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Original Article

The changes of red blood cell viscoelasticity and sports anemia in male 24-hr ultra-marathoners

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Abstract

Background: In endurance sports, stress, dehydration and release of chemical factors have been associated with red blood cell (RBC) alterations of structure and function, which may contribute to sports anemia, a well-observed phenomenon during long-distance running. Until now, the investigation of the changes of viscoelastic properties of RBC membrane, a decisive factor of RBC deformability to avoid hemolysis, is lacking, especially in an Oriental population.

Methods: nineteen runners were prospectively recruited into our study. Hematological parameters were analyzed before and immediately after the 2015 Taipei 24H Ultra-Marathon Festival, Taiwan. Video particle tracking microrheology was used to determine viscoelastic properties of each RBC sample by calculating the dynamic elastic modulus G′(f) and the viscous modulus G″(f) at frequency f = 20 Hz.

Results: Haptoglobin, RBC count, hemoglobin, hematocrit, mean cell hemoglobin, plasma free hemoglobin and unsaturated iron-binding capacity values of the recruited runners showed a statistically significant drop in the post-race values. Blood concentration of reticulocyte and ferritin were significantly higher at post-race compared with pre-race. 15 out of the 19 runners had a concurrent change in the elastic and the viscous moduli of their RBCs. Changes in the elastic and the viscous moduli were correlated with changes in the RBC count, hemoglobin and hematocrit.

Conclusion: Viscoelasticity properties, the elastic modulus G′(f) and the viscous modulus G″(f) of RBCs are associated with endurance exercise-induced anemia.

Keywords: Clinical sports medicine; Red blood cell; Sports anemia; Ultra-marathon; Viscoelastic properties

Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

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1. Introduction

Exercise associated anemia, or decrease in red blood cell (RBC) levels, is a widely observed phenomenon during long-distance running.[1−3] Hemolysis has been proposed as a mechanism for exercise associated anemia, with numerous hematological abnormalities being described in runners after strenuous exercise. Studies supporting the phenomenon of exercise induced hemolysis report decrease in haptoglobin and increase in free circulating plasma hemoglobin levels, reticulocytes, and increased ferritin levels.[3−5] Several recent studies have reported conflicting results reporting a modest presence of hemolysis by measuring similar physiological parameters with various methods.[6−8] The proposed term "Foot strike hemolysis", defined as a shearing force causing RBC corpuscular destruction resulting from the athlete's feet striking on hard surfaces frequently, has also been called into question.[4,6] While the causes of fluctuations in RBC levels are far from settled, it is generally accepted that RBC levels do change during prolonged exercise.[9] Whether or not this is purely secondary to fluid status changes, or do RBC characteristics (such as half-life, rheology, size and shape) play a role in determining RBC levels, is still largely unknown.

During high intensity endurance sports, the body undergoes dehydration and release of chemical factors into the blood stream. This phenomenon as well as changes in rheology (increased blood flow and speed) can theoretically alter with RBC structure and function.[10−12] Blood viscosity has been reported no changes after endurance sports such as cycling.[13] Other studies report structural changes in RBC membrane skeleton following marathon running.[14,15] Altered RBC osmotic resistance and increased susceptibility to chemical and physical stress after prolonged endurance exercise have also been reported in different studies.[16] These evidence prompted us to hypothesize that microscopic changes in RBC properties such as viscosity and elasticity may be present in athletes following endurance sports.

We thus designed an experimental study to specifically assess the viscoelastic properties of RBC in runners before and after a 24-hr ultra-marathon.

2. Methods

2.1. Study design and population

Twenty-five experienced male ultra-marathon runners participating in the event known as the 2015 Taipei 24H Ultra-Marathon Festival, in Taipei, Taiwan volunteered for this study. Approval was obtained from the TMU- Joint Institutional Review Board (201309022). All subjects provided written consent to participate in the study. The competition began at 3 pm February 13, 2015 and ended at 3 pm on February 14, 2015. Ultimately, the data of 19 male runners were included in the analysis; One runner who had history of anemia (hemoglobin <13 g/dL), 1 runner who didn't run more than 2/3 of the average kilometers of all finishers and 4 runners who didn't finish the 24-h race were excluded. All runners ran around a 668-m park trail, and they were permitted to rest and to ingest water and food freely. Before the competition, all runners were required to complete a questionnaire for demographic data and information on medical and training history. The body weight of each of the 19 subjects was measured 30 min before and immediately after the race.

2.2. Laboratory assessment

Using sterile techniques, blood (20 mL) was drawn from an antecubital vein from each subject 1 week before and immediately after the race. All specimens were refrigerated and transported to the laboratory within 4 h of sampling. Red blood cell (RBC) count, hemoglobin, hematocrit, mean cell volume (MCV), mean cell hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width, coefficient of variation (RDW-CV) and reticulocyte were performed on a Coulter LH 750 Hematology Analyzer (Beckman Coulter, Miami, FL, USA), which was based on impedance detection for counting and sizing the blood cells. The evaluated plasma haptoglobin was assayed by the rate nephelometry on an Immage 800 Analyzer (Beckman Coulter, Fullerton, CA, USA). Free plasma hemoglobin was tested with a HemoCue Plasma/Low Hb System (HemoCue, Lake Forest, CA, USA) utilizing the azide-methemoglobin method. Ferritin levels were determined by an Architect I-2000 Analyzer (Abbott Diagnostics, Abbott Park, IL, USA), which used a chemiluminescent microparticle immunoassay. Iron and unsaturated iron-binding capacity (UIBC) were measured on a Modular E170 Analyzer (Roche Diagnostics, Mannheim, Germany) using an electrochemiluminescence immunoassay.

2.3. Viscoelastic analysis

Particle Tracking Microrheology (PTM) has been established as an important tool for the study of the viscoelasticity of living cells for more than a decade[17−24]; however, only a few studies deal with RBC viscoelasticity measurements by PTM.[21,23] On the measurements of RBC viscoelasticity, the experimental results may vary by one to two orders of magnitude depending on the specific approach to measure either the global stretching, bending, or torsional modes of the RBC deformations or local membrane fluctuation.[17,18] In our VPTM approach, we used fluorescent nanoparticles attached to the RBC membrane to probe the local RBC membrane fluctuation to deduce the elastic and the viscous moduli (via the Generalized Stokes−Einstein equation),[17,21,22] which are expected to be dominant by the viscoelasticity of the RBCs spectrin network underneath the lipid bilayer.[23] Specifically, Video particle tracking microrheology (VPTM) was used to track, record, and analyze the Brownian motion of 100 nm diameter fluorescence polystyrene beads attached to the membrane of the RBC samples to deduce the dynamic elastic modulus $G'(f)$ and the viscous modulus $G''(f)$ of each RBC sample, for frequency (f) in the range of 0.2 Hz−100 Hz. The sample preparation and the experimental procedures were as

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