Araştırma Makalesi *Research Article*

Pamukkale Tıp Dergisi
Pamukkale Medical Journal

doi:https://dx.doi.org/10.31362/patd.615270

A bout of upper body wingate anaerobic power and capacity test alters blood rheology in untrained individuals

Tek seans uygulanan üst ekstremite wingate anaerobik güç ve kapasite testi sedanter bireylerde kan reolojisini değiştirir

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Gönderilme tarihi:04.09.2019 Kabul tarihi:03.01.2020

Abstract

Purpose: The aim of this study was to investigate the acute effects of a single bout of upper body wingate anaerobic power and capacity test (WAnT) on hemorheology (erythrocyte deformability, aggregation) and total oxidant/antioxidant status (TOS/TAS) in untrained individuals.

Materials and methods: Fourteen sedentary healthy men (mean age 21.86±0.55 years) performed upper body 30 s Wingate Test. Blood samples were obtained before and immediately after the exercise within 1 minute. Hemorheological parameters were determined by an ektacytometer. TOS/TAS were measured using a commercial kit and blood lactate concentration was determined by a lactate analyzer.

Results: There were statistically significant decrements in RBC deformability determined at 9 shear stresses between 0.3 and 30 Pa (p<0.05). The exercise protocol induced a statistically significant increment in erythrocyte aggregation index (AI, p=0.001) and decrement in aggregation half time (t½, p=0.003). A single session of exercise resulted in acute statistically significant increments in TOS (p=0.034), hematocrit (p=0.007) and blood lactate concentrations (p=0.0001). The rise observed in TAS and the decrement in oxidative stress index did not reach statistically significant level (p>0.05).

Conclusion: These data show that one session of upper body WAnT has acute effects on circulation by reducing red blood cell (RBC) deformability and increasing erythrocyte aggregation. Altered hemorheological parameters may be explained by the oxidative stress enhancing effect of exercise.

Key words: Anaerobic performance, anaerobic test, erythrocyte deformability, red blood cell aggregation, oxidative stress.

Ünver F, Kılıç Toprak E, Alemdaroğlu BU, Kılıç Erkek Ö, Özdemir Y, Oymak B, Küçükatay V, Baki Yağcı A, Bor Küçükatay M. A bout of upper body wingate anaerobic power and capacity test alters blood rheology in untrained individuals. Pam Med J 2020;13:248-256.

Özet

Amaç: Çalışmamızın amacı sedanter bireylerde tek seans uygulanan üst ekstremite Wingate Anaerobik Güç ve Kapasite Testi (WAnT)'ın hemoreoloji (eritrosit deformabilite, aggregasyon) ve total oksidan/antioksidan durum (TOS/TAS) üzerine etkilerini araştırmaktır.

Gereç ve yöntem: 30 sn'lik üst ekstremite Wingate testine 14 sedanter sağlıklı erkek (ort yaş 21,86±0,55 yıl) birey dahil edilmiştir. Egzersizden önce ve egzersiz bitiminde 1 dk içinde kan örnekleri toplanmıştır. Hemoreolojik parametreler ektasitometre ile değerlendirilmiştir. TOS/TAS ticari bir kit aracılığıyla ve kan laktat konsantrasyonu ise laktat analizörü ile ölçülmüştür.

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Bulgular: 0,3-30 Pa arasında 9 farklı kayma kuvvetinde ölçülen eritrosit deformabilitesinde istatistiksel olarak anlamlı bir değişiklik saptanmıştır (p<0,05). Uygulanan egzersiz protokolüyle eritrosit agregasyon indeksinde istatistiksel olarak anlamlı bir artış (AI, p=0,001), agregasyon yarı zamanında anlamlı bir azalma belirlenmiştir (t½, p=0,003). Tek seans uygulanan egzersiz TOS (p=0,034), hematokrit (p=0,007) ve kan laktat konsantrasyonlarında (p=0,0001) akut bir artışa yol açmıştır. TAS'da gözlenen artış ve oksidatif stres indeksindeki azalma istatistiksel olarak anlamlı seviyeye ulaşmamıştır (p>0,05).
Sonuç: Verilerimiz, tek seans uygulanan üst ekstremite WAnT'ın eritrositlerde deformabiliteyi azalttığını ve agregasyonu arttırdığını göstermektedir. Hemoreolojik parametrelerdeki değişiklikler, egzersizin oksidatif stresi arttırmasıyla açıklanabilmektedir.

Anahtar kelimeler: Anaerobik performans, anaerobik test, eritrosit deformabilitesi, eritrosit agregasyonu, oksidatif stres.

Ünver F, Kılıç Toprak E, Alemdaroğlu BU, Kılıç Erkek Ö, Özdemir Y, Oymak B, Küçükatay V, Baki Yağcı A, Bor Küçükatay M. Tek seans uygulanan üst ekstremite wingate anaerobik güç ve kapasite testi sedanter bireylerde kan reolojisini değiştirir. Pam Tıp Derg 2020;13:248-256.

Introduction

The upper body Wingate anaerobic power and capacity test (WAnT) consists of arm-cranking at maximal speed against a constant force, predetermined to yield maximal mechanical power. It was suggested for coaches to objectively evaluate and monitor the upper body power of especially competitive swimmers, canoeists and athletes with lower extremity problems using wheelchairs [1, 2]. Recently, WAnT with resting intervals, was used as a high-intensity interval exercise; to lead an effective improvement in the performance of not only recreational and elite athletes, but also non-trained individuals [3]. It is also kind of a powerful tool that uses time effectively to improve fitness and cardiometabolic health in sedentary individuals and clinical populations [4-6].

There are significant differences in neuromuscular and cardiovascular functions between the upper and lower body at rest and during exercise [7, 8]. The upper body is reported to have a higher percentage of type II fibers [9] and extract less oxygen during exercise compared to the lower body [7]. Significant differences in peak and mean power have been also reported between the upper and lower body even when normalized for active muscle mass [10]. Therefore, the contribution of the energy systems to upper body WAnT can be different from the lower body during a cycle of WAnT. The upper body WAnT was shown to have 11.4%±1.4% aerobic, 60.3%±5.6% anaerobic lactic and 28.3%± 4.9% anaerobic alactic components [11].

Heavy exercise has been reported to induce significant acute alterations in blood composition and rheology. The athletic capacity of the individual also plays a significant role [12-15]. Blood flow, red blood cell (RBC) aggregation and deformability are main components of hemorheology. The ability of the entire RBC to distort is critical to perform its function and is also a determinant of cell survival time in the circulation [16]. Erythrocyte aggregation tends to increase blood viscosity in low shear flow and disturbs the passage in capillary circulation [17]. In most studies on untrained individuals, the acute effect of exercise is decreased blood fluidity which is determined by its composition (plasma protein levels and hematocrit) and the rheological properties of RBC [12-15]. 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 [15].

Erythrocyte deformability and RBC aggregation are also closely related to exercise induced oxidative stress. Although alterations in blood rheological properties following lower body WAnT in healthy sedentary individuals were determined in one study [15], acute hemorheological and oxidative stress responses to upper body WAnT remain unknown. Clarification of this issue may provide significant contributions to our understanding of adaptations following acute heavy exercise sessions. Hence, the present study aimed to determine the acute effects of a single bout of upper body WAnT on hemorheology and total oxidant/antioxidant status in healthy young

subjects for the first time. In the light of the above mentioned data, the hypothesis of this study was one session of upper body WAnT affects blood rheology acutely and oxidative stress plays role in this alteration.

Materials and methods

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Additionally, the approval of experimental procedures was provided by the University Ethics Committee where the study was carried out (60116787-020/44167) (07/04/2017). Written informed consent was obtained from each participants after a detailed written and oral explanation of the potential risks and benefits resulting from this study participation.

Subjects

Fourteen healthy male volunteers participated to the study with a mean age of 21.86±0.55 years (20-28), a mean body height of 176.29±1.57 cm (168-188) and mean body weight of 73.86±2.09 kg (62.0-85.6). The subjects were not exercising regularly. Exclusion criteria consisted of the following: 1) engagement in regular physical activity; 2) engagement in upper extremity exercise program; 3) detection of systemic disease; 4) use of medication; 5) having a medical condition that would limit exercise participation.

Assesment of body composition

Subjects were asked to adhere to the following pretest requirements: (a) no exercise within 6 months before participating and throughout the study (b) no eating or drinking for 4-8 hours before testing and (c) no alcohol consumption throughout the experimental period. Their height, weight, body mass index (BMI, kg/m^2) and percentage of body fat were estimated using bioimpedance method by the TANITA body composition analyzer (TANITA Corporation of America, BC-418A).

Ultrasound determined muscle thickness

Participants were in a supine position on a medical bench with their elbows extended and relaxed. All measurements were performed at a fixed point (marked with a permanent marker to ensure that the same area was used for measurements), corresponding to one-third of the distance between the medial epicondyle and the coracoid process, respectively. Thus, thicknesses of m. biceps brachii and m. brachialis were measured. All ultrasound measurements were performed by the same trained sonographer and with the scanning head placed perpendicular to the skin, measuring the distance in cm from the superficial to the profound part of the muscle fascia. Three successive measurements were performed of which the average value is presented.

Exercise protocol

A standard period of anaerobic exercise, based on the upper body WAnT, was performed by the subjects at Pamukkale University Faculty of Sport Sciences and Technology. The test was performed on a mechanically braked cycle ergometer (891E, Monark, Vansbro, Sweden). Firstly, the subjects performed 5 minute warm up for a period of 5 min at 50-60 rpm speed with no load and they performed four sprints for 3 seconds in last two minutes and no static stretching [18]. One minute after the warm-up period, the participants were asked to pedal as fast as they could. When the pedalling rate reached approximately 160-170 rpm, the resistance was applied and the subjects performed 30 seconds all-out cycle against 7.5 gr per kg body weight extra loading. Subjects were verbally encouraged during the test [19]. The computerized system calculated power outputs at 1-second intervals. Subjects' peak power (Peak Power, determined as the highest value over a 5-second period of testing), minimum power (minimum power, determined as the lowest value over a 5-second period of testing), average power (average power, determined as the average power throughout the 30 seconds of testing), and fatigue index (Fatigue Index, the maximum anaerobic power-minimum anaerobic power/max anaerobic powerX100 over a 5-second period) were calculated for each test [20].

Samples and measurements

Blood samples were obtained before and immediately after the exercise within 1 min. Venous blood samples of 10 ml were drawn by venipuncture from antecubital vein of the subjects in supine position during rest into standart tubes containing EDTA (1,5 mg/ml) after 8 hours of fasting. Samples were appropriately transported to the Physiology Laboratory and

hemorheological tests were performed within 3 hours in accordance with “new guidelines for hemorheological laboratory techniques” [21]**.** For the determination of oxidative stress parameters, blood samples were centrifuged at 6250 rpm. for 6 min. The serum layer was separated and stored at -80°C until being used for the analysis.

Erythrocyte deformability measurements

RBC deformability was determined at various fluid shear stresses by laser diffraction analysis using an ektacytometer (LORCA; RR Mechatronics, Hoorn, The Netherlands). The system has been described elsewhere in detail [22]. Briefly, a low hematocrit (Hct) suspension of RBC in an isotonic viscous medium (4% polyvinylpyrrolidone -PVP- 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. The viscosity of the PVP suspending medium was 22.5. 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 Pascal (Pa) and similar patterns of RBC deformability alterations were obtained between groups at all stress levels. All measurements were carried out at 37°C.

Erythrocyte aggregation measurements

RBC aggregation was also determined by LORCA as described elsewhere [22]. 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 aggregation index (AI) and the aggregation half time (t½) 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 and blood was fully oxygenated before the measurements.

Determination of Total Oxidant Status (TOS)

The serum total oxidant status (TOS) was measured using a novel automated colorimetric measurement method for TOS developed by Erel [23]. In this method, oxidants present in the sample oxidize the ferrous ion O-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion makes a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules (e.g., lipids, proteins) present in the sample. The assay is calibrated with hydrogen peroxide, and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 equiv/L/mg protein).

Measurement of Total Antioxidant Status (TAS)

The total antioxidant status (TAS) of the serum was measured using a novel automated colorimetric measurement method for TAS developed by Erel [24]. In this method the hydroxyl radical, the most potent biological radical, is produced by the Fenton reaction and reacts with the colorless substrate O-dianisidine to produce the dianisyl radical, which is bright yellowish-brown in color. Upon the addition of a plasma sample, the oxidative reactions initiated by the hydroxyl radicals present in the reaction mix are suppressed by the antioxidant components of the tissues, preventing the color change and thereby providing an effective measure of the tissue TAS. The results are expressed as the mmol Trolox/ mg protein.

Calculation of oxidative stress index

The ratio of TOS to TAS is referred as oxidative stress index (OSI). The OSI is calculated according to the following Formula;

OSI (arbitrary unit)=TOS (μmolH_2O_2 Equiv. /L) /TAS (mmol Trolox Equiv./L) X 100 [25].

Measurement of blood lactate concentrations

Lactate concentrations of the subjects were determined by a lactate analyzer (Lactate Plus L^+ Nova Biomedical USA) before and 3 minutes following the exercise session using 5 μL of blood obtained from their earlobe.

Statistical analysis

As a result of the power analysis we performed before the study, if the effect size of the difference between pre- and post-exercise measurements would be d=0.7, we need at least 14 people for %95 confidence and %80 power. With 14 participants, the results showed us that, we reached %80 power with %95 confidence. Continuous variables were expressed as mean ± standard error (SE). Shapiro-Wilk tests were used for testing normality. For pairwise comparisons; if parametric test conditions were satisfied, paired samples t test was used and if parametric test conditions were not satisfied Wilcoxon signed rank test was used. Spearman correlation analysis was used for the relation among variables. All statistical analyses were performed by SPSS, 21.0. *p* values <0.05 were accepted as statistically significant.

Results

Demographic data, muscle thickness and upper body want data of the subjects

Demographic variables, flexor muscle thicknesses (*m. biceps brachii* and *m. brachialis*) and upper body WAnT data of the subjects are described in Table 1.

Erythrocyte deformability and aggregation

A single session of acute upper body WAnT applied herein resulted in statistically significant decrements in RBC deformability determined at 9 shear stresses between 0.3 and 30 Pa (Table 2). Table 2 shows that the exercise protocol also induced a statistically significant increment in erythrocyte aggregation index (AI, p=0.001) and decrement in aggregation half time (t½, p=0.003). The augmentation observed in AI is in concordance with the decline in t½, and together indicate increment of RBC aggregation.

Oxidative-antioxidative status, blood lactate concentrations and hematocrit

TOS, TAS, OSI, blood lactate concentrations and Hct of the subjects in response to upper body WAnT are presented in Table 3. A single session of exercise resulted in statistically significant acute increments in TOS (p=0.034), Hct (p=0.007) and blood lactate concentrations (p=0.0001). The rise observed in TAS and the decrement in OSI did not reach statistically significant level (p>0.05).

Table 1. Demographic data, muscle thickness and upper body WAnT data of the subjects.

Parameters	Mean±SE
Age (years)	21.86±0.55
Body height (cm)	176.29±1.57
Body weight (kg)	73.86±2.09
BMI (kg/cm^2)	23.71±0.38
Percentage body fat (%)	14.41±0.8
Right arm flexor muscle (m. biceps brachii and m. brachialis) thickness (cm)	2.45±0.05
Left arm flexor muscle (m. biceps brachii and m. brachialis) (cm)	2.35±0.07
Peak power (watt)	593.95±36.78
Average power (watt)	306.82±14.34
Minimum power (watt)	128.89±8.10
Fatigue index (%)	77.60±1.80

Values are expressed as means±SE; n=14 BMI: body mass index, WAnT: Wingate anaerobic power and capacity test.

Table 2. Hemorheological parameters of the study population.

	Before upper body WAnT	After upper body WAnT	*p*
Erythrocyte deformability (EI) measured shear stress (Pa)			
0.30	0.058±0.003	0.049±0.002*	**0.022**
0.53	0.126±0.004	0.109±0.003*	**0.012**
0.95	0.225±0.005	0.204±0.004*	**0.007**
1.69	0.335±0.006	0.296±0.016*	**0.011**
3.0	0.439±0.005	0.420±0.004*	**0.020**
5.33	0.516±0.003	0.502±0.003*	**0.011**
9.49	0.561±0.002	0.549±0.002*	**0.001**
16.87	0.592±0.002	0.577±0.003*	**0.0001**
30.00	0.607±0.003	0.579±0.012*	**0.001**
Erythrocyte aggeration values			
AI (%)	56.226±2.256	62.026±2.414*	**0.001**
t ½ (sn)	3.218±0.336	2.521±0.291*	**0.003**

Values are expressed as means ± SE; n=14 WAnT: Wingate anaerobic power and capacity test, EI: elongation index, Pa: pascal, AI: aggregation index, t ½: aggregation half time. * $p<0.05$: difference from pre-exercise value.

Table 3. TOS, TAS, OSI, blood lactate concentrations and hematocrit values of the subjects.

	Before upper body WAnT	After upper body WAnT	*p*
TOS (μmol H_2O_2 Equiv. /L)	3.99±0.28	4.81±0.50*	**0.034**
TAS (mmol Trolox Equiv./L)	1.05±0.16	1.34±0.15	0.158
OSI (arbitrary unit)	0.75±0.37	0.44±0.08	0.875
Lactate (mmol/L)	1.65±0.12	8.85±0.48*	**0.0001**
Hematocrit (%)	49.85±0.76	52.28±0.77*	**0.007**

Values are expressed as means ± SE; n=14 WAnT: Wingate anaerobic power and capacity test, TOS: The total oxidant status, TAS: The total antioxidant status levels, OSI: The oxidative stress index. * $p<0.05$: difference from pre-exercise value.

Correlations of the data

Post-exercise analysis showed that there was a negative correlation between fatigue index and blood lactate concentration (p=0.002, r=-0.773) (correlation data were not shown). On the other hand, there were no statistically significant correlations between muscle thickness and parameters of blood rheology and oxidative stress (p>0.05).

Discussion

Main findings

Results of the current study show for the first time that, a single bout of upper body WAnT acutely alters blood rheology (decreases RBC deformability and increases erythrocyte aggregation) in untrained individulas. The upper body WAnT applied also results in increment of oxidative stress, Hct, and nearly 5-fold augmentation in blood lactate concentrations.

Suggested mechanisms

The participants perceived the performed upper body WAnT experience as strenuous and barely tolerable. Increased blood lactate concentrations also clearly indicate the high level of metabolic load induced by this exercise protocol. In line with our results, it was previously shown that strenuous exercise is followed by a series of alterations in hemodynamic conditions. For instance, increased flow and shear forces within the circulation would be expected to lead to recruitment of sequestered RBC in various circulatory beds. Plasma volume alterations and

increased damage and/or removal of circulating RBC may also contribute to these alterations [12, 13].

Similar to our results, exercise is known to cause hemoconcentration [26-27] which leads increased RBC aggregation [28]. Mechanical properties of RBC are dependent on the proper metabolic conditions and a normal homeostasis in their microenvironment [17]. Oxidative stress is accepted as one of the determinants of erythrocyte deformability and aggregation [29]. Exercise-induced oxidative stress may increase RBC aggregation by increasing plasma fibrinogen levels [30]. Several reports indicate exercise induced acute enhancements in fibrinogen concentrations [30-31]. Thus, it can be speculated that increased plasma fibrinogen concentration might-at least partly-be responsible for the augmentation observed in RBC aggregation.

Physical activity was shown to lead oxidative stress via increasing metabolic processes which can be reversed by antioxidant defence system [32]. Acute exercise is usually known to yield increment in oxidative stress [33], while prolonged exercise induces antioxidant defence system [34]. Alterations in oxidative stress parameters following the upper body WAnT has not been investigated before. In the current study, we did not determine the individual enzyme levels, but showed the total oxidant / antioxidant status of the organism. The rise observed in erythrocyte aggregation and the decline in RBC deformability following upper body WAnT can also be explained by the increased oxidative stress level [29]. The fatigue Index, which can be defined as the percentage of power output drop throughout the test from the maximal power output was negatively correlated with blood lactate concentration. This data may indicate that the individual producing less lactate during the test loses more power.

Practical applications

The upper body WAnT protocol used in this study is a well-known type of heavy anaerobic exercise which may therefore be accepted as a model of unexpected, forced heavy physical exercise that might be encountered by any person under demanding conditions [1]. Although the anaerobic lactic system is the predominant energy system during the upper body WAnT, the aerobic system also provides a small contribution to the overall energy requirements [11]. Here, we present that a single bout of upper body WAnT may acutely alter blood rheological properties in sedentary subjects by decreasing RBC deformability and increasing erythrocyte aggregation. This may lead a hemorheological extra load. On the other hand, there are studies in the literature that discuss the potential benefits of acute rigid RBC [35, 36]. It is worthy to note that an acute decrease in RBC deformability as observed in our study can be beneficial due to (i). increment of RBC transit time to pass through capillaries [35, 37], and; (ii). reduction of the distance between vessel wall and the erythrocyte [35, 38]. There is also evidence that the increased blood viscosity during exercise might provide enhanced stimulation of the endothelial production of vasoactive substances that would promote tissue perfusion [36]. Our results also show that antioxidant therapy can be recommended before heavy physical exercise episodes.

Strengths and limitations

Current study presents short term responses to upper body WanT in untrained healthy individuals for the first time in the literature. Decrement of RBC deformability was also shown immediately after lower body WAnT for at least 12 hours. On the contrary, erythrocyte aggregation was found to be reduced after lower body WAnT [15]. RBC aggregation was assessed by a photometric aggregometer in this previous study [15]. On the other hand, time course of alterations in response to upper body WanT was not studied in the current study.

RBC deformability was measured between shear stresses of 0.3 and 30 Pa, if measurements were extended to higher shear stresses (50 Pa) this would allow us to ensure that a ceiling effect does not impact the results. Additionally, the physiological meaning of the hemorheological alterations observed following upper body WanT remains unclear. Our participants were untrained healthy male volunteers. The rheological changes may indicate flow redistribution in some parts of the microcirculation and should have a physiological meaning that is unknown at this time. Moderate impairments in blood fluidity may not impair blood flow but adapt it to specific conditions [35,

36]. To get more relevant information about the effects of high intensity anaerobic exercises, the alterations in hemorheological parameters and oxidative stress indices of sportsmen in response to WAnT may also be investigated.

Conflicts of interest: The authors declare no potential conflicts of interest.

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Ethics approval: Pamukkale University Non-Interventional Clinical Research approved by the Ethics Committee (Date: 07/07/2017, number: 60116787-020/44167).

This study was supported by Pamukkale University Scientific Research Projects Coordination Unit through project number 2017HZDP028.

Acknowledgements

The 1st and the 2nd authors contributed equally to this work. The research was conducted by Pamukkale University; Laboratory of Physiology and Laboratory of Sport Sciences Faculty. Part of this work was presented as poster at 43rd National Physiology Congress, Denizli, 7-10 September 2017, Acta Physiologica, Special Issue Turkish Society of Physiological Sciences Volume 221, Supplement S713, September 2017, Page 102.