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Effect of aerobic interval training on erythrocyte rheological and hemodynamic functions in heart failure patients with anemia

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ABSTRACT

Background: Anemia disturbs hemorheological/hemodynamic properties, whereas aerobic interval training (AIT) achieves a superior aerobic fitness in patients with heart failure (HF). This study investigated whether AIT influences functional capacity by modulating hemorheological/hemodynamic functions in HF patients with/without anemia.

Methods: Sixty HF patients were divided into non-anemic (HF-NA, hemoglobin≧12 g/dL in women/≧13 g/dL in men; n = 30) and anemic (HF-A, hemoglobin<11 g/dL in women/<12 g/dL in men; n = 30) groups, and 30 normal counterparts were enrolled as a control group. These HF patients performed AIT (3-minute intervals at 40% and 80%VO_{2peak}) on a bicycle ergometer for 30 min/day, 3 days/week for 12 weeks. Erythrocyte rheological and central/peripheral hemodynamic characteristics were determined by slit-flow ektacytometer and bioreactance-based device/near infrared spectrometer, respectively.

Results: In both HF-NA and HF-A groups, the AIT regimen 1) reduced blood senescent/spherical erythrocyte counts, 2) diminished the values of critical shear stresses for disaggregation and half-maximal deformation of erythrocytes, 3) enhanced cardiac output during exercise, 4) heightened VO_{2peak} and O_2 uptake efficiency slope (OUES), and 5) decreased plasma myeloperoxidase and interleukin-6 levels. However, AIT increased the amounts of blood distributed to the frontal cerebral lobe and vastus lateralis muscle during exercise in HF-NA group but not in HF-A group. Additionally, HF-A group exhibited fewer the enhancements of VO_{2peak} and OUES caused by AIT than HF-NA group did.

Conclusion: AIT improves aerobic capacity and efficiency by depressing aggregability and enhancing deformability of erythrocytes in patients with HF. However, anemic comorbidity attenuates the adaptations of cerebral/muscular hemodynamic responses to exercise following this regimen.

1. Introduction

Anemic comorbidity is associated with an adverse prognosis in patients with heart failure (HF) [1–3]. An increased rigidity of erythrocyte has been identified in patients with anemia [4]. Patients with cardiovascular diseases were frequently accompanied by increased erythrocyte aggregability, which may heighten tendency for vascular thromboembolism [5,6]. Moreover, anemia-related hemorheological dysfunctions may lead to reduced aerobic capacity in patients with HF [7]. Hence, a feasible therapeutic strategy for improving erythrocyte rheological function needs to further develop in HF patients with anemia.

Aerobic interval training (AIT) is a more effective modality for improving functional capacity than traditional endurance training in patients with HF [8,9]. Authors' recent study further demonstrated that AIT was superior to moderate continuous training for enhancing central/peripheral hemodynamic efficiencies in patients with HF [9]. However, no study has investigated whether AIT influences the rheological properties of erythrocyte in HF patients with anemia. Moreover, the relationship between changes in erythrocyte rheological function and aerobic fitness caused by AIT has not yet been established. Elevated oxidative stress and pro-inflammatory status substantially may influence the development and progression in chronic HF [10,11]. A clinical investigation has demonstrated that enhanced erythrocyte adhesiveness/aggregation corresponded to low-grade inflammation in patients with cardiovascular disorders [12]. Some studies have showed that AIT effectively suppressed productions of peroxide and pro-inflammatory cytokine in people with metabolic syndrome or HF [9,13]. Accordingly, we hypothesize that AIT influences aerobic fitness in HF patients with

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anemia by modulating erythrocyte rheological properties, which is associated with changes of circulatory redox status.

To answer the above questions, this investigation evaluated the effects of AIT on 1) erythrocyte geometric shape and senescence, 2) erythrocyte aggregability and deformability under shear flows, 3) central (cardiac) and peripheral (cerebral/muscular) hemodynamic responses to exercise, 4) aerobic capacity and efficiency, and 5) circulatory peroxide and pro-inflammatory cytokine levels in HF patients with/without anemia. The purpose of this study was to clarify whether AIT improves aerobic fitness by enhancing hemorheological or/and hemodynamic function(s) in HF patients with/without anemia.

2. Methods

2.1. Subjects

This study enrolled 60 patients diagnosed with HF from the Department of Cardiology, Chang Gung Memorial Hospital, Taiwan. The HF was diagnosed if the patients had (i) a left ventricular ejection fraction (LVEF) ≤40% or (ii) LVEF>40% with episodes of acute pulmonary edema after excluding other non-cardiogenic etiologies. These patients with HF belonged to New York Heart Association functional classes II to III and had received optimal treatment for at least 12 months according to American Heart Association/American College of Cardiology guidelines. Exclusion criteria included the presence of atrial fibrillation/flutter, second/third degree heart block, history of life-threatening ventricular arrhythmias, recent unstable angina, myocardial infarction or coronary revascularization (<4 weeks), uncontrolled diabetes mellitus, severe chronic obstructive pulmonary disease, or symptomatic cerebrovascular disease within 12 months, collagen vascular disease, alcohol or drug abuse during the previous 12 months or significant renal or hepatic disease. Moreover, all HF patients had not received cardiac resynchronization therapy and left ventricular assist devices. Subjects were divided into the non-anemic (HF-NA, Hb≧12 g/dL in women and ≧13 g/dL in men; n=30) and anemic (HF-A, Hb<11 g/dL in women and <12 g/dL in men; n=30) groups. Additionally, normal control (NC) group was carefully selected to recruit 30 subjects who had no anemia (Hb≧12 g/dL in women and ≧13 g/dL in men) and cardiopulmonary/hematological disorders. The investigation was performed according to the Helsinki declaration, and was approved by the Institutional Review Board of Chang Gung Memorial Hospital, Taiwan. All subjects provided informed consent after the experimental procedures were explained.

2.2. Graded exercise test

Subjects performed a graded exercise test on a bicycle ergometer (Ergoselect 150P, Germany) [9,14]. Each subject was instructed to fast for at least 8 h and to refrain from exercise for at least 24 hours before the test. All subjects arrived at the testing center at 9:00 AM to eliminate diurnal effects. The exercise test comprised 2 min of unloaded pedaling followed by a continuous increase in work-rate of 10 W per minute until exhaustion (progressive exercise to peak oxygen consumption, VO_{2peak}). Minute ventilation (V_E), oxygen consumption (VO_2), and carbonic dioxide production (VCO_2) were measured breath by breath using a computer-based system (MasterScreen CPX, Cardinal-health Germany). Heart rate (HR) was determined from the R–R interval on a 12-lead electrocardiogram, mean arterial pressure (MAP) was measured using an automatic blood pressure system (Tango, SunTech Medical, UK), and arterial O_2 saturation was monitored by finger pulse-oximetry (model 9500, Nonin Onyx, Plymouth, Minnesota). The VO_{2peak} was defined by the following criteria: (i) VO_2 increased by less than 2 mL/kg/min over least 2 min, (ii) HR exceeded 85% of its predicted maximum, (iii) the respiratory exchange ratio exceeded 1.15, or (iv) some other symptom/sign limitations, as described in the guidelines of the American College of Sports Medicine for exercise testing [15]. Additionally, the O_2 uptake efficiency slope (OUES) was derived from the slope of a logarithm plot of V_E versus VO_2 [16,17]. Ventilation and VCO_2 responses, obtained during the period between the start of exercise and the peak, were used to calculate the V_E-VCO_2 slope using least squares linear regression ($y = m \cdot x + b$, m = slope) [16,17].

2.3. Exercise training

Both HF-NA and HF-A groups performed supervised hospital-based AIT regimen on a bicycle ergometer (Ergoselect 150P, Germany) for 30 min/day, 3 days/week for 12 weeks as described in our previous study [9]. These subjects warmed up for 3 min at 30% of VO_{2peak} [≈30% heart rate reserve (HRR); ≈30% ($HR_{peak} - HR_{rest}$) + HR_{rest}] before exercise for five 3-minute intervals at 80% of VO_{2peak} (≈80% HRR). Each interval was separated by a 3-minute exercise at 40% of VO_{2peak} (≈40% HRR). The exercise session was terminated by a 3-minute cool-down at 30% of VO_{2peak} (≈30% HRR). Each subject used a HR monitor (Tango, SunTech Medical, UK) to obtain the assigned intensity of exercise. Borg 6-to-20 scale was used to assess the rate of perceived exertion during and after each exercise session. The work-rate of bicycle ergometer was adjusted continuously to ensure that the intensity of exercise matched the target HR throughout the training period [9]. The rates of compliance with the HF-NA and HF-A subjects were 96.7% and 93.3%, respectively.

2.4. Cardiac hemodynamic measurements

A noninvasive continuous cardiac output monitoring system (NICOM) Cheetah Medical, Wilmington, Delaware) was used to evaluate cardiovascular hemodynamic response to exercise, which analyzes the phase shift (ΔΦ) created by alternating electrical current across the chest of the subject, as described in our previous studies [9,14,18]. Stroke volume (SV) was estimated using the following equation: $SV = C \times VET \times d\Phi/dtmax$, where C is a constant of proportionality, and VET denotes the ventricular ejection time, as determined using the NICOM and electrocardiogram signals. The CO, MAP, and total peripheral resistance (TPR) were then calculated using the following equation: CO = SV×HR; MAP = [(2×diastolic blood pressure) + systolic blood pressure]/3; TPR = MAP/CO [12,13,17].

2.5. Cerebral and muscular hemodynamic measurements

Two pairs of near infrared (NIR) probes (Oxymon, Artinis, The Netherland) were attached to each subject to monitor light absorption across the left frontal cortex region (FC) and vastus lateralis muscle (VL) during exercise testing, as described in our previous studies [9,14,18]. The Beer–Lambert law was used to calculate micromolar changes in tissue oxygenation (Δ[O_2Hb] and Δ[HHb]) using received optical densities from the two NIR wavelengths of 780 and 850 nm. Total Hb concentration (Δ[THb]) was calculated as the sum of Δ[O_2Hb] and Δ[HHb] [19]. Because Hb concentration in blood can influence the results obtained from NIR signals, an adjusted change in regional blood volume was calculated using the following equation: Δ[THb] determined by NIR spectrometry/[Hb] in blood. Since Δ[HHb] is closely associated with changes in venous oxygen content and is less sensitive to Δ[THb] than is Δ[O_2Hb], the Δ[HHb] provides a highly sensitive measure of relative tissue de-oxygenation owing to O_2 extraction [19]. Data were recorded at 10 Hz and filtered using a Savitzky–Golay smoothing algorithm before analysis.

2.6. Erythrocyte isolation and shape

Twenty mL of blood samples was transferred to polypropylene tubes that contained sodium citrate (3.8 g/dL: 1 vol. to 9 vol. of blood) (Sigma). Hematologic parameters were measured using an automatic blood cell counter (Sysmax SF-3000, GMI Inc.). Erythrocytes were isolated from whole blood by centrifugation (1000 *g* for 15 min at room temperature) followed by three washing steps in Ringer solution containing 125 mM NaCl, 5 mM KCl, 1 mM $MgSO_4$, 32 mM HEPES, 5 mM glucose and 1 mM $CaCl_2$ (osmolality = 300 mOsm/L, pH = 7.4) (Sigma). The erythrocyte count was then adjusted using Ringer solution to 1×10^4 cells/μL.

Erythrocyte shape was assessed by FACScan flow cytometric techniques (Becton Dickinson), as described a previous study [7,20]. Briefly, the FACScan histograms showed a typically bimodal distribution of erythrocytes, which reflected biconcave erythrocytes as essentially two populations of cells in the flow cytometric analysis. This study fixed two gates of interest for these histograms: R1 and R2. Two median values (M1 and M2) were calculated for each predetermined gate of interest (R1 and R2). The M2:M1 ratio termed the spherical index (SI). Additionally, the second Pearson coefficient of dissymmetry (PCD), which represented asymmetry of global histogram, was calculated using the following equation: 3×(mean − median/standard deviation A less negative PCD value or a smaller SI was associated with a more spherical shape of erythrocyte [7,20].

2.7. Iron metabolism

Serum iron and total iron-binding capacity (TIBC) were measured colorimetrically (Thermo Fisher Scientific). Transferrin saturation (TS) was calculated as a ratio of serum iron to TIBC, multiplied by 100 and expressed in %. Serum ferritin was measured by commercially available ELISA kid (Assaypro). Iron deficiency was principally defined as serum ferritin<100 μg/L or serum ferritin 100–300 μg/L with serum transferrin saturation<20% [21,22].

2.8. Reticulocyte count

The erythrocyte suspensions (1×10^4 cells/μL) were incubated with saturating concentrations of monoclonal anti-human CD71 antibody conjugated with phycoerythrin (DB Pharmingen™) and Vybrant Dyecycle Green (Invitrogen) in the dark for 30 min at 37 °C, and then twice washed using Ringer solution. Finally, the count of CD71-positive reticulocytes obtained from 50,000 erythrocytes was measured using a two-color FACScan flow cytometer (Becton Dickinson) [7,23].

2.9. Senescence-related molecules on erythrocyte

The erythrocyte suspensions (1×10^4 cells/μL) were incubated with saturating concentrations (10 μg/mL) of monoclonal anti-human CD47 antibody (BioLegend) or monoclonal anti-human CD147 antibody (*e*Bioscience) that was conjugated with fluorescein isothiocyanate (FITC) in the dark for 30 min at 37 °C. Erythrocytes that had been treated with FITC-conjugated anti-rabbit IgG control antibody were utilized to correct for background fluorescence (*e*Bioscience). The mean fluorescence intensity obtained from 10,000 erythrocytes was measured using FACScan flow cytometry (Becton Dickinson) [7].