Effects of Short Duration Supramaximal Exercise on Plasma Atrial Natriuretic Peptide Concentrations in Healthy Subjects

Sağlıklı Kişilerde Kısa Süreli Supramaksimal Egzersizin Plazma Atriyal Natriüretik Peptid Düzeyine Etkisi

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ABSTRACT Objective: Atrial natriuretic peptide (ANP) is a hormone with biological effects, including natriuresis, diuresis, and vasodilation. The aim of the study was to determine plasma ANP, hemotocrit and blood lactate concentrations after supramaximal exercise in healthy subjects. **Material and Methods:** The study group was consisted of 15 male volunteers; mean age 20.3 ± 2.2 years. Venous blood samples for measuring plasma ANP, blood hematocrit and blood lactate concentrations were taken from the subjects prior to 30 second supramaximal exercise and 5th and 15th minute after the end of exercise. Plasma ANP concentrations were measured by enzyme immunoassay method. **Results:** When compared to pre-exercise plasma ANP concentrations (55.39 ± 14.15 pg/ml), significant increase was observed both at 5th minute (93.47 ± 21.83 pg/ml) and 15th minute (104.29 ± 23.55 pg/ml) after the end of exercise (< 0.01). Plasma ANP concentrations were inversely related with hematocrit concentrations prior to exercise (r= -0.56, p= 0.02), at 5th minute (r= -0.76, p= 0.01), and at 15th minute (r=-0.66, p=0.07) after the end of exercise. There was no significant relationship between ANP and lactate concentrations at 5th minute (r= -0.34, p= 0.21) and 15th minute after the end of exercise (r= -0.24, p= 0.38). **Conclusion:** The study demonstrated that plasma ANP concentrations increased in recovery period following short duration supramaximal exercise. Exercise periods with short duration but high intensity could be an effective factor for high concentrations of plasma ANP levels in exercise recovery. Plasma ANP and hemotocrit concentrations increased in recovery period following supramaximal exercise. On the other hand, our study demonstrated that hematocrit concentrations were inversely related with the plasma ANP levels before and after the exercise.

Key Words: Atrial natriuretic peptide (ANP); exercise; hematocrit; lactate

ÖZET Amaç: Atriyal natriüretik peptid (ANP), natriürez, diürez ve vazodilatasyon gibi biyolojik etkileri olan bir hormondur. Bu çalışmanın amacı sağlıklı kişilerde supramaksimal egzersiz sonrası plazma ANP, hemotokrit ve kan laktat konsantrasyonlarını belirlemektir. **Gereç ve Yöntemler:** Çalışma grubu yaş ortalamaları 20.3 ± 2.2 olan, 15 gönüllü erkek katılımcıdan oluştu. Plazma ANP, kan hematokrit ve kan laktat konsantrasyonlarını ölçmek için katılımcıların kan örnekleri 30 saniye süren supramaksimal egzersiz öncesinde ve egzersiz sonrası 5. ve 15. dakikalarda alındı. Plazma ANP düzeyi enzim immunoassey metodu kullanılarak ölçüldü. **Bulgular:** Egzerzis öncesi plazma ANP düzeylerinin (55.39 ± 14.15 pg/ml), egzersiz sonrası 5. dakika (93.47 ± 21.83 pg/ml) ve 15. dakika (104.29 ± 23.55 pg/ml) ile karşılaştırıldığında anlamlı düzeyde artmış olduğu gözlendi (< 0.01). Plazma ANP düzeyi egzersiz öncesi (r= -0.56, p= 0.02), egzersiz sonrası 5. dakika (r= -0.76, p= 0.01) ve egzersiz sonrası 15. dakikada (r= -0.66, p= 0.07) hematokrit düzeyi ile ters ilişkili bulundu. Egzersiz sonrası 5. dakikada (r= -0.34, p= 0.21) ve 15. dakikada (r= -0.24, p= 0.38) plazma ANP ve laktat konsantrasyonları arasında anlamlı ilişki saptanmadı. **Sonuç:** Çalışmamız plazma ANP düzeyinin kısa süreli supramaksimal egzersizi takiben dinlenim döneminde artmış olduğunu gösterdi. Kısa süreli fakat yüksek yoğunluklu egzersiz periyodu, egzersiz sonrası dinlenimde saptanan yüksek plazma ANP konsantrasyonları için etkin bir faktör olabilir. Supramaksimal egzersizi takiben dinlenim periyodunda plazma ANP ve hematokrit konsantrasyonları arttı. Diğer yandan çalımamız hematokrit konsantrasyonunun egzersiz öncesi ve sonrası plazma ANP düzeyleri ile ters ilişkili olduğunu gösterdi.

Anahtar Kelimeler: Atriyal natriüretik peptit (ANP); egzersiz; hematokrit; laktat

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Atrial natriuretic peptide (ANP) is a natriuretic, diuretic and vasodilator hormone that is synthesized by cardiac myocytes.[1] ANP produces a wide range of physiological effect such as modulation of body fluid, anti-hypertrophy, anti-inflamation.[2] Alterations in plasma concentrations of ANP following submaximal and maximal exercise have been investigated in various studies.[3-6] However, the responses of plasma ANP levels to supramaximal exercise are not known. Supramaximal exercise could be described as high intensity exercise which requires effort greater than maximal aerobic capacity and is usually achived by many sport activities such as football, volleyball, basketball, wrestling and tennis. These periods may enhance plasma ANP levels and may cause different biological effects.

ANP has been reported to have negative inotropic effects on heart.[7] The effects of this hormone on cardiac contractility are suggested to be concentration dependant.[8] Therefore, understanding the alterations of ANP concentrations with different intensities of exercise is considered to be important.

A recent study has suggested that ANP could be a factor for the difference of blood hematocrit concentrations in exercise recovery.[9] It has been shown that blood hematocrit concentration increases following the exercise.[10,11] Only a few minutes of heavy exercise is adequate for higher hematocrit concentrations.[12] The increases in plasma viscosity and hematocrit have been attributed to exercise-induced haemoconcentration.[13] The main reason of exercise induced hemoconcentration is explained as the fluid shift to the intracellular space due to cells rich in lactate that may increase cell osmolality.[12] Knowledge about the relationship between ANP and hematocrit concentrations that is an indicator of hemoconcentration after exercise is limitted.

The aim of the present study was to demonstrate the alteration of plasma ANP, hemotocrit and lactate concentrations after short duration supramaximal exercise, and secondly to investigate the relationship between plasma ANP and hematocrit concentrations.

MATERIAL AND METHODS

STUDY POPULATION

Fifteen healthy male undergraduate students attending physical education department volunteered to participate in the study. The ages of all subjects ranged from 18 to 26 years (mean 20.3 ± 2.2 year). All subjects were nonsmokers and took no medication. No subjects were declared any illnesses. Subjects were excluded from the study if they had had any family history of sudden death or any personal history of hypertension, diabetes or syncope. Participants underwent to a comprehensive physical examination. Details of the study were explained to each subject and signed informed consent was obtained. The study was approved by the Local Ethics Committee of Trakya University Faculty of Medicine.

STUDY DESIGN

Height, weight, body fat, blood pressure measurements and ECG recordings (Cardioline Delta 1 Plus, Bologna, Italy) of the subjects were performed just before the exercise test. Body fat measurements were made by bioelectrical body impedance analysis (BIA) using a body fat analyzer (model Tanita TBF300 Japan).

Venous blood samples were taken from the subjects to determine ANP, hematocrit and blood lactate concentrations. Before the exercise began, an indwelling polyethylene catheter inserted into the antecubital vein. Blood samples were taken just before the exercise, 5th and 15th minute after the end of exercise. The subjects were waited at sitting position until the third blood sample was taken. After each blood sample was taken, blood lacate levels were measured immediately by using a portable blood analyzer (Lactate-pro, Arkray, KDK Corporation, Kyoto, Japan). The rest of blood sample was divided into two tubs. One of the tubes contained ethylenediaminetetraacetic acid and it was centrifuged at 3.000 × g for 10 minutes at 4°C to remove plasma. Plasma samples were stored at -80°C until analysis. Plasma ANP concentrations was measured by an enzyme immunoassay human kit (a- Atrial Natriuretic Polypeptide (1-28) EIA Kit) according to the instructions on the manufacturer

(Phoenix Pharmaceuticals California, USA). The range of assay was 0-25 ng/ml and the lineer range was 0.07-1.56 ng/ml. The sensitivity of the assay was 0.07 ng/ml and the intra- and inter-assay coefficients of variation were lower than 5% and 14%, respectively. The second tub was a heparinized capillary tube. It was filled with the venous blood and sealed at the bottom for hemotocrit measurement. The tubes were centrifuged at 1.000 × g for 5 minutes and the height of the red cell column is measured as a percent of the total blood column.

EXERCISE TEST

The Wingate cycle ergometer test is used to determine subject's anaerobic capacity. In some studies, this test was used to demonstrate the responses to supramaximal exercise.[10] The Wingate test consisted of a 30-s supramaximal cycling against a resistance load.[14] Each test was performed on a Monark cycle ergometer (Model 894-E, Sweden) and for each participant the load was calculated as 0.075 kg x.kg^{-1} body mass. The subjects warmed up by pedaling for 3 minutes against a 30 watt load. After 5 minutes rest period, by the command "start" the subject began pedaling as fast as possible against a predetermined work load until the end of the test period. Strong motivation was given verbally to subjects to maintain maximal pedaling rate during the test. The data were used to calculate peak power and mean power as in previous studies.[14]

STATISTICAL ANALYSIS

Based on data from a previous report,[15] the intention was to detect a difference between the ANP concentration before exercise (6.78 ± 3.26 fmol/ml) and 15th minute ANP concentration after the exercise (13.54 ± 7.27 fmol/ml), with a= 5% and power= 80% suggested in a study population of 13 subjects.

The numeric results were expressed as mean ± SD, and categorical results were expressed as a number (percentage). Normality distribution of the variables was tested by using One Sample Kolmogorov Smirnov test. Comparison of before exercise, 5th minute after the exercise, and 15th minute after the exercise scores of patients was evaluated by repeated measures analysis of variance (ANOVA) test for normal distributed data, Freidman ANOVA test for non-normal distributed data, and then Bonferroni post-hoc test was used when the significance difference was obtained. The Pearson or the Spearman rank correlation analysis was performed to examine the relationships between variables.

A p-value < 0.05 was considered statistically significant. Statistica 7.0 (StatSoft Inc., Tulsa, OK, USA) was used for statistical analyses.

RESULTS

Mean weight of the subjects was 80.9 ± 13.8 kg (range: 61.0-117.0), mean height was 181.8 ± 8.2 cm (range: 168.0-197.0), mean body mass index was 24.4 ± 3.4 kg/m^2 (range: 21.0-33.0) and mean body fat ratio was 16.4 ± 5.8 % (range: 11.0-31.0). No abnormality was found in systolic and diastolic blood pressures and ECG recordings in subjects. Mean heart rate was 66.27 ± 8.57 beats per minute, mean systolic and diastolic blood pressure was 117.00 ± 7.74/73.00 ± 5.27 mmHg prior to exercise. The peak power (715.4 ± 152.5), mean power (574.5 ± 123.9) and anaerobic fatigue (0.34 ± 0.1) values recorded during Wingate test.

The ANP, hematocrit and lactate concentrations were determined before exercise, 5 and 15 minutes after exercise test (Table 1). Statistically significant differences were obtained amongst pre-exercise, 5th minute and 15th minute values in plasma ANP, hematocrit and lactate concentrations. When compared to pre-exercise levels, plasma ANP concentrations were found to increase at 5th and 15th minutes following the exercise.

In this study, an inverse correlation (r= -0.56, p= 0.02, Figure 1) between pre-exercise ANP and hemotocrit concentrations was found. On the other hand, strong negative correlation were demonstrated between ANP and hematocrit concentrations at 5th minutes after exercise (r= -0.76, p= 0.01, Figure 2) and 15th minutes after exercise (r= -0.66, p= 0.07, Figure 3). There was no significant correlation between pre-exercise ANP and lactate concentrations (r= 0.16, p= 0.56). Similarly, no significant relationship was shown between ANP and lactate concentrations at 5th minute (r= -0.34, p=

TABLE 1: Plasma atrial natriuretic peptid (ANP), lactat and hemotocrit concentrations of subjects.

	Pre-exercise	5th minutes after the end of exercise	15th minutes after the end of exercise		P
ANP pg/ml	55.39 ± 14.15	93.47 ± 21.83[a]	104.29 ± 23.55[a,b]	F= 103.9; df= 2	<0.001
Lactat mmol/lt	1.50 ± 1.25	8.15 ± 1.83[a]	5.6 ± 1.69[a,b]	Freidman χ^2= 28.1; df= 2	<0.001
Hematocrit %	45.2 ± 2.37	48.1 ± 2.97[a]	46.9 ± 1.50[a,b]	F= 27.3 ; df= 2	<0.001

Data are expressed as the mean ± SD

[a]: p< 0.001 when compared with pre-exercise

[b]: p< 0.001 when compared with 5th minutes after the end of exercise.

0.21) and 15th minute after the end of exercise (r= -0.24, p= 0.38).

DISCUSSION

The alterations of plasma ANP concentrations following short duration supramaximal exercise were examined in the present study. Our results indicated that plasma ANP levels increased in healthy subjects following the supramaximal exercise. The changes of plasma ANP after exercise shows different results depending on intensity, type and duration of exercise. Plasma ANP levels did not change significantly after mild intensity exercise according to a previous study.[16] On the other hand, it has been demonstrated that plasma ANP concentrations were increased up to 30% following the maximal aerobic exercise.[4] Mandroucas et al[3] reported 96 % increase in plasma ANP concentrations following maximal exercise. They have shown that in comparison to submaximal level, maximal exercise was the main stimulus for elevated plasma ANP concentrations. In our study, 69% and 88% increase was present in plasma ANP concentrations at 5th minutes and 15th minutes after the end of supramaximal exercise, respectively. Our results indicated that exercise intensity appeared to be an effective factor for ANP secretion.

Post exercise blood samples were taken at 5th and 15th minutes after the end of exercise (recovery) in the present study. Therefore, blood samples were assessed at two different durations in recovery. ANP concentrations were higher at both the 5th and 15th minutes than resting values (Table 1). A previous study, which compare plasma ANP concentrations at 10th minute following the exercise with immediately after the end of exercise reported significant reduction.[3] However, our findings were not in agreement with previous observations suggesting an immediate decrease in

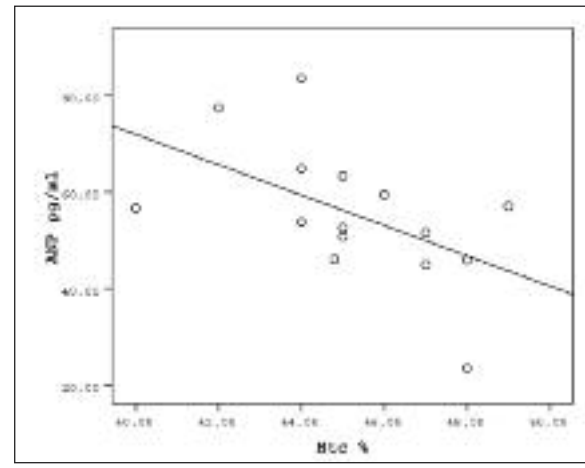

FIGURE 1: Correlations of plasma atrial natriuretic peptid (ANP) with hemotocrit (Htc) concentrations before exercise (r= -0.56, p= 0.02).

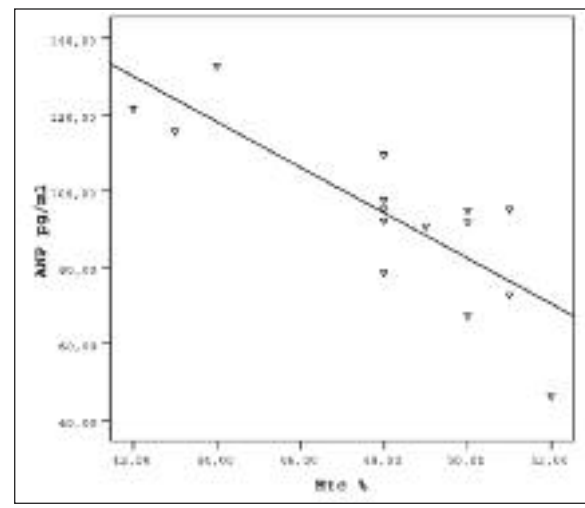

FIGURE 2: Correlations of plasma atrial natriuretic peptid (ANP) with hemotocrit (Htc) concentrations five minute after the end of exercise (r= -0.76, p= 0.01)

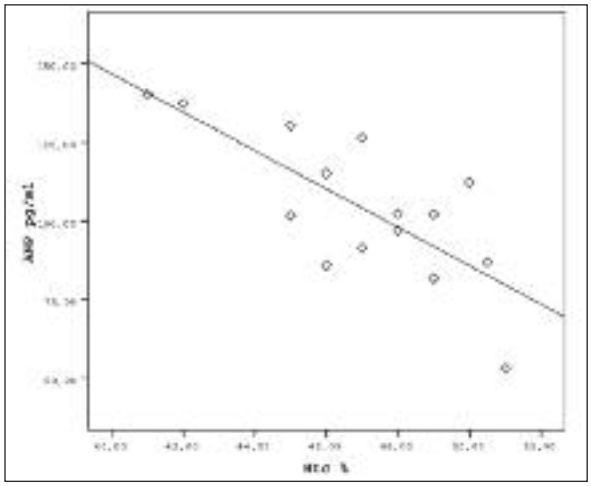

FIGURE 3: Correlations of plasma atrial natriuretic peptid (ANP) with hemotocrit (Htc) concentrations fifteen minute after the end of exercise (r= -0.66, p= 0.07)

plasma ANP concentrations.[3,17] According to the present study, short duration supramaximal exercise causes the increase in ANP secretion to continue for at least 15 minutes in recovery period.

Several factors may be responsible for the ANP secretion during and subsequent to the exercise. The increase in plasma norepinephrine have been suggested as an important factor for the release of ANP in response to exercise in healthy subjects.[18] It has been reported that an increased norepinephrine concentration was significantly related with an increased ANP level after short-term exercise testing.[16,18] On the other hand, it is known that ANP inhibits sympathetic nerve activity.[19-20] Therefore, ANP secretion following the exercise may play a feedback modulator role in the regulation of sympathetic activity.

The type of exercise may be accepted as another factor affecting the alterations in plasma ANP concentrations. In a recent study, increase in plasma ANP concentrations have been found to be different in arm exercise from leg exercise.[21] In addition, a significant postural influence was reported on plasma ANP concentrations.[21] In our study, all subjects have made leg exercise in a sitting posture in order to avoid any alteration that may result from different postures and the use of different extremities following exercise.

Secondly, we investigated whether there was a relationship between plasma ANP and hematocrit concentrations before and after exercise. Surprisingly, plasma ANP concentrations were negatively associated with hemotocrit concentrations both before and after exercise in our study. It was found in the present study that the higher is the ANP level in blood samples, the lower are the hemotocrit levels. Negative association between plasma ANP and hemotocrit concentrations following the exercise was more pronounced than the values obtained before the exercise. Despite a medium association (= -0.56, p= 0.02) that was shown before exercise, strong negative correlations were obtained at 5th minute (r= -0.76, p= 0.01) and 15th minute after the end of exercise (r= -0.66, p= 0.07) between plasma ANP and hemotocrit concentrations. However, hemotocrit increase was observed together with ANP increase at 5th minute after exercise. At the 15th minute hemotocrit level tended to decrease despite ANP increase, which may indicate that negative relation between ANP and hemotocrit is independent from hemotocrit change induced by exercise. Similar to our study, Fukuta et al.[22] firstly demonstrated that ANP concentrations were inversely related with plasma hemoglobin concentrations and anemia was an independent predictor for eleveted plasma ANP concentrations in coronary artery patients. According to this study, the relationship between ANP and hemoglobin was independent from other cardiac and noncardiac determinants of natriuretic peptide concentrations, including left ventricul end-diastolic pressure and body mass index.[22]

Physiological alterations such as acidosis occur during and subsequent to exercise. Mandroukas et.al. [3] reported an increase in plasma ANP levels together with higher blood lactate levels after exercise. Similarly, ANP and lactate levels were found to increase at 5th minute after exercise in our study. However, our results showed no marked relation between plasma ANP and lactate concentrations

The effect of supramaximal exercise on ANP, hematocrit and lactate concentrations were investigated using Wingate test. However, there are seve-

ral limitations in the present study. First, we could not determine plasma viscosity and red blood cell rigidity that were suggested to be other physiological adaptive modificators in exercise induced hemoconcentration.[23] Secondly, determining the plasma K^+ ion concentrations could be helpful to understand whether the extracelluler fluid volume decrease in exercise induced hemoconcentration.[12]

In conclusion the present study demonstrated short duration and high intensity exercise could be an effective factor for high concentrations of plasma ANP levels in recovery period of exercise. Plasma ANP concentrations could not decrease at 15th minute after the exercise. In addition to plasma ANP, hemotocrit concentrations increased in recovery period following supramaximal exercise. On the other hand, according to the findings of our study, hematocrit concentrations were inversely related with the plasma ANP concentrations both before and after the exercise. Our study suggests that higher ANP secretion subsequent to exercise could not seem to be related with blood lactat levels.

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