INTRODUCTION

Erythrocytes are a group of blood cells circulating the body and affecting the circulatory fluid dynamics. The mechanical properties of erythrocytes are important determinants of tissue perfusion [32]. The aggregability of erythrocytes is one of these properties, which plays a major role in haemodynamics. Erythrocyte aggregation is the reversible adhesion of erythrocytes. The physiological importance of erythrocyte aggregation in circulation is probably its tendency to increase the blood viscosity in low shear flow and to disturb the passage in capillary circulation [14]. Erythrocyte hyperaggregation may lead to local consequences: decrease in blood flow rate particularly in post-capillary vessels, decrease in the volume concentration of erythrocytes in capillaries, change in erythrocyte rheological parameters, increase in peripheral resistance, tendency to develop venous thrombosis, damage to tissues as a result of anoxia [31].

Erythrocytes are highly deformable, and this physical property plays a pivotal role in facilitating blood flow, particularly in the microcirculation [15]. The deformability of erythrocytes is important with respect to the ability to pass capillaries with a smaller diameter than theirs and the reduction of blood viscosity under high shear rates. If erythrocytes were rigid, blood at high haematocrit levels would be solid and stiff. Decreased deformability may contribute to shortened lifespan of red blood cell, thus leading to onset of anemia. Deformability of erythrocyte depends on erythrocyte geometry, membrane flexibility and internal cytoplasmic viscosity. Impairment of erythrocyte physical and mechanical properties may significantly affect blood fluidity and tissue perfusion [15]. The erythrocyte deformability, an important haemorheological parameter contributing to the exchange of metabolic products with the tissue environment, is thus attributed to the various constituents of the membrane and haemoglobin. Any variation in these may result in the impaired functioning of erythrocytes [32]. Free radicals are known to play a vital role in tissue damage, and they have adverse effects on erythrocytes [3]. In the resting state, the body is equipped with both non-enzymatic and enzymatic antioxidant reserves to prevent the potentially harmful effects of free radicals. Antioxidant defence systems preserve homeostasis for normal cell function at rest. Rheological properties of erythrocytes...
are affected by oxidant attacks after exercise and large quantities of the ambient oxygen consumption [39]. In addition to the emergence of free radicals from mitochondrial leakage owing to enhanced oxygen consumption, ischemia-reperfusion process and leukocyte activation may also contribute to oxidative stress during exercise, especially in extra muscular tissues (heart, liver, brain) and erythrocytes [22].

Exercise may affect erythrocyte deformability and aggregation [13,15]. A large number of studies have tested the effect of running on the erythrocyte rheological properties. However, it has been demonstrated that traumatic damage of red cells due to their compression in the foot plantar circulation is likely to be important in sports like running [8]. Therefore, we chose swimming exercise for the exercise model in this study. Moreover, the effect of swimming on blood rheology is unknown. The 400-meter freestyle swimming is an event in which both aerobic and anaerobic processes contribute to the energy release [27]. Blood lactate concentration increases after 400-meter freestyle swimming [25,27]. Increased lactate levels may affect erythrocyte rheological properties [33]. The present study was performed to investigate the effects of acute swimming exercise on erythrocyte aggregation and deformability.

**MATERIALS AND METHODS**

Participants. Seventeen male university swimmers participated in the study. The age, height, weight, and body fat ratio of the participants were as follows: age = 20.82±0.6 years, height = 179.35±1.5 cm, weight = 72.21±1.9 kg, body fat ratio = 13.18±0.8%. Mean performance for the 400-meter was 325.20±7.5 s. They have at least 4 years of experience in swimming and a weekly training volume of 30 km. All the swimmers have 6 training sessions per week. This study was approved by the clinical and laboratory investigation ethic committee of the Dokuz Eylül University, Faculty of Medicine. All swimmers were informed in detail about the experimental procedure and signed an informed consent statement. They were also advised to avoid heavy exercise for 24 h preceding study.

Experimental design. Exercise sessions were performed at same interval of the day (between 8.00 and 9.00 a.m.) after a warm-up. The water temperature was kept at 26-27°C. For 400-meter freestyle swimming, participants were asked to swim as fast as they could. On arrival at the pool for swimming, swimmers rested for 20 min and then a venous blood sample (5 mL) was collected from antecubital vein. It has been found that blood lactate concentration is maximal level at the fifth minute of recovery after 400-meter swimming [25]. Therefore, one more venous blood sample (5 mL) was collected at the fifth minute of recovery after 400-meter swimming in this study. Blood lactate concentration, erythrocyte lipid peroxidation and plasma protein oxidation levels, erythrocyte deformability and aggregation, and several haematological parameters were investigated after swimming and compared with pre-exercise values.

**Measurement of blood lactate.** Blood samples were analyzed for lactate concentration with an automated lactate analyzer (Yellow Springs Instruments Model 23L).

**RESULTS**

Hematological parameters and blood lactate concentration. Some major haematological indices were analyzed to determine whether
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All swimmers were in normal hematological ranges. The results of hematological measurements are listed in Table 1. The mean value of erythrocyte count, Hct, Hb concentration and MCV of swimmers were in normal range. Erythrocyte count, Hct, and MCV of swimmers increased after swimming (p<0.01). Hb concentration was not change with 400-meter swimming. Blood lactate concentration was 1.06±0.1 mmol/L at rest and significantly increased to 13.02±0.7 mmol/L after swimming (p<0.001).

Plasma protein oxidation and erythrocyte lipid peroxidation. Erythrocyte malondialdehyde and plasma total sulfhydryl concentrations are shown in Table 2. Concentration of erythrocyte malondialdehyde as an indicator of lipid peroxidation and concentration of plasma sulfhydryl as an indicator of protein oxidation were unchanged with swimming.

Erythrocyte deformability and aggregation. Erythrocyte deformability indices are presented in all shear stresses in Fig. 1. Erythrocyte deformability was not change after swimming. Erythrocyte aggregation indices are presented in Fig. 2. Erythrocyte aggregation was found to be increased after swimming (p<0.01). There were not correlations between blood lactate and erythrocyte hemorheological properties before and after swimming.

DISCUSSION

In this study, erythrocyte aggregation and deformability were investigated following acute swimming. Erythrocyte deformability indices were not change whereas erythrocyte aggregation indices were found to be increased after swimming. To our knowledge, this is the first report about the effects of acute swimming on erythrocyte deformability and aggregation in swimmers.

In the healthy body, the range of antioxidant defense mechanisms should be adequate to protect against oxidative damage. It was demonstrated that physical exercise increases the production of reactive oxygen species, and both membrane and cytoplasmic structures of the erythrocytes may be affected by such oxidant attack [5,35]. Many studies report increased lipid peroxidation and protein oxidation in different tissues after exercise [21,28]. In the present study, erythrocyte malondialdehyde and plasma sulfhydryl levels were unchanged with acute swimming. These results may result from protective antioxidant mechanisms that develop with regular training. Aerobic, anaerobic and mixed exercise causes an enhanced free radical production. In the same way, humans have an adaptive reaction with an increased mobilization of a variety of enzymatic and non-enzymatic antioxidants in cells or in plasma [16].

### TABLE 1. THE MEAN VALUES OF HEMATOLOGICAL VARIABLES BEFORE AND AFTER SWIMMING

<table>
<thead>
<tr>
<th>Sampling time</th>
<th>Erythrocyte count (10⁶/µL)</th>
<th>Hct</th>
<th>Hb (g/dL)</th>
<th>MCV (fL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before swimming</td>
<td>5.00 ± 0.07</td>
<td>44.74 ± 0.63</td>
<td>14.70 ± 0.18</td>
<td>89.29 ± 0.81</td>
</tr>
<tr>
<td>After swimming</td>
<td>5.15 ± 0.08*</td>
<td>46.83 ± 0.75*</td>
<td>14.82 ± 0.24</td>
<td>91.00 ± 0.87*</td>
</tr>
</tbody>
</table>

Note: *p < 0.01 compared to before swimming. Values are mean ± SEM. Hb, hemoglobin; Hct, hematocrit; MCV, mean corpuscular volume.

### TABLE 2. ERYTHROCYTE MALONDIALDEHYDE AND PLASMA TOTAL SULFHYDRYL CONCENTRATIONS BEFORE AND AFTER SWIMMING

<table>
<thead>
<tr>
<th>Sampling time</th>
<th>Plasma total SH (µmol/L)</th>
<th>Erythrocyte MDA (nmol/g Hb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before swimming</td>
<td>514.2 ± 18.7</td>
<td>33.76 ± 2.40</td>
</tr>
<tr>
<td>After swimming</td>
<td>478.9 ± 24.6</td>
<td>35.21 ± 2.06</td>
</tr>
</tbody>
</table>

Note: Values are mean ± SEM. SH, Sulfhydryl; MDA, Malondialdehyde.
In the hemorheological studies, determination of the erythrocyte deformability and aggregation facilitates the evaluation of rheological behaviours of the erythrocytes. Previous studies examining the effect of exercise on red blood cell deformability have produced conflicting results [15]. In some studies a considerable decrease was reported [17,26,40] while in others no change [24,34]. Conflicting results may be due to differences in the exercise models [40]. For example, sports like running can cause traumatic damage of red cells due to their compression in the foot plantar circulation [8]. In this study, we chose swimming exercise for exercise model. Some factors may impair the normal functioning of erythrocytes [32]. Reaction to heavy exercise may even involve an inflammatory response [10], including the activation of leukocytes [35,38]. Oxidant attacks may affect rheological properties of erythrocyte after exercise [39]. Some studies have demonstrated that lactate anion may enter into red blood cells [29,30,33]; and change blood rheology [12,23,36]. It was proposed that an exercise-associated increase in blood lactate could be the physiological mechanism responsible for the diminished red blood cell deformability. This assumption was based on the observations that the in vitro addition of lactate shrinks red blood cells and decreases their flexibility and filterability, and moderate lactic acidosis during moderate-intensity exercise coincided with a temporary increase in red blood cell rigidity [15]. Muscles produce lactic acid during 400-meter swimming, which leads to lactate accumulation in the blood [25,27]. Similarly, in the present study, blood lactate concentration increased considerably after swimming, but we could find no correlation between blood lactate levels and erythrocyte deformability indices before and after swimming. It has been shown that red blood cell deformability increase with regular training [15]. In this study, erythrocyte deformability was not change after acute swimming. It can be due to training-related improvements in blood rheology [8].

Some studies show that the aggregation of erythrocytes is a complex dynamic process affected by cellular and plasma factors [32]. The mechanisms of the erythrocyte aggregation could not be explained satisfactorily. Few studies have investigated the changes in red blood cell aggregation during exercise and conflicting data can be found in the literature. Conflicting results may be due to differences in the methods determining the aggregation index [37]. Connes et al. [13] demonstrated that red blood cell aggregation was not changed by a progressive and maximal exercise test in well trained endurance athletes. Yalcin et al. [40] found that erythrocyte aggregation was decreased after exercise in untrained subjects, but the onset of this decrease was delayed by 30 minutes. Bouix et al. [6] reported increased erythrocyte aggregation in rugby players in response to an aerobic exercise test. Hardeman et al. [19] determined that erythrocyte aggregation index increased immediately after exercise in athletes. Similarly, the data obtained in the present study indicate that erythrocyte aggregation increases after swimming exercise. There are some evidences about the mechanisms underlying erythrocyte aggregation in exercise. For example, Connes et al. [13] found that lactate is not able to change erythrocyte aggregation in trained athletes. Similarly, in the present study, we could find no correlation between blood lactate levels and erythrocyte aggregation indices before and after swimming. It has been previously demonstrated that activated leukocytes may induce structural and functional alterations in the neighbouring erythrocyte [11] altering erythrocyte aggregability [5,9] after exercise. It was reported that maximal exercise induces a significant increase in plasma fibrinogen [2]. Increased red blood cell aggregation is generally believed to be related to increased plasma levels of fibrinogen and other acute-phase reactants [4,15].

**CONCLUSIONS**

In conclusion, in this study we found that erythrocyte aggregation increased after acute swimming. Blood lactate concentration increased considerably after swimming, but we could find no correlation between blood lactate levels and erythrocyte rheological properties. In addition to, erythrocyte malondialdehyde, plasma sulfhydryl levels and erythrocyte deformability were unchanged with acute swimming. These results can be due to training-related improvements in blood rheology and protective antioxidant mechanisms. The findings obtained from the present study have shown that further studies are required to thoroughly clarify the effects of swimming on erythrocyte rheology. In particular, one should investigate whether there will be a time-dependent change in erythrocyte rheology after swimming, and the effect of swimming in various distances on rheological parameters.

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**REFERENCES**

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