



# Resting Heart Rate is Not Associated with Oxidative Stress in Healthy Adults

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## ABSTRACT

**Introduction:** Although resting heart rate (RHR) and oxidative stress are risk factors for cardiovascular morbidity and mortality, the association between them has not been fully understood. The aim of the present study was to investigate the relationship between RHR and oxidative/antioxidative stress markers.

**Patients and Methods:** The study consisted of 56 healthy volunteers (33 males; mean age: 44.1 ± 9.0 years). Subjects were divided into two groups according to heart rate quartiles: lower two quartiles as group 1 (n= 29) and upper two quartiles as group 2 (n= 27). We measured total oxidant status (TOS), total antioxidant capacity (TAC), oxidative stress index (OSI), and ceruloplasmin (CP) levels of subjects.

**Results:** There was no any significant difference in baseline clinical characteristics and laboratory measurements between the groups (p> 0.05 for all variables). Mean RHRs were 71.3 ± 4.6 in group 1 and 82.4 ± 4.1 in group 2. The TAC, TOS, OSI, and CP levels were similar between the two groups (p> 0.05 for all variables). We did not detect any significant association between RHR and oxidative/antioxidative parameters.

**Conclusion:** RHR was not associated with TOS, TAC, OSI, and CP levels in our study.

**Key Words:** Resting heart rate; oxidative stress; total oxidant status; total antioxidant status; oxidative stress index; ceruloplasmin

## Sağlıklı Erişkinlerde İstirahat Kalp Hızı Oksidatif Stresle İlişkili Değildir

### ÖZET

**Giriş:** Her ne kadar istirahat kalp hızı (İKH) ve oksidatif stres, kardiyovasküler morbidite ve mortalite için risk faktörleri olsa da bunlar arasındaki ilişki tam olarak anlaşılamamıştır. Bu çalışmamızda, İKH ile oksidatif/antioksidatif stres belirteçleri arasındaki ilişkiyi araştırmayı amaçladık.

**Hastalar ve Yöntem:** Çalışma 56 sağlıklı gönüllüden (33 erkek; ortalama yaş: 44.1 ± 9.0 yıl) oluşmaktaydı. Gönüllüler kalp hızı çeyrekliklerine göre iki gruba bölündüler: alt iki çeyreklik grup 1 (n= 29) ve üst iki çeyreklik grup 2 (n= 27). Hastaların total oksidan kapasitesi (TOK), total antioksidan kapasitesi (TAK), oksidatif stres indeksi (OSI) ve serum seruloplazmin düzeylerini ölçtük.

**Bulgular:** Bazal klinik karakteristikler ve laboratuvar ölçümleri açısından iki grup arasında önemli bir fark yoktu (p> 0.05 tüm değişkenler için). Ortalama İKH grup 1'de 71.3 ± 4.6 iken grup 2'de 82.4 ± 4.1 idi. TOK, TAK, OSI ve seruloplazmin düzeyleri her iki grupta benzerdi (p> 0.05 tüm değişkenler için). İKH ve oksidatif/antioksidatif stres parametreleri arasında anlamlı bir ilişki saptayamadık.

**Sonuç:** Çalışmamızda İKH, TOK, TAK, OSI ve seruloplazmin düzeyleri ile ilişkili bulunmadı.

**Anahtar Terimler:** İstirahat kalp hızı; oksidatif stres; total oksidan kapasitesi; total antioksidan kapasitesi; oksidatif stres indeksi; seruloplazmin

## INTRODUCTION

Resting heart rate (RHR) is associated with cardiovascular morbidity and mortality, independently of other risk factors, for example, age, smoking, hypertension, and diabetes in some clinical studies<sup>(1-5)</sup>. In human and animal studies, it was reported that an increased heart rate is associated with atherosclerosis<sup>(6-8)</sup>. An abnormality of the autonomic nervous system, such as sympathetic overactivity, is thought to be the underlying mechanism<sup>(9,10)</sup>. Experimental and clinical studies suggested the favorable effect of heart rate reduction in the progression of atherosclerosis<sup>(7,11,12)</sup>.

Oxidative stress is known as dysfunction in balance between reactive oxygen species (ROS) production and antioxidant activity. It was associated with the pathogenesis of many

*Cite this article as: Baysal SS. Resting heart rate is not associated with oxidative stress in healthy adults. Koşuyolu Heart J 2019;22(1):20-4.*

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Submitted: 09.07.2018

Accepted: 26.11.2018

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diseases, such as coronary artery disease (CAD)<sup>(13-15)</sup>. It was demonstrated that lowering heart rate reduces the formation of ROS in rats<sup>(16)</sup>. However, there are limited studies that investigated the relationship between heart rate and oxidative stress.

In the present study, we aimed to investigate the relationship between RHR and oxidative/antioxidative stress markers.

## PATIENTS and METHODS

### Study Design and Setting

A total of 56 healthy individuals whose ages were between 18 and 65 years were enrolled randomly into the study. All subjects underwent a detailed medical evaluation including clinical history, physical examination, routine laboratory panel, electrocardiography (ECG), and echocardiography. A 24-hour Holter monitoring was performed by the Promedic HECG-12 Holter management system including Ambulatory ECG Systems software running under Microsoft Windows. All patients were instructed not to exercise, smoke, and drink alcohol or coffee during Holter recording. Recordings were analyzed by two cardiologists blinded to the study. RHR was determined by the mean of the three lowest heart rates obtained from day time (09:00-22:00 h) recordings. Nighttime heart rate was excluded due to concerns regarding the influence of diurnal variation<sup>(17)</sup>. Subjects were divided into two groups according to quartiles of RHR as per most previous heart rate studies. Group 1 consisted of subjects with lower two quartiles heart rate between 60 and 79 beats/min, and group 2 consisted of subjects with upper two quartiles heart rate between 80 and 94 beats/min. Exclusion criteria were CAD, significant valvular heart disease, heart failure (left ventricular ejection fraction < 40%), inflammatory diseases (acute or chronic), smoking, use of any medical drugs that have an impact on heart rate, and hepatic, thyroid, and renal disorders. The ethics committee of Harran University approved the study (7405 9997.050.01.04/107). Written informed consent was obtained from all of the study population.

Complete hematological count, glucose level, lipid profile, liver enzyme level, and creatinine concentration were analyzed in peripheral venous blood samples collected after 12 h of fasting. All biochemical parameters were determined using the Abbott Diagnostics C8000i auto-analyzer (Abbott, Wiesbaden, Germany). Samples were obtained by centrifugation at 3000 rpm for 15 min and stored at -80°C for analysis of oxidative stress biomarkers, total oxidant status (TOS), total antioxidant capacity (TAC), oxidative stress index (OSI), and ceruloplasmin (CP).

### Measurement of Plasma TOS and TAC

The serum TAC and TOS levels were determined by a novel automatic method as developed by Erel<sup>(18,19)</sup>. TAC was calculated by measuring the antioxidative power of the sample

against the hydroxyl radical-initiated reactions. Oxidants, present in the sample, oxidize ferrous ion-dianisidine complex to ferric ion that is colored with xylenol orange in the acidic medium to be measured by spectrophotometer to calculate TOS. TAC is expressed as 1 mmol Trolox equivalent/l where 1  $\mu\text{mol H}_2\text{O}_2$  equivalent/L is used to state TOS.

### Oxidative Stress Index

The ratio of TAC to TOS is defined as OSI, expressed as percentage (%). For calculation, TAC units were changed to mmol/L, and the OSI value was calculated according to the following formula: OSI (arbitrary unit) = TOS (mmol  $\text{H}_2\text{O}_2$  equiv/L)/TAC (mmol Trolox equiv/L).

### Ceruloplasmin

The enzyme activity of CP was determined according to Erel's method<sup>(19)</sup>. Using this assay, ferrous ion is oxidized to ferric ion via CP ferroxidase activity. CP levels are expressed as units per gram protein (U/L).

### Statistical Analysis

All statistical analyses were performed by using SPSS for Windows software (ver. 22.0; SPSS Inc., Chicago, IL, USA). The Shapiro-Wilk test was used to evaluate the normality of distributions of continuous variables. The independent samples t-test was used to compare normally distributed continuous variables, and the Mann-Whitney U test was used to compare non-normally distributed continuous variables. Descriptive statistics were expressed as mean and standard deviation for normally distributed variables and median and minimum-maximum values for non-normally distributed variables. Pearson's correlation coefficients were used for normally distributed variables. A p-value < 0.05 was considered as statistically significant in all analyses.

## RESULTS

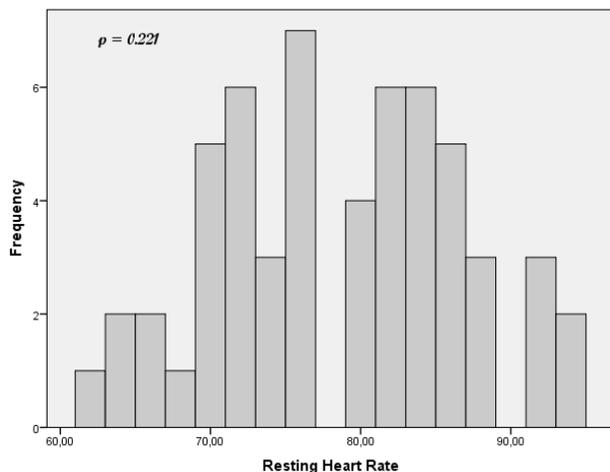
There were 29 (17 male and 12 female) patients in group 1 and 27 (16 male and 11 female) subjects in group 2. The clinical features and laboratory parameters of the study population are presented in Table 1. These groups were similar with respect to age, gender, body mass index (BMI), lipid panel, creatinine, fasting glucose, high-sensitivity C-reactive protein (hs-CRP), and hemoglobin levels. Mean RHRs were  $71.3 \pm 4.6$  in group 1 and  $82.4 \pm 4.1$  in group 2. RHR was normally distributed (Figure 1).

Oxidative and antioxidative stress marker levels are shown in Figure 2. We did not find any significant difference in TOS, TAC, OSI, and CP levels between the groups (p= 0.77, p= 0.69, p= 0.77, and p= 0.54, respectively). When we performed correlation analysis, we did not see any significant correlation between RHR and oxidative/antioxidative stress biomarkers (Table 2).

**Table 1. Baseline clinical and laboratory features of the study population**

	Group 1 (n= 29)	Group 2 (n= 27)	p
Resting heart rate (beats/min)	71.3 ± 4.6	82.4 ± 4.1	< 0.001
Age, years	44.4 ± 9.4	43 ± 8.7	0.11
Female, n (%)	12 (41)	11 (40)	0.71
BMI, kg/m <sup>2</sup>	26.3 ± 2.0	25.6 ± 1.4	0.12
Total cholesterol, mg/dL	196 (165-252)	190 (92-290)	0.42
LDL, mg/dL	100 (76-139)	104 (69-156)	0.77
HDL, mg/dL	39.2 ± 5.7	39.2 ± 8.4	0.99
Triglyceride, mg/dL	156 (58-351)	169 (96-480)	0.72
Fasting glucose, mg/dL	88 (72-100)	91 (75-103)	0.27
Creatinine, mg/dL	0.78 (0.70-0.95)	0.80 (0.65-1.0)	0.30
Hemoglobin, g/dL	15.2 (13.6-17)	15.1 (13.6-17)	0.70
hs-CRP, mg/dL	0.48 ± 0.24	0.43 ± 0.20	0.40

BMI: Body mass index, HDL: High-density lipoprotein, hs-CRP: High-sensitivity C-reactive protein, LDL: Low-density lipoprotein.



**Figure 1.** Histogram of the resting heart rate distribution of the study population.

## DISCUSSION

In our study, we did not find any significant relationship between RHR and oxidative/antioxidative stress parameters. In addition, RHR was not significantly related with age, gender, and BMI.

There is strong evidence proving that an increase in RHR leads to an increased risk of cardiovascular morbidity and mortality especially in patients with hypertension and metabolic syndrome and in the geriatric population<sup>(1,3,4,20,21)</sup>. It was also reported that an increased heart rate is associated with atherosclerosis independently of other risk factors<sup>(6,9)</sup>. An increased heart rate accumulates the power and frequency of the tensile stress on the arterial wall and prolongs the exposure

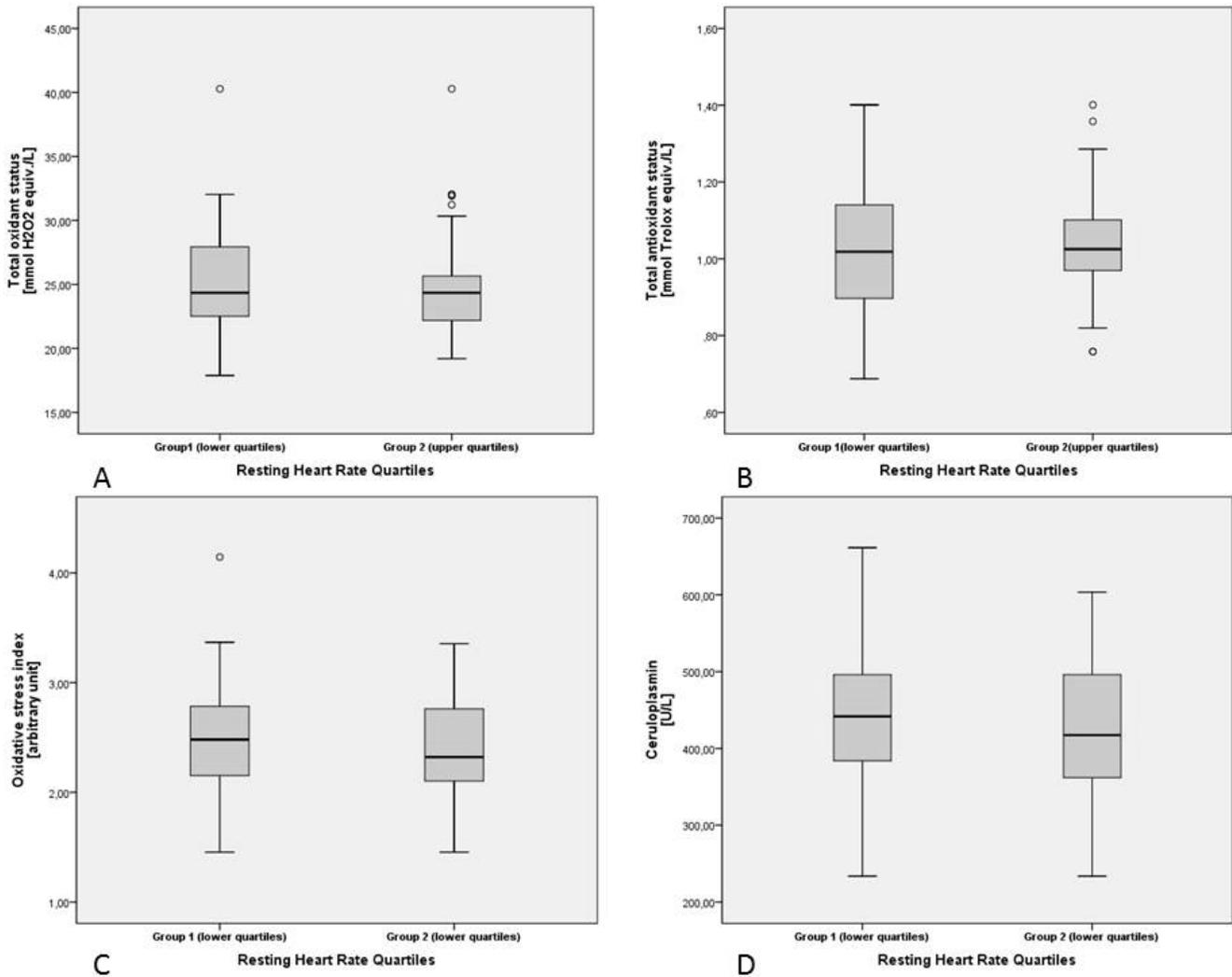
of the coronary endothelium to the systolic low and oscillatory shear stress. These make changes in the structure and function of endothelial cells, promoting atherosclerosis<sup>(6,22,23)</sup>.

An increased heart rate could affect the cardiovascular system in different mechanisms. It may increase myocardial oxygen demand and decrease coronary blood flow by reducing diastolic filling time. It may also reflect autonomic nervous system abnormalities, such as increased sympathetic tone that could lead to cardiovascular morbidity and mortality in ischemic conditions<sup>(6,8,10)</sup>.

Oxidative stress is caused by an increased production and insufficient elimination of ROS. Although the underlying exact mechanism has not been fully discovered, oxidative stress is thought to have a significant function in the pathogenesis of many disorders, such as cardiovascular diseases, hypertension, Parkinson's disease, and Alzheimer's disease<sup>(13-15)</sup>.

There are limited studies investigating the association between RHR and oxidative stress. In an animal study, it was demonstrated that heart rate reduction decreased vascular oxidative stress, improved endothelial function, and inhibited the atherosclerotic plaque formation. The underlying mechanism of this finding is that reduced heart rate decreased superoxide release and lipid peroxidation<sup>(16)</sup>. In contrast with the previous study, in our study, we did not find any association between oxidative/antioxidative stress markers and RHR. This could be explained by a relatively small sample size of our study.

In conclusion, RHR was not significantly associated with oxidative/antioxidative stress parameters (TOS, TAC, OSI, and CP).



**Figure 2.** Oxidative/antioxidative (A: Total oxidant status, B: Total antioxidant status, C: Oxidative stress index, D: Ceruloplasmin) concentration of the study population grouped by heart rate quartiles.

**Table 2. Correlation between resting heart rate and other variables**

	Coefficient*	P
Age, years	-0.153	0.26
BMI, kg/m <sup>2</sup>	-0.213	0.11
Total oxidant status	-0.030	0.82
Total antioxidant status	0.068	0.61
Oxidative stress index	-0.108	0.42
Ceruloplasmin	-0.078	0.56
hs-CRP, mg/dL	-0.254	0.46

\*From Pearson’s correlation analysis.

BMI: Body mass index, hs-CRP: High-sensitivity C-reactive protein.

**Limitations**

Cross-sectional design, relatively small sample, and lack of evaluating heart rate variability are the main limitations of our study. Another limitation of the present study is that patients are not questioned for psychiatric disorders, exercise status, and dietary and social background variables, such as economic and education status that could affect the RHR.

**Acknowledgments**

I thank Harran University Biochemistry Department for analysis of oxidative/antioxidative stress biomarkers.

**CONFLICT of INTEREST**

The author reported no conflict of interest related to this article.

**AUTHORSHIP CONTRIBUTIONS***Concept/Design:* SB*Analysis/Interpretation:* SB*Data Acquisition:* SB*Writing:* SB*Critical Revision:* SB*Final Approval:* SB**REFERENCES**

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