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The Effects of Stretching Training on Erythrocyte Rheological Properties and Anaerobic Performance in Healthy Young Males

Genç sağlıklı erkeklerde esneklik antrenmanının eritrosit reolojik özelliklerine ve anaerobik performansa etkileri

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Abstract

Purpose: Stretching exercise training is used to improve flexibility and performance. This study aimed to investigate the short and long-term effects of stretching exercise on flexibility, performance, red blood cell (RBC) deformability and aggregation.

Materials and methods: 7 young male subjects (mean age 20.71±0.52 years) participated in the study. Stretching protocol consisted of 3 repetitions for 60 seconds, 3 days/week, applied for 6 weeks. Blood samples were obtained before and immediately after the first stretching exercise and on the last day of the program. Flexibility measurement was performed, as well as counter movement, squat jump, sprint tests. Hemorheological parameters were determined using an ektacytometer.

Results: A significant elevation in flexibility and decrement in sprint time were observed. Following an acute insignificant elevation immediately after the exercise on the first day, RBC deformability was slightly decreased on the last day after the exercise. RBC aggregation significantly decreased immediately after the stretching exercise on the first day, while post-exercise elevation was detected on the 6th week.

Conclusion: The results of the current study indicate that the stretching exercise training used to develop physical capacity of the athletes contributes to blood flow and thus, athletic performance of the individual by inducing acute increments in RBC deformability and through decrements in erythrocyte aggregation.

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Key words: Stretching, RBC deformability, erythrocyte aggregation, anaerobic performance test, flexibility.

Özet

Amaç: Germe egzersizi, fleksibilite ve performansı geliştirmek için kullanılmaktadır. Bu çalışmada, germe egzersizinin eritrosit deformabilite ve agregasyonu, fleksibilite ve performans üzerindeki kısa ve uzun süreli etkilerinin incelenmesi amaçlanmıştır.

Gereç ve yöntem: Çalışmaya ortalama yaşları 20.71±0.52 yıl olan 7 genç, erkek denek katılmıştır. Germe protokolü 60 saniyelik, 3 tekrarlı, haftada 3 gün olmak üzere 6 hafta uygulanmıştır. Kan örnekleri ilk egzersizden önce, hemen sonra ve 6. haftanın sonunda egzersiz bitiminde alınmıştır. Deneklere ters yönde çömelme ve sıçrama ile esneklik ölçümleri ve sprint testleri uygulanmıştır. Hemoreolojik parametreler ektasitometre aracılığıyla ölçülmüştür.

Bulgular: Deneklerin esneklik ölçümlerinde istatistiksel olarak önemli bir yükselme, sprint zamanında ise azalma gözlenmiştir. İlk gün egzersiz sonrası eritrosit deformabilitesinde istatistiksel olarak önemli olmayan akut bir artış gözlenmiştir. 6. hafta sonunda son egzersizi takiben ise eritrosit deformabilitesinde hafif bir düşüş tespit edilmiştir. Eritrosit agregasyonu ilk egzersiz sonrası istatistiksel olarak anlamlı düzeyde azalmış, 6. haftada egzersiz sonrası ise artmıştır.

Sonuç: Çalışmamızın sonuçları, sporcuların fiziksel kapasitelerini geliştirmek için uygulanan germe egzersiz eğitiminin, kan akımının düzenlenmesine ve eritrosit deformabilitesinde akut artışlar, agregasyonunda ise azalmalar sağlayarak bireylerin atletik performansına katkıda bulunduğunu göstermektedir.

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Anahtar sözcükler: Esneklik, eritrosit deformabilitesi, eritrosit agregasyonu, anaerobik performans testi, fleksibilite.

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Introduction

Flexibility (range of motion) is known as an important component of the physical fitness for development of many athletes [1]. Stretching is a widespread and useful modality for improving flexibility and is an integral part of many sports performed by individuals prior to engaging in physical activity [2,3]. Stretching as part of a warm-up (pre-exercise stretching) and cool-down (post-exercise stretching), has been recommended in many textbooks for the claimed purposes of reducing risk of injury, decreasing post-exercise muscle soreness and enhancing athletic performance [4,5].

Although pre-event static stretching is suggested by professional organizations and continues to be part of the warm-up protocol for most young athletes [6,7], there are conflicting results; a number of authors have reported that an acute bout of pre-exercise static stretching may actually reduce anaerobic performance in adults through decreasing force and power [8,9], while some others suggest the opposite [4,10]. On the other hand, there are only a few studies in the literature investigating chronic effects of stretching training on athletic performance and they report elevations in strength performance [11,12].

Deformability and aggregability of red blood cells (RBC) are main components of hemorheology [13]. Any alterations in RBC structural and mechanical properties may affect oxygen transfer to the actively used tissues, influencing athletic performance [14]. Deformability of RBCs is one of the key factors in the perfusion of capillaries, whereas RBC aggregation affects the fluidity of blood in larger blood vessels [15]. The short and long term effects of various exercise types on hemorheology have been well defined and reviewed [14,16,17]. Exercise induced alterations on blood rheology depend on the type, duration, intensity of exercise and the athletic capacity of the individual also plays a significant role [14,17].

Although previous studies have presented evidences about the relationship between some biochemical markers, hormones and flexibility exercise; to our knowledge, no information is available about hemorheological responses to flexibility exercise and training which are widely used before and after sports for improvement of physical capacities of athletes and reducing injury. Therefore, the present study was designed to primarily explore the short term (acute) and long term (chronic) effects of flexibility training on RBC deformability and aggregation in healthy young males, further providing a feasible strategy for developing an appropriate exercise regimen that minimizes the risk of hemorheological disorders. However, the second goal of this study was to investigate how flexibility training (chronic) affects anaerobic performance (speed and force) in healthy young males.

Material and Methods

Subjects

7 male volunteer students of School of Sport Science and Technology (mean age 20.71±0.52 years; mean body height 180.25±1.99 cm; mean body weight 74.28±4.56 kg; mean body mass index 22.71±1.18 kg/m^2) with no apparent health problems participated in the study. They were all nonsmokers and warned not to use alcohol or any medication during the study. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki. The approval of experimental procedures were provided by Pamukkale University Ethics Committee and also written consent forms were obtained from all subjects, who were completely informed about the study.

Measurement of flexilibility

Firstly, all participants performed a warm-up with 5 min running and afterwards sit and reach test was assessed with a Flexion-D Takei Physical Fitness Test Standing Trunk Flexion Meter, Japan. Each participant was standing on the Standing Trunk Flexion Meter with legs stretched out straight ahead and bend forward with straight legs and reach down along the measuring line as far as possible. Next, the countermovement (CMJ) -squat jump was measured.

Vertical Jump Measurements

Vertical jump performance was assessed using a portable force platform (Newtest, Finland). Players performed countermovement (CMJ) and squat jumps (SJ) according to the protocol described by Bosco et al. [18]. Before testing, the players performed self-administered submaximal CMJs and SJ (2-3 repetitions) to get familiar with the testing procedures. They were asked to keep their hands on their hips to prevent any influence of arm movements on the vertical jumps and to avoid coordination as a confounding variable in the assessment of leg extensors [19]. Each subject performed

3 maximal CMJs and SJs, with approximately 2 minutes recovery in between. Players were asked to jump as high as possible; the best score was recorded in centimeters [19].

10 and 30 m Sprint Test

Subjects performed 2 maximal 30 m sprints (with 10 m split times also recorded) on the basketball court. There was a recovery period of 3 minutes between the 30 m sprints. Prior to each sprint, players performed a warmup consisting of 5 minutes of jogging. Time was measured using an electronic timing system (Prosport TMR ESC 2100, Tumer Engineering, Ankara, Turkey).

Flexibility Training

The full exercise program was completed in Pamukkale University, Faculty of Sport Sciences. The lower-body stretching program was performed 3 days per week, for 6-weeks. All sessions were supervised. Exercise sessions included the performance of 3 sets of 60 seconds, 3 repetitions approximately for 25 minutes. The program included a variety of common static stretches for the leg extension (quadriceps), leg curl (hamstring), hip flexion (iliopsoas) and plantar flexors (gastrocnemius, soleus). Each muscle was held in the stretched position for 60 seconds, followed by a rest time of 20 seconds and this was repeated 3 times for each limb. The exercises were performed with both limbs (Figure 1).

Figure 1. Stretching positions. **A**) Stretching for the knee flexors and hip extensors **B**) Stretching for the hip flexors **C**) Stretching for the plantar flexors **D**) Stretching for the knee extensors.

Blood sampling

Blood samples were collected from the right antecubital veins of the subjects after the exercise on the 1st and 6th week (last day of the exercise).The measurements obtained on the 1st week before the exercise session was evaluted as basal value. Hemorheological parameters were carried out within 3 hours after blood collection.

Erythrocyte Deformability Measurements

RBC deformability was determined at various fluid shear stresses by laser diffraction analysis using an ectacytometer (LORCA; RR Mechatronics, Hoorn, The Netherlands). A low hematocrit (Hct) suspension of RBC in an isotonic viscous medium (4% polyvinylpyrrolidone 360 solution; MW 360 kD; Sigma P 5288; St. Louis, MI) was sheared in a Couette system composed of a glass cup and a precisely fitting bob, with a gap of 0.3 mm between the cylinders. A laser beam was directed through the sheared sample, and the diffraction pattern produced by the deformed cells was analyzed by a microcomputer. On the basis of the geometry of the elliptical diffraction pattern, an elongation index (EI) was calculated as $EI = (L - W)/(L + W)$, where L and W are the length and width of the diffraction pattern, respectively. EI values were determined for 9 shear stresses between 0.3 and 30 Pa and similar patterns of RBC deformability alterations were obtained between groups at all stress levels. All measurements were carried out at 37°C [20].

Determination of Erythrocyte Aggregation

Erythrocyte aggregation was also measured by LORCA as described elsewhere [20]. The measurement is based on the detection of laser back-scattering from the sheared (disaggregated), then unsheared (aggregating) blood, performed in a computer-assisted system at 37°C. Backscattering data are evaluated by the computer and the aggregation index (AI), the amplitude (AMP) which is the total extent of aggregation, the aggregation half time (t1/2) are calculated on the basis that there is less light backscattered from aggregating red cells. Aggregation measurements were determined using RBCs in autologous plasma adjusted to 40% Hct. Blood was fully oxygenated before measurements.

Statistical Analyses

Statistical analyses were performed using the computer software SPSS version 10.0 (Statistical Package for Social Sciences). Results were expressed as means ± standard error (SE) and minimum-maximum values. Statistical comparisons between two measurements were done with Wilcoxon Signed Rank Test. *p* values<0.05 were accepted as statistically significant.

Results

All subjects completed test protocols without problems. At the beginning of the study there were no statistically significant differences in physical characteristics and hemorheological parameters of the subjects. The subjects' blood variables which were measured in the current study were all within normal limits. Power analyses have shown that, when 7 subjects participated to the study 90% power is achieved with 95% confidence. The physical characteristics data of the subjects are demonstrated in Table 1. At the end of the six-week static stretching program, while a statistically significant elevation in flexibility was observed (initial =11.15±2.15 cm, end =14.47±1.91cm) (p=0.018), decrements in 10 m sprint time (initial =1.75±0.026 sec, end =1.66 ±0.034 sec) (p=0.018)and 30 m sprint time (initial =4.46±0.072 sec, end =4.38±0.067 sec) (p=0.017) were observed. On the other hand, statistically insignificant performance changes in countermovement jump (CMJ) height (initial =32.42±1.25 cm, end =33.71±0.83 cm) (p=0.202) and squat jump height (initial =32.28 ± 1.14 cm, end =33.57±1.37 cm) (p=0.201) after the static stretching program of six-weeks were also determined (Table 2).

Alterations in RBC deformability (i.e., the elongation index, EI) in response to the flexibility exercise training applied herein were measured at 9 shear stresses between 0.3 and 30.0 pascal (Pa) (Table 3). Although the flexibility exercise applied induced increments in RBC deformability at all shear stresses acutely, none of them were found to be statistically significant. Table 3 shows that, RBC deformability tended to slightly decrease compared to 1st week after exercise values after a stretching program of six-weeks, except for the one measured at 0.30 Pa. The decrements were more prominent at 5.33-16.87 Pa, but still not statistically significant.

A statistically significant acute decrease in RBC aggregation index (AI) (p=0.028), increase in aggregation half time ($t_{1/2}$) (p=0.028) and a non-significant decrease in aggregation amplitude (AMP) was observed after the static stretching exercise on the first day of the program (Figure 2-4). After a stretching program of six-weeks although AI and AMP increased

Table 1. Physical characteristic data of the subjects

	mean ± SE	min - max
Age (years)	20.71±0.52	18-22
Weight (kg)	74.28±4.56	62-98
Height (cm)	180.25±1.99	173-187
Body Mass Index (kg/m^2)	22.71±1.18	20-28.65

Table 2. Flexibility, countermovement (CMJ) - squat jump (SJ), 10-30 m sprint time, test data of the subjects

	1st week	6th week	
	mean±SE (min-max)	mean±SE (min-max)	p
Flexibility (cm)	11.15±2.15 (5.40-2.80)	14.47±1.91 (8.2-23.90)	0.018
Countermovement jump (CMJ) (cm)	32.42±1.25 (28-38)	33.71±0.83 (30-37)	0.202
Squat jump (SJ) (cm)	32.28±1.14 (29-37)	33.57±1.37 (27-39)	0.201
10m-sprint time (s)	1.75±0.026 (1.62-1.85)	1.66±0.034 (1.49-1.79)	0.018
30m-sprint time (s)	4.46±0.072 (4.12-4.77)	4.38±0.067 (4.09-4.67)	0.017

Table 3. RBC elongation index (EI) of the subjects

	Basal value	1st week After exercise	6th week After exercise
EI (0.30 Pa)	0.037±0.003	0.038±0.001	0.051±0.011
EI (0.53 Pa)	0.051±0.004	0.060±0.004	0.059±0.006
EI (0.95 Pa)	0.124±0.006	0.132±0.007	0.127±0.010
EI (1.69 Pa)	0.225±0.008	0.231±0.008	0.230±0.011
EI (3.00 Pa)	0.332±0.007	0.338±0.007	0.333±0.011
EI (5.33 Pa)	0.430±0.007	0.435±0.007	0.429±0.013
EI (9.49 Pa)	0.507±0.005	0.510±0.006	0.495±0.011
EI (16.87 Pa)	0.561±0.004	0.562±0.005	0.551±0.010
EI (30.00 Pa)	0.597±0.005	0.600±0.004	0.597±0.007

EI: Elongation Index.
Values are expressed as mean±SE.

and $t_{1/2}$ decreased, the alteration was only statistically significant for AMP compared to first week after exercise values (*p*=0.043). Taken together, the statistically significant increments observed in AI and AMP are in concordance with the decrement observed in $t_{1/2}$ and indicate enhancement in RBC aggregability.

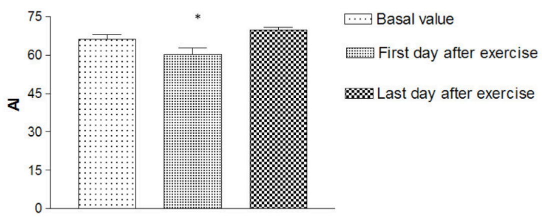

Figure 2. RBC aggregation index (AI) (%) values of the subjects. Values are expressed as mean ± SE. * $p < 0.05$, vs. basal value.

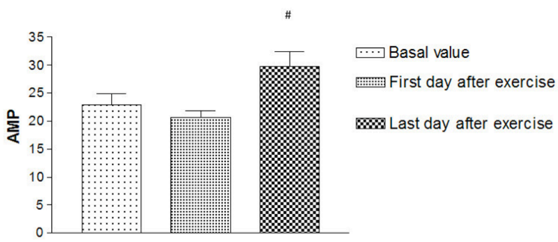

Figure 3. RBC aggregation amplitude (AMP) (au) values of the subjects. Values are expressed as mean ± SE. $^{\#}p < 0.05$, vs. first week after exercise values.

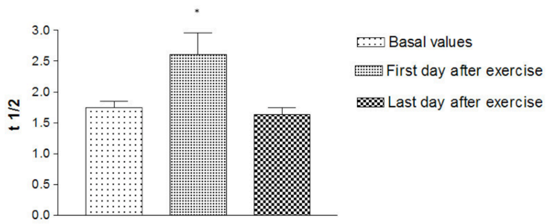

Figure 4. RBC aggregation half time ($t_{1/2}$) (sec) values of the subjects. Values are expressed as mean ± SE. **p* < 0.05, vs. basal value.

Discussion

Flexibility is an important component of well-being for development of many athletes and stretching is a widespread and favorable procedure for increasing flexibility. Notwithstanding the widespread acceptance and use of stretching exercises as a component of exercise training, there is limited research documenting the benefits derived from regular stretching training program. Hunter and Kokkonen reported that flexibility training increases vertical jump and/or sprint performance [11,12]. Dintiman found that 8 weeks of static stretching and sprint training increase flexibility and running speed significantly [21]. The results of the current study indicating static stretching training has positive effects on anaerobic power and flexibility support reports mentioned above. On the other hand, there are also experiments showing that flexibility training doesn't increase range of motion (ROM), or sprint and vertical jump performance in women track and field athletes [22]. The results of our study, which is the first one in the literature investigating the immediate and late effects of static stretching training on hemorheological parameters, also demonstrate that stretching exercise induces acute increments in RBC deformability and decrements in aggregability. On the contrary, 6 weeks of stretching training causes increments in RBC aggregation.

Erythrocyte deformability, or the capacity of the cell to change its shape under applied stress, is important for performing its function of oxygen delivery, and is a determinant of the cell survival time in the circulation. Since the small vessels of the microcirculation have a diameter less than that of the resting erythrocytes, cells must deform markedly during circulation [23]. Findings of the studies investigating the effects of exercise on RBC deformability differ depending on (a) the type, duration, intensity of the exercise (b) athletic capacity of the individuals and (c) the method used to measure RBC deformability [14]. Although decrement of RBC deformability after exercise has been described in subjects with low physical fitness [17,24], there is accumulating data about acute post-exercise increments in RBC deformability in physically active subjects [25,26].

In the current study, acute non-significant increases in RBC deformability at all shear stresses measured were observed after the static stretching exercise on the 1st day. Subjects in our study, were not engaged in any regular flexibility training, but were physically active (football, folk-dance, running). This may be an explanation for the augmentation of RBC deformability in concordance with the above mentioned results. Lopes et al. have demonstrated that blood lactate concentration was increased acutely in response to 30 min of static stretching in physical active male subjects [27]. Lactate is known to impair RBC deformability in untrained subjects but improves it in trained individuals [24]. Although blood lactate concentration was not measured in the current study, it can be speculated that the possible acute increment of lactate in our physically active young students may at least partially be responsible for the acute increment of RBC deformability. On the other hand, divergent literature data are found concerning lactate as a potential antioxidant agent by preventing lipid peroxidation [28]. These factors may explain the increment of RBC deformability in spite of the elevation of blood lactate concentration. After 6 weeks of flexibility training although slight decreases occurred in RBC deformability compared to the after exercise values of the 1st week, these decrements were not statistically significant, thus the obtained values still being higher than basal values between 0.30-3 Pa. The decrements in RBC deformability as a result of stretching training of 6 weeks were more prominent at higher shear stresses (>5.33 Pa). It can be suggested that although acute stretching exercise may help tissue oxygenation through increasing RBC deformability, this favorable effect is not observed after 6 weeks of stretching training especially at higher shear stresses which are usually observed at the muscle tissue capillary level.

RBC aggregation which is the reversible adhesion of adjacent RBCs was also measured in the current study [14,16]. Concerning aggregation alterations in response to exercise, conflicting results were reported: some studies have shown no change [16], an increase, in association or not with a rise in plasma fibrinogen level [25], or a decrease [15,17]. Although the reasons for incongruent findings are unclear, they may again be related to the differences in 1) the population tested (athletes, sedantary individuals, population with disease) 2) the exercise performed 3) the technique used to measure aggregation. In the current study, we have used ektacytometry which is a sensitive and well standardized technique and gives a quantitative assessment of the RBC aggregation and deformability processes. The stretching activities typically recommended to be included in training programs are of a lower magnitude than those used to achieve the reported strength gains. As far as we know, our study is the first one in the literature exploring the acute and chronic effects of stretching exercise on RBC aggregation. In the current study RBC aggregation was found to be inhibited during the early phase after the static stretching exercise. This inhibition might be explained by the alterations in surface properties of RBCs. Additionally, it is known that there is a negative correlation between erythrocyte aggregation and blood lactate concentrations [29]. The acute decrement in RBC aggregation in response to stretching exercise may be related with enhancements of blood lactate concentrations as discussed above. However, the chronic effects of static stretching training on RBC aggregation are quite different; we have observed increase in the aggregation indexes. Although no parameter to support our suggestion was determined herein, it can be speculated that this enhancement may be due to the the acute-phase response related to inflammatory process induced by stretching training of 6 weeks [30].

Pre and post-exercise stretching is regularly performed with the claimed purpose of preventing injury and muscle soreness or even enhancing performance. Although hemorheological alterations in response to various exercise types are well-known [14,16,17], these alterations may be masked by the effects from the stretching protocols. There is no information about the acute and long term effects of stretching exercises on RBC deformability and aggregation in the literature. The findings of the current study indicate that the static stretching which is commonly performed prior to exercise and athletic events by inducing increment in RBC deformability and decrement in erythrocyte aggregation acutely may serve as a favorable mechanism to increase perfusion and muscle oxygenation for the upcoming exercise and thus may be recommended for cardiovascular fitness. On the other hand, a 6 week program of flexibility training induces impairs erythrocyte deformability and induces enhancement of RBC aggregability. It is worthy to emphasize that alterations in hemorheological parameters in response to different stretching training models (active, dynamic, sport –specific stretching) as well as differing stretching protocols [muscle groups, time of stretch, length of program participation) on diverse populations (inflexible, recreational, and/or aging athletes) and athletic activities (e.g., swimming, running) may also be studied in order to observe which of them are more effective and can be suggested. Additionally, to get more relevant information about the health aspects of stretching training, the alterations in hemorheological parameters of a group of patients with cardiovascular pathologies in response to stretching may also be investigated.

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Conflict of interest: The authors declare that they have no conflicts of interest.

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