral blood mononuclear cells (PBMC), which include lymphocytes and monocytes, are responsible for energy-related homeostatic and specialized activities, such as cell growth and cytokinesis, dependent on energy supply [32]. In this line, disorders in energy homeostasis maintenance and altered redox state in PBMC have been connected to different metabolic- and cardiovascular-related conditions, such as obesity, diabetes, atherosclerosis, coronary artery disease and stroke [6,14,36]. With regard to obesity, it is suggested that the mitochondria plays a central role in individual susceptibility to obesity via the mitochondrial–nuclear interaction process [18]. Moreover, it is known that oxidative stress is induced by estrogen depletion in the postmenopausal process, since this hormone presents antioxidant properties [17]. Fortunately, the PBMC redox state has been shown to

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be modulated by environmental factors, such as exercise [9,23], although the effects of regular exercise on redox status-related markers in PBMC of postmenopausal women have not been reported yet. Therefore, this study was designed to evaluate the effects of an active lifestyle on bioenergetics, redox status and antioxidant enzyme activities in PBMC of overweight/obese postmenopausal women.

Materials and methods

Study population and design

Volunteers were recruited through advertisements on the Universidade Federal de Santa Maria main campus, in the institutional website and in local newspapers. Thirty-five postmenopausal women aged 45–64 years and with body mass index (BMI) of >25 kg/m^2 were classified as sedentary (n = 12) or active (n = 23). Physical activity status was determined by self-reported ≥30 min of moderate-intensity aerobic exercise (e.g., swimming, brisk walking, jogging, cycling, hiking, dancing) resulting in mild perspiration and increased respiratory rates over three times per week in the last three months as active [4,12]. Likewise, sedentary participants were classified as sedentary with self-reported ≤15 min of moderate aerobic exercise per week [4]. Volunteers signed an informed written consent before enrolment in the study, which was approved by the Ethics Committee of Universidade Federal de Santa Maria (Permit number 0032.0.243.000–07). The ethical standards set forth in the Declaration of Helsinki for clinical settings have been respected throughout the study.

Menopause was defined as the self-reported absence of menses for at least 12 months [37]. A medical history screening was performed and volunteers with renal, liver, heart diseases, recent infections, muscular or joint disability, and also those taking hormonal replacement therapy or antioxidant supplementation were excluded from the study.

Anthropometric and functional tests

Body mass index and height, waist and hip circumferences were evaluated by standardized protocols. BMI was calculated by dividing weight in kilograms by the height in meters squared. Systolic and diastolic blood pressure was assessed by a digital sphygmomanometer (Omron, Kyoto, Japan) after the participants sat quietly in a padded chair for 5 min. Cardiorespiratory fitness was assessed by a progressive load treadmill test (Inbramed KT 10.100 ATL, Porto Alegre, Brazil), according to Bruce's modified protocol [11]. Participants were verbally encouraged to perform the maximum effort during the test. A computerized gas analyzer (MedGraphics Cardiorespiratory Diagnostic Systems, Saint Paul, USA) was used to determine maximum oxygen uptake (VO_2max), which was calibrated before each test. VO_2max was determined by attaining at least three of the following criteria: (1) respiratory exchange ratio greater than 1.1; (2) heart rate over 90% of age-predicted maximum; (3) a plateau in VO_2 with increasing workload and/or (4) volitional fatigue [19].

Blood sampling and PBMC separation

Participants were asked to fast (water *ad libitum*) for 12 h and refrain from caffeinated beverages and exercise in the 48 h prior to drawing of blood. The blood samples were taken in the morning (07:00–08:30 a.m.) to avoid the confounding diurnal variation of oxidative stress biomarkers in white cells as previously registered [10]. Blood samples were collected from the antecubital vein into 4 mL ethylenediamine tetraacetic acid (EDTA) tubes and routinely centrifuged. The PBMC separation was performed immediately after blood collection and the plasma was aliquoted and frozen during the assay. The PBMC were separated from whole blood by density gradient centrifugation with a Histopaque®-1077 solution (Sigma-Aldrich, St. Louis, USA), as previously described [9] with brief modifications. For each sample, four 15-mL centrifuge tubes were used to layer 16 mL of blood onto 8 mL of Histopaque®-1077. The suspension was centrifuged for 30 min at 225 ×*g* at room temperature. The layer of PBMC was removed by manual pipetting, washed one time in phosphate buffered saline (PBS) (136 mM NaCl, 2.7 mM KCl, 7.8 mM Na_2HPO_4, 1.7 mM KH_2PO_4; pH 7.4) and centrifuged for 10 min at 450 ×*g*. After, cell supernatants were discarded and the PBMC pellets were dried out with lysing solution (150 mM NH_4Cl, 10 mM $NaHCO_3$, 1 mM EDTA), and after, centrifuged for 3 min at 300 ×*g*. The pellets were frozen at −80 °C for further analysis.

Peripheral blood mononuclear cells assays

Electron transport chain enzymes

The samples were frozen and thawed five times to fully expose the enzymes to substrates and achieve maximal activities. The activity of complex I (NADH dehydrogenase) was measured by following the oxidation of NADH [7,26]. PBMC supernatants were added to a solution containing a 35 mM potassium phosphate buffer (pH 7.4) and 0.065 mM 2,6-dichloroindophenol (DCIP) in a final volume of 1 mL. The reaction was initiated with the addition of 0.15 mM NADH. Absorbance at 600 nm was monitored for 3 min to follow the NADH oxidation rate, and the activity was determined using an extinction coefficient of 6.22 mM^{-1} cm^{-1}. The activity of complex II (succinate dehydrogenase) was determined by following the reduction of DCIP by succinate [21,26]. The reaction mixture consisted of a 50 mM potassium phosphate buffer pH 7.0, 1 mM KCN, 0.05 mM DCIP, 16 mM succinate and 30 μL of PBMC supernatants. Absorbance changes were followed at 600 nm for 3 min, using an extinction coefficient of 19.1 mM^{-1} cm^{-1} for DCIP.

Methyl-tetrazolium (MTT) reduction levels

The most MTT reduction involves extramitochondrial NADH and NADPH-dependent mechanisms, being an index of the dehydrogenase enzymes functions implicated in cellular viability [5]. The MTT assay was carried out as previously described [5] with slight modifications. Resuspended PBMC were incubated in a buffer containing succinate (5 mM) and MTT (0.5 mg/mL) for 30 min at 37 °C. MTT reduction reaction was stopped by the addition of 1 mL of dimethylsulfoxide (DMSO). The formed formazan (purple) levels were determined spectrophotometrically, reported as the difference in absorbance between 570 and 630 nm and corrected by the protein content.

Estimation of ROS production

Reactive oxygen species (ROS) production was estimated in PBMC with the fluorescent probe, 2′,7′-dichlorofluorescein diacetate (DCFH-DA) as previously described [13,16]. After PBMC resuspension in PBS, aliquots of 2.5 mL were incubated in the presence of DCFH-DA (5 μm) at 37 °C for 60 min. The DCFH-DA is enzymatically hydrolyzed by intracellular esterases to form nonfluorescent DCFH, which is then rapidly oxidized to form highly fluorescent 2′,7′-dichlorofluorescein (DCF) in the presence of ROS. DCF fluorescence intensity is proportional to the amount of ROS formed. Fluorescence was measured using excitation and emission wavelengths of 480 and 535 nm, respectively. A calibration curve was established with standard DCF (0.1 nm to 1 mm), and ROS levels were expressed as nmol/mg of protein.

Reduced (GSH) and oxidized glutathione (GSSG) content

GSH and GSSG levels were determined with fluorescence detection after reaction of the deproteinized supernatants containing H_3PO_4/NaH_2PO_4-EDTA or H_3PO_4/NaOH-EDTA, respectively, with *O*-phthalaldehyde (OPT) [28]. In brief, PBMC were resuspended in 700 μL phosphate buffer (100 mM NaH_2PO_4, 5 mM EDTA, pH 8.0) and 250 μL H_3PO_4 4.5% and were rapidly centrifuged at 100,000 ×*g* and 4 °C (Hitachi TL-100 ultracentrifuge, Tokyo, Japan) for 30 min. For GSH determination, 100 μL of supernatant was added to 1.8 mL phosphate buffer and 100 μL OPT. After thorough mixing and incubation at room temperature for 15 min, the solution was transferred to a quartz cuvette