Time course of hemorheological alterations after heavy anaerobic exercise in untrained human subjects

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EXERCISE MAY INDUCE SUDDEN death in both athletes and sedentary people (1, 17, 23, 26), and such events have been reported among all age groups (e.g., children, young adults, mid-age groups, elderly) (1, 23, 33). Sudden deaths have been reported during both early and late stages of exercise or after exercise (1, 2, 21, 23), and Albert et al. (1) accepted sudden death events that occurred within 30 min after exercise as “exercise related.” Some of these events could be related to underlying diseases (e.g., cardiovascular), yet in some situations postmortem examinations failed to reveal findings specific to the cause of such deaths (15, 23, 29). Nevertheless, exercise-related sudden deaths are usually reported as cardiovascular events (23, 33, 39), with arrhythmias, myocardial ischemia and/or infarction, and other vascular problems (e.g., atherosclerotic blood vessels, microcirculatory problems) the most frequent manifestations of these events (29, 32, 39).

Heavy exercise has been reported to induce acute significant alterations in blood composition and rheology (10, 31, 38), with the effects of exercise on blood rheology dependent on the type, duration, and intensity of exercise, and the athletic capacity of the individual also plays a significant role. In most studies on untrained individuals, the acute effect of exercise was found to be decreased blood fluidity (10, 31, 41). The fluidity of blood is determined by its composition (plasma protein levels, hematocrit) and the rheological properties of blood cells, especially red blood cells (RBC); because blood is a shear-thinning, non-Newtonian fluid, blood fluidity is also affected by flow conditions. Hence, increased blood pressure and the resulting increase of flow and shear forces during strenuous exercise may compensate for any deterioration in blood fluidity. However, immediately after the cessation of exercise hemodynamic factors return to resting levels, thereby normalizing the shear forces; hemorheological alterations that continue to exist after this hemodynamic normalization may contribute to tissue perfusion problems.

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Although hemorheological alterations induced by various exercise models have been reported, the time course of these alterations has not been investigated in detail. In particular, most studies have focused on the immediate effects noted after cessation of exercise. However, it can be hypothesized that hemorheological alterations may continue for prolonged periods after exercise and thus could have adverse effects well past the acute event. This study was thus designed to test this hypothesis by investigating the time course of hemorheological and related hematological alterations after heavy anaerobic exercise in sedentary male adults.

MATERIALS AND METHODS

Subjects. Ten apparently healthy male volunteers, 20–36 yr of age, were included in the study; the subjects were nonsmokers and were not exercising regularly. The experimental procedures were explained to all subjects, and their written consents to participate in the study were obtained. The subjects were asked to have a light, standard breakfast in the morning of the day of the experiment, then to report to the exercise laboratory at 9:00 AM. Their body fat was estimated by bioimpedance method by use of the TANITA body composition analyzer (TANITA Corporation of America), after which they carried out the exercise protocol described below.

Exercise protocol. A standard period of anaerobic exercise, based on the Wingate anaerobic power test (24), was performed by the subjects at the Akdeniz University Sports Sciences Research and Application Center. The test was performed on a bicycle ergometer equipped with an optical sensor enabling computerized monitoring of the cycling speed. The subjects were asked to warm up for a period of 3 min at 60-rpm speed with a load of 1.5 kg. After the warm-up period, the subjects were asked to cycle at their maximum speed and to maintain this speed for 30 s after 75 kgkgs extra loading. The computerized system calculated the maximum speed, the equivalent distance cycled during the 30-s period, and power outputs at various intervals of the test. A fatigue index was also calculated on the basis of the differences in power output at the start and end of the test period.

Blood sampling. Blood samples were obtained before the start of exercise and were repeated at 15-min intervals during the first hour, then at 2, 4, 8, 12, and 24 h after exercise. Blood samples were anticoagulated with EDTA (1.5 mg/ml), and all analyses were performed within 4 h after blood sampling. RBC and white blood cell (WBC) counts, as well as differential WBC counts, were obtained by using an electronic hematology analyzer (Cell-Dyn 3500R, Abbott Diagnostics Division). Blood lactate concentration was measured by a lactate analyzer using BM-Lactate test strips (Accusport, Boehringer Mannheim, Diagnostics & Biochemicals).

RBC deformability measurements. RBC deformability (i.e., the ability of the entire cell to adopt a new configuration when subjected to mechanical forces) was determined, at 37°C and at a fluid shear stress of 1.58 Pa, by laser diffraction analysis using an ektyctometer (LORCA, RR Mechatronics, Hoorn, The Netherlands). This system calculates an average RBC elongation index, where a higher elongation index indicates greater cell deformability (19).

RBC aggregation measurements. RBC aggregation was assessed by using a photometric aggregometer interfaced to a digital computer (5); this system is based on the increase of light transmission through a RBC suspension consequent to aggregation. The system reports a dimensionless index that increases with increased extent of aggregation.

RBC aggregation was measured for cells in autologous plasma and for cells resuspended in isotonic PBS (290 mosM/kg, pH = 7.4) containing 1% Dextran 500 (molecular weight of 500,000, Sigma Chemical, St. Louis, MO). The measurements made by use of this standard dextran solution reflect the intrinsic tendency of RBC to aggregate (3). The hematocrit of the samples used for aggregation measurements was adjusted to 0.4 l/l, with all measurements done at room temperature (20 ± 2°C).

Quantitation of granulocyte respiratory burst. Granulocytes were isolated from whole blood by using polysucrose density gradients (Histopaque 1119; Sigma Chemical) as described elsewhere (9). Isolated granulocytes were washed with and then suspended in PBS at a cell count of 2.5 × 10^6 per milliliter. The granulocyte suspension was then incubated in the dark at 37°C for 25 min in the presence of 10–6 mol/l 2′,7′-dichlorofluorescein diacetate (D-6883; Sigma Chemical). The fluorescence of dichlorofluorescein diacetate was determined in granulocyte suspensions (10^6 per ml in Krehs phosphate buffer) by a spectrofluorometer (Schimadzu RF-5000, Schimadzu, Tokyo, Japan) at 485-nm excitation and 510-nm emission wavelengths (22), with the intensity of the fluorescence directly related to the degree of activation of granulocytes (i.e., granulocyte respiratory burst).

Statistics. Results are expressed as means ± SE. Statistical comparisons between groups were done by repeated-measures ANOVA followed by Newman-Keuls post hoc test, with P values <0.05 accepted as statistically significant.

RESULTS

The anthropometric parameters of the study group are presented in Table 1. Body mass index, body fat, and lean body mass were within normal limits. During the performance of the Wingate protocol, the average power output was 7.8 ± 0.8 W/kg and the fatigue index was 45.4 ± 13.1%.

At the end of the Wingate anaerobic test, blood lactate concentration increased to a mean value of 10 mmol/l from a preexercise value of 0.9 mmol/l (Table 2). The blood lactate concentration then returned to the preexercise level at 120 min. The anaerobic exercise period immediately induced a significant, 8–10% increment in RBC counts that then returned to control at 15 min postexercise (Fig. 1). However, during the later portion of the monitoring period, there was a drop in RBC counts; starting at the second hour after the end of exercise significant decrements in RBC counts were observed (Fig. 1). Mean corpuscular volume was 87.7 ± 1.2 fl before the exercise, and no significant alterations were observed during the followup period.

RBC deformability was found to be significantly reduced immediately after the anaerobic exercise episode and for 12 h after exercise, then to return to control levels by 48 h postexercise.
levels at 24 h (Fig. 2). RBC aggregation in plasma was also significantly reduced after the exercise, with the onset of this reduction delayed until 30 min; reduced aggregation was observed during the first 12 h (Fig. 3).

A similar pattern of reduced RBC aggregation was observed for RBC resuspended in the 1% Dextran 500 solution, although the significant onset was delayed until 45 min and was observed only up to 8 h (Fig. 4).

WBC counts increased significantly immediately after cessation of anaerobic exercise, then fell to control levels for the first hour (Fig. 5). However, there was a second phase of increase that started at 120 min after the end of exercise and persisted until 4 h. The composition of the WBC population was also affected by exercise, such that the percentage of granulocytes increased starting at 45 min after the anaerobic test and lasted for >3 h (Fig. 6). Additionally, the respiratory burst of the granulocyte population was found to be enhanced during the first 4 h with the increase above control significant at the 60-min time point (Fig. 7).

**DISCUSSION**

The Wingate protocol used in this study is a well-known model of heavy anaerobic exercise (24), and it was observed that all 10 subjects perceived the performed exercise experience as strenuous and barely tolerable. Blood lactate concentrations increased more than 10-fold, thus clearly indicating the high level of metabolic load induced by this exercise protocol. Other groups have also reported similar increments in lactate levels after Wingate protocol in untrained adults (18). This strenuous anaerobic period of exercise performed by untrained individuals can therefore be accepted as a model of unexpected, forced heavy physical exercise that might be encountered by any person under demanding conditions.

Strenuous exercise was followed by a series of changes in RBC and WBC counts that may partly be explained by altered hemodynamic conditions. That is, increased flow and shear forces within the circulation would be expected to lead to recruitment of sequestered RBC in various circulatory beds (3, 25, 41) and of WBC from the marginal pool (40). Plasma volume alterations (10, 31, 38) and increased damage and/or removal of circulating RBC (4, 25, 37) may also contribute to these alterations. At present, we are unable to determine which of these factors contributed importantly to the observed changes (Figs. 1, 5, and 6).

The results of this study also indicate mechanical deterioration of RBC as indicated by decreased elongation indexes (Fig. 2), with the significant impairment of RBC deformability noted immediately after the strenuous exercise. The increased blood lactate concentration at the 5-min time point might be an explanation for this immediate impairment of RBC deformability inasmuch as Brun et al. (10) demonstrated a significant correlation between blood lactate and RBC rigidity after exercise. However, the mechanical impairment of RBC continued long after blood lactate levels had returned to resting levels.
concentrations returned to normal values (Fig. 2, Table 2).

Significant decrements in RBC aggregation in both autologous plasma and the standard dextran aggregating medium were slightly delayed and were then observed for up to 8–12 h (Figs. 3 and 4). Decreased aggregation in plasma may result from alterations of both plasma composition and RBC properties (28, 35), and thus a decreased fibrinogen concentration related to plasma volume expansion after exercise might be responsible for such an alteration (35). However, RBC aggregation measurements in a standard aggregating medium (1% Dextran 500) also revealed a similar pattern of alteration, suggesting that cellular alterations also play role in decreased aggregation after heavy exercise. Impaired RBC deformability would also be expected to reduce RBC aggregability because rigid cells exhibit lower levels of aggregation (35). Alternatively, RBC surface properties that play role in aggregation might be altered by oxidation and/or proteolytic enzymes released by activated granulocytes (6).

Our results for RBC deformability are consistent with other literature data indicating impaired RBC deformability after strenuous exercise (8, 11, 16, 30, 41, 42). However, literature data on alterations of RBC aggregation are not consistent: increased (8, 10), unaltered (16), or decreased (41) RBC aggregation have been reported to occur after exercise. This inconsistency does not seem to be surprising, because the exercise models, the properties of subjects (e.g., the degree of training), and the hemorheological methods used in these prior studies vary widely.

Granulocyte activation may play a role in the hemorheological effects of strenuous exercise. Published literature reports also support the role of an inflammatory response to strenuous exercise as contributing to hemorheological deteriorations, and the inflammatory effects of strenuous exercise have been compared with those occurring in sepsis (13, 36). The most likely cause of the inflammatory response after strenuous exercise is generalized muscle damage (34), and the existence of such a response has been reported by a large number of publications (e.g., 27, 34). Because an inflammatory response has been associated with RBC mechanical alterations (6), granulocyte activation can thus be considered to be at least one of the factors responsible for the hemorheological alterations observed in this study. Note, however, that the data obtained in the present study do not directly identify a causal relationship between WBC and RBC alterations; possible patho-

Fig. 4. RBC M measured in 1% solution of Dextran 500 (molecular weight 500,000) before and after the anaerobic exercise bout (means ± SE; n = 10; difference from value measured before exercise: *P < 0.05).

Fig. 6. Percentage of granulocytes in total WBC count before and after the anaerobic exercise bout (means ± SE; n = 10; difference from value measured before exercise: *P < 0.05, †P < 0.01, ‡P < 0.001).

Fig. 5. White blood cell (WBC) counts before and after the anaerobic exercise bout (means ± SE; n = 10; difference from value measured before exercise: †P < 0.01).

Fig. 7. Granulocyte respiratory burst measured as fluorescence of 2',7'-dichlorofluorescein diacetate (means ± SE; n = 10; difference from value measured before exercise: *P < 0.05).
physiological mechanisms underlying RBC-WBC interactions are discussed in detail elsewhere (6). Note also that in addition to inducing RBC mechanical alterations, activated granulocytes may directly influence microcirculatory hemodynamics (20) because granulocyte activation is associated with significant rigidification of these cells (12) and activated granulocytes may block microcirculatory circuits (20). Together with their increased number, activated and rigid granulocytes may be among the factors that contribute to postexercise circulatory problems. In overview, this study demonstrates that impaired RBC deformability may continue up to 12 h after a single period of strenuous exercise. RBC deformability is one of the factors determining the fluidity of blood and hence the hydrodynamic resistance in blood vessels (14). Furthermore, it is a major rheological factor affecting microcirculatory and capillary blood flow, and thus reduced deformability may limit tissue perfusion, especially in individuals with geometrically challenged vascular systems and diminished local perfusion pressures (7). During heavy exercise, the circulation and local perfusion pressures are markedly enhanced, easily balancing any extra hemorheological load related to RBC mechanical deterioration. However, after hemodynamic activity and perfusion pressures return to preexercise levels, this hemorheological extra load may not be well tolerated and tissue perfusion problems might be encountered. Therefore, this prolonged effect of heavy exercise on RBC deformability may well explain the cardiovascular problems encountered during the early and late recovery periods after heavy exercise episodes (1, 17, 23, 26).

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REFERENCES


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