Resting heart rate is associated with novel plasma atherosclerosis biomarkers

Yu Jiang[1], Suhang Shang[2], Liangjun Dang[2], Chen Chen[2], Shan Wei[2], Ling Gao[2], Jin Wang[2], Kang Huo[2], Jingyi Wang[3], Haixia Lu[4], Qiumin Qu[2]

[1]Department of Neurology, 2nd Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China
[2]Department of Neurology, 1st Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China
[3]Huxian Hospital of Traditional Chinese Medicine, Xi'an, China
[4]Institute of Neurobiology, Xi'an Jiaotong University Health Science Center, Xi'an, China

Related article
by Lippi et al.

Correspondence to:
Qiumin Qu, MD, PhD,
Department of Neurology,
1st Affiliated Hospital of Xi'an Jiaotong University,
277 West Yanta Rd, Xi'an 710061, China,
phone: +86 29 8532 4083,
e-mail: quqiumin@xjtufh.edu.cn

DOI: 10.33963/KP.a2022.0188

Received:
March 23, 2022

Accepted:
July 19, 2022

Early publication date:
August 4, 2022

ABSTRACT

Background: Resting heart rate (RHR) is a strong predictor of adverse cardiovascular outcomes. Both soluble low-density lipoprotein receptor-related protein-1 (sLRP1) and soluble receptor for advanced glycation end products (sRAGE) are novel plasma biomarkers for atherosclerosis. In this study, we examined the potential associations between RHR and plasma sLRP1 and sRAGE levels and whether any associations might be modified by apolipoprotein E (*APOE*) ε4 carrier status.

Methods: This cross-sectional study included 941 apparently healthy adults aged 40 years or older. Plasma sLRP1 and sRAGE levels were measured by a commercial enzyme-linked immunosorbent assay. *APOE* gene polymorphisms were analyzed by a polymerase chain reaction and Sanger sequencing.

Results: RHR was a significant determinant of log-transformed sLRP1 (β = 0.004; 95% confidence interval [CI], 0.002–0.007; $P = 0.001$) and log-transformed sRAGE (β = 0.005; 95% CI, 0.002–0.007; $P < 0.001$) independently of age, sex, body mass index, blood pressure, blood glucose, blood lipids, lifestyle, and medical history. Additionally, *APOE* ε4 carrier status was inversely associated with log-transformed plasma sLRP1 level (β = –0.072; 95% CI, –0.130 to –0.015; $P = 0.01$) and did not modify the relationship between RHR and plasma sLRP1 level.

Conclusions: An elevated RHR was associated with increased sLRP1 and sRAGE values, which was not modified by *APOE* genotype. The underlying mechanism of this effect may be relevant to the progression of atherosclerosis.

Key words: apolipoprotein E, atherosclerosis, coronary heart disease, heart rate

INTRODUCTION

Resting heart rate (RHR) is a routinely collected vital sign that is easily and noninvasively measured without requiring special training or equipment. A large body of evidence indicates that an elevated RHR can be considered an important determinant of atherosclerosis and a strong predictor of adverse cardiovascular outcomes [1]. However, the mechanisms underlying these correlations are not fully understood.

Low-density lipoprotein receptor-related protein-1 (LRP1) is a large multifunctional type 1 transmembrane receptor that participates in multiple physiological and pathological processes, including lipid and glucose metabolism, inflammation, atherosclerosis, acute myocardial infarction, myocardial ischemia-reperfusion injury, and adverse left ventricular remodeling [2, 3]. LRP1 β-chain proteolysis results in the release of soluble LRP1 (sLRP1), which can be detected in the plasma. Various *in vitro* models suggested that sLRP1 is a biologically active mediator of several cellular processes linked with atherosclerotic disease [4, 5]. More recently,

WHAT'S NEW?

Resting heart rate (RHR) was independently and positively associated with plasma soluble low-density lipoprotein receptor-related protein-1 (sLRP1) and plasma soluble receptor for advanced glycation end products (sRAGE) levels, and this association was not modified by *APOE* ε4 carrier status. These data suggest a relationship between RHR and atherosclerosis that may be both relevant to the pathology of coronary heart disease (CHD) and clinically useful for identifying patients who are at risk or in the early stages of CHD.

increasing evidence supports the hypothesis that sLRP1 is a novel biomarker for atherosclerosis and is associated with the development of coronary artery disease (CAD) [5, 6].

The receptor for advanced glycation end products (RAGE), which is found on the surface of numerous cells, is also involved in atherosclerosis development via activation of the inflammatory and oxidative stress pathways [7]. The soluble isoforms of RAGE (sRAGE), formed from the cleavage of the native membrane receptor and present in the circulation, can limit these signaling pathways by binding RAGE ligands as decoy receptors. In fact, sRAGE levels were reportedly higher in coronary disease or higher atherosclerotic burden cases than in control subjects in some studies [8], and elevated sRAGE levels are associated with an increased risk of cardiovascular disease [9]. Thus, both sLRP1 and sRAGE are associated with coronary atherosclerosis, but little is known about the association between RHR and plasma levels of sLRP1 and sRAGE.

The human apolipoprotein E (*APOE*) gene exists as three polymorphic alleles including ε2, ε3, and ε4; this polymorphism is among common genetic factors responsible for inter-individual differences in lipid and lipoprotein levels [10]. In several meta-analyses, the ε4 allele was associated with a moderately increased risk of ischemic heart disease, whereas the ε2 allele was associated with reduced risk [10, 11]. Some studies also suggested that *APOE* ε4 carriers are particularly prone to developing disseminated coronary lesions or are at an increased risk of coronary heart disease (CHD)-related mortality [12]. Moreover, synergistic effects were observed between the ε4 allele carrier state and some traditional risk factors, which largely increases an individual's risk of CAD [13].

The primary purpose of this analysis was to explore the relationship between RHR and plasma sLRP1 and sRAGE. We also examined whether any associations might be modified by *APOE* ε4 carrier status. We examined these questions using samples and data from a population-based cross-sectional study of middle-aged and elderly Chinese people.

METHODS

Participants

This study was conducted in the Qubao Village between October 2014 and March 2015 and described in detail elsewhere [14]. Briefly, the inclusion criteria were as follows: (1) age 40 years or older; (2) permanent resident living in Qubao Village for more than 3 years; and (3) consent to participate in the study. Individuals were excluded from the study if they had an acute or chronic infection, or severe cardiac, pulmonary, hematological, hepatic, renal disease, or tumors. Subjects who were taking antihypertensive, antidiabetic, antilipidemic, antithrombotic, nonsteroidal anti-inflammatory drugs, contraceptives, or vitamins were also excluded to avoid the confounding influence of these drugs on RHR and plasma atherosclerosis biomarkers. Subjects for whom at least one RHR, plasma sLRP1, or sRAGE concentration, *APOE* genotype, or covariable measurement was missing were also excluded, leaving an analytic sample of 941 subjects (Figure 1).

RHR measurements

RHR measurements were performed after each blood pressure reading in duplicate and then averaged. Before the measurement, the patients rested for at least 5 minutes and had avoided exercise, caffeine, alcohol, and tobacco use in the previous 12 hours. Blood pressure was measured using a manual mercury sphygmomanometer with a regular adult cuff on the right arm before breakfast in the morning (8:00–10:00 AM), and RHR was measured on the right radial artery for 1 minute by palpation.

Quantification of plasma atherosclerosis biomarkers

Overnight fasting venous blood samples were collected in ethylenediaminetetraacetic acid vacutainer tubes and centrifuged at 3000 × g for 10 minutes at 20°C immediately, and the plasma was separated and stored at –80°C until use. Plasma concentrations of sLRP1 and sRAGE were detected with commercially available quantitative enzyme-linked immunosorbent assay kits (Yuanye Co., Shanghai, China). Measurements were performed using an RT-6000 analyzer (Rayto Co., Shenzhen, China) at 450 nm while sample concentrations were calculated from the standard curve. Duplicate measurements were performed, and the average values were included in the analysis.

APOE genotype

Genomic deoxyribonucleic acid (DNA) was extracted from peripheral blood leukocytes using a TIANamp Genomic DNA Kit (Tiangen Co., Beijing, China). *APOE* gene polymorphisms, rs7412 and rs429358, which determine the *APOE* alleles ε2, ε3, and ε4, were analyzed by a polymerase chain reaction and Sanger sequencing (Sangon Co., Shanghai, China).

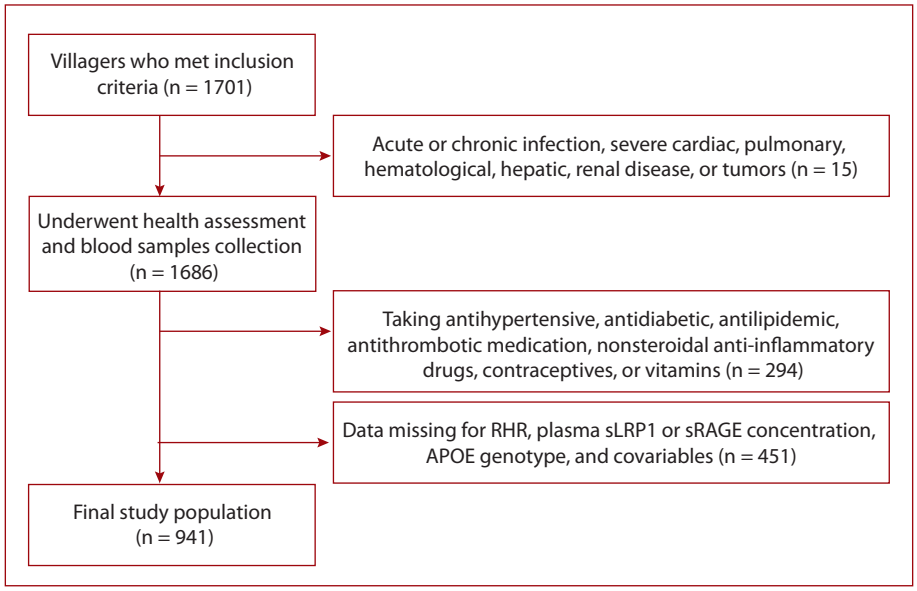

Figure 1. Flow chart showing the selection process for the present study

Abbreviations: RHR, resting heart rate; sLRP1, soluble low-density lipoprotein receptor-related protein-1; sRAGE, soluble receptor for advanced glycation end products

Demographic, clinical, and biological data collection

A standardized questionnaire was administered to collect the participants' demographic data (age, sex, and education years) and clinical data, including medical history (hypertension, diabetes mellitus, dyslipidemia, CHD, and transient ischemic attack [TIA], or stroke), medication use (antihypertensive, antidiabetic, antilipidemic, antithrombotic medication, nonsteroidal anti-inflammatory drugs, contraceptives, and vitamins), and lifestyle habits (smoking, drinking alcohol, and physical activity level). Body weight and height were measured, and body mass index (BMI) was calculated as weight/height squared (kg/m^2). Biochemical parameters such as fasting blood glucose, serum total low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, total cholesterol, and triglycerides were determined from non-fasting blood samples. A history of CHD was defined as any of the following: (1) a previous diagnosis of myocardial infarction; (2) known angina pectoris; (3) a history of hospitalization for unstable angina; or (4) previous coronary revascularization. The definitions of hypertension, diabetes, and hyperlipidemia were the same as in our previous study [14].

Statistical analysis

Continuous variables are presented as mean (standard deviation) or median (interquartile range), while categorical variables are presented as numbers (percentage). RHR values were grouped into thirds of their distribution. One-way analysis of variance (ANOVA) or the Kruskal-Wallis and χ^2 tests were used for the group comparisons. Next, a *post hoc* Bonferroni adjusted test was used to analyze differences in plasma sLRP1 and sRAGE levels in the lowest, medium, and highest tertiles. Differences between *APOE* ε4 non-carriers and *APOE* ε4 carriers were compared using an unpaired Student t-test, the Mann-Whitney U-test, or the χ^2 test as appropriate.

The binary logistic regression analysis was performed in the univariable and multivariable analyses to identify risk factors for CHD. In particular, three explanatory variables with a *P*-value of <0.1 were included in the multivariable logistic regression model. Because of their non-Gaussian distribution, plasma levels of triglycerides, fasting blood glucose, sLRP1, and sRAGE were log-transformed before the statistical analysis. Correlations between RHR and plasma biomarkers were examined using partial rank correlation coefficients, with adjustment for age and sex, and then additionally for BMI, systolic blood pressure, diastolic blood pressure, fasting blood glucose, triglyceride level, total cholesterol level, high-density lipoprotein cholesterol level, smoking, drinking, physical activity level, TIA or stroke, and CHD.

Multiple linear regression models were used to examine the associations between RHR and plasma levels of sLRP1 and sRAGE. Given that the *APOE* ε4 allele may exert differential influences on plasma atherosclerosis biomarkers, another linear regression model that included RHR and *APOE* ε4 carrier status was simultaneously applied. Furthermore, when RHR and *APOE* ε4 carrier status were associated with plasma atherosclerosis biomarkers, the interaction term (RHR × *APOE* ε4 carrier status) was included in the multivariable linear regression model to investigate the interaction effects after mean centering RHR and *APOE* ε4 carrier status.

All statistical analyses were performed using SPSS Statistics version 20.0 (IBM Corporation, Armonk, NY, US). Statistical significance was defined as a two-sided *P*-value of 0.05.

RESULTS

General characteristics of the study subjects

A total of 941 participants (59.7% women) aged 40–85 years (mean [standard deviation, SD] age, 54.7 [10.0] years) ultimately participated in our study. The patients were divided into three groups according to RHR tertile; the cut-offs were ≤71 beats per minute (bpm) for the first third, 72–77 bpm for the second, and ≥78 bpm for the third. Participants in the highest RHR tertile were more likely to have hypertension, CHD, and higher diastolic blood pressure than those in the lowest RHR tertile. In contrast, they were less likely to be male or to smoke. Participants in the lowest, medium, and highest RHR tertiles were similar in terms of *APOE* ε4 carrier status and other demographic, clinical, and biological data (Table 1).

Association between RHR and CHD

Considering that an increase in RHR may induce cardiovascular events, next we investigated the risk factors for CHD to clarify the effect of RHR on CHD. In the bivariable analysis, age, hypertension, a lack of physical activity, and the RHR category were identified (Table 2). However, a further analysis of three explanatory variables in the multiple logistic regression model showed that age, lack of physical activity, and the RHR category were significantly associated with CHD. Specifically, participants with the highest RHR had nearly four times higher odds of developing CHD than those with the lowest RHR (OR, 3.926; 95% CI, 1.303–11.832) (Table 3).

Association between RHR and plasma atherosclerosis biomarkers

In the present study, we found that there were significant differences in the plasma levels of sLRP1 and sRAGE among the three groups (Figure 2A–B). While the medium and highest RHR groups did not differ significantly from each other in plasma sLRP1 level (medium RHR vs. highest RHR, 315.29 [160.66–498.67] vs. 329.93 [168.33–462.47] ng/ml; $P = 1.00$), both groups showed a statistically significantly elevated mean sLRP1 concentration relative to the lowest RHR group (lowest RHR vs. medium RHR, 240.94 [135.35–416.72] vs. 315.29 [160.66–498.67] ng/ml; $P = 0.001$; lowest RHR vs. highest RHR, 240.94 [135.35–416.72] vs. 329.93 [168.33–462.47] ng/ml; $P = 0.001$, respectively). In addition, only the plasma concentration of sRAGE in the highest RHR group was significantly higher than in the lowest RHR group (lowest RHR vs. highest RHR, 1307.84 [759.03–2275.31] vs. 1724.45 [941.90–2766.62] pg/ml; $P = 0.001$). A similar trend, though not statistically significant, was observed in the medium RHR group (lowest RHR vs. medium RHR, 1307.84 [759.03–2275.31] vs. 1536.11 [821.06–2649.88] pg/ml; $P = 0.07$), whereas the medium and highest RHR groups did not differ significantly from each other (medium RHR vs. highest RHR,

Table 1. Characteristics of the total study population and that stratified by the resting heart rate category

Characteristics	Total (n = 941)	Lowest RHR tertile (n = 279)	Medium RHR tertile (n = 323)	Highest RHR tertile (n = 339)	*P*-value
Age, years	54.7 (10.0)	54.3 (9.6)	55.0 (10.0)	54.7 (10.4)	0.67
Male, n (%)	379 (40.3)	137 (49.1)	127 (39.3)	115 (33.9)	0.001
Education, years	7 (5–9)	8 (5–9)	7 (5–9)	7 (4–8)	0.42
Hypertension, n (%)	369 (39.2)	90 (32.3)	124 (38.4)	155 (45.7)	0.003
Diabetes mellitus, n (%)	58 (6.2)	11 (3.9)	21 (6.5)	26 (7.7)	0.15
Dyslipidemia, n (%)	461 (49.0)	129 (46.2)	155 (48.0)	177 (52.2)	0.30
CHD, n (%)	30 (3.2)	4 (1.4)	7 (2.2)	19 (5.6)	0.006
TIA or stroke, n (%)	42 (4.5)	12 (4.3)	14 (4.3)	16 (4.7)	0.96
Active smoking, n (%)	278 (29.5)	99 (35.5)	92 (28.5)	87 (25.7)	0.03
Alcohol drinking, n (%)	143 (15.2)	51 (18.3)	48 (14.9)	44 (13.0)	0.19
Lack of physical activity, n (%)	146 (15.5)	38 (13.6)	50 (15.5)	58 (17.1)	0.49
BMI, kg/m^2	24.9 (3.1)	24.7 (2.8)	24.9 (3.1)	25.1 (3.2)	0.15
RHR, bpm	75.2 (8.6)	66.4 (4.4)	74.5 (1.7)	83.2 (7.6)	<0.001
Systolic blood pressure, mm Hg	129.4 (17.2)	128.1 (16.2)	128.8 (16.9)	131.0 (18.1)	0.08
Diastolic blood pressure, mm Hg	80.6 (9.8)	79.8 (9.2)	80.0 (9.3)	82.0 (10.6)	0.007
Fasting blood glucose, mmol/l	5.35 (5.05–5.69)	5.29 (5.05–5.61)	5.37 (5.03–5.71)	5.41 (5.06–5.75)	0.09
Triglycerides, mmol/l	1.41 (1.01–1.96)	1.42 (1.00–1.94)	1.37 (1.01–1.93)	1.45 (1.05–2.09)	0.21
Total cholesterol, mmol/l	5.01 (0.97)	4.93 (0.93)	5.00 (0.93)	5.07 (1.03)	0.20
LDL-C, mmol/l	3.29 (0.91)	3.25 (1.00)	3.26 (0.79)	3.34 (0.92)	0.38
HDL-C, mmol/l	1.41 (0.31)	1.40 (0.29)	1.43 (0.32)	1.39 (0.32)	0.25
APOE ε4+ carrier status, n (%)	148 (15.7)	42 (15.1)	47 (14.6)	59 (17.4)	0.56

Data are shown as mean (standard deviation), median (interquartile range), or number (percentage)

Abbreviations: BMI, body mass index; CHD, coronary heart disease; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TIA, transient ischemic attack; other — see Figure 1

Table 2. Univariable logistic regression analysis of coronary heart disease and other variables

Characteristic	CHD (n = 30)	No CHD (n = 911)	Univariate analysis		
			OR	95% CI	*P*-value
Age, years	62.2 (11.4)	54.4 (9.9)	1.070	1.035–1.107	<0.001
Sex					
Male, n (%)	12 (40.0)	367 (40.3)	0.988	0.470–2.076	0.98
Female, n (%)	18 (60.0)	544 (59.8)	1.000	reference	
Education, years	6 (3–8)	7 (5–9)	0.927	0.836–1.028	0.15
Hypertension					
Yes, n (%)	17 (56.7)	352 (38.6)	2.077	0.996–4.328	0.05
No, n (%)	13 (43.3)	559 (61.4)	1.000	reference	
Diabetes mellitus					
Yes, n (%)	3 (10.0)	55 (6.0)	1.729	0.509–5.878	0.38
No, n (%)	27 (90.0)	856 (94.0)	1.000	reference	
Dyslipidemia					
Yes, n (%)	13 (43.3)	448 (49.2)	0.790	0.379–1.646	0.53
No, n (%)	17 (56.7)	463 (50.8)	1.000	reference	
TIA or stroke					
Yes, n (%)	3 (10.0)	39 (4.3)	2.484	0.722–8.544	0.15
No, n (%)	27 (90.0)	872 (95.7)	1.000	reference	
Smoking					
Yes, n (%)	8 (26.7)	270 (29.6)	0.863	0.380–1.963	0.73
No, n (%)	22 (73.3)	641 (70.4)	1.000	reference	
Alcohol drinking					
Yes, n (%)	2 (6.7)	141 (15.5)	0.390	0.092–1.656	0.20
No, n (%)	28 (93.3)	770 (84.5)	1.000	reference	
Lack of physical activity					
Yes, n (%)	12 (40.0)	134 (14.7)	3.866	1.820–8.209	<0.001
No, n (%)	18 (60.0)	777 (85.3)	1.000	reference	
BMI, kg/m^2	24.7 (3.0)	24.9 (3.1)	0.974	0.863–1.099	0.67
RHR					
Lowest tertile, n (%)	4 (13.3)	275 (30.2)	1.000	reference	
Medium tertile, n (%)	7 (23.3)	316 (34.7)	1.523	0.441–5.258	0.51
Highest tertile, n (%)	19 (63.3)	320 (35.1)	4.082	1.372–12.143	0.01
APOE ε4+ carrier status					
Yes, n (%)	3 (10.0)	145 (15.9)	0.587	0.176–1.960	0.39
No, n (%)	27 (90.0)	766 (84.1)	1.000	reference	

Abbreviations: CI, confidence interval; OR, odds ratio; other — see Figure 1 and Table 1

Table 3. Multivariable logistic regression analysis of coronary heart disease and other variables

Characteristic	OR	95% CI	*P*-value
Age	1.060	1.024–1.097	0.001
Lack of physical activity	2.746	1.245–6.059	0.01
RHR			0.01
Lowest tertile	1.000	reference	
Medium tertile	1.451	0.415–5.066	0.56
Highest tertile	3.926	1.303–11.832	0.02

Abbreviations: see Figure 1 and Table 2

1536.11 [821.06–2649.88] vs. 1724.45 [941.90–2766.62] pg/ml, $P = 0.46$).

Partial rank correlation analyses and multiple linear regressions were performed to test these associations further when RHR was assessed as a continuous variable. After adjusting for age and sex, both plasma sLRP1 level and plasma sRAGE level were positively correlated with RHR ($r = 0.108$; $P = 0.001$; $r = 0.121$; $P < 0.001$, respectively) (Figure 3A–B), an additional adjustment in the multivariable analysis produced similar results (Figure 3C–D). As shown in Table 4, a multiple linear regression adjusted for age and sex revealed that RHR was positively associated with both log-transformed sLRP1 and log-transformed sRAGE (β = 0.004; 95% CI, 0.002–0.007; $P = 0.001$; β = 0.005; 95% CI, 0.002–0.007; $P < 0.001$, respectively). Furthermore, after adjusting for age, sex, BMI, blood pressure, fasting blood glucose, blood lipids, lifestyle, and medical history, RHR was still independently and positively associated with log-transformed sLRP1 and log-transformed sRAGE (β = 0.004; 95% CI, 0.002–0.006; $P = 0.002$; β = 0.005; 95% CI, 0.002–0.007; $P < 0.001$, respectively).

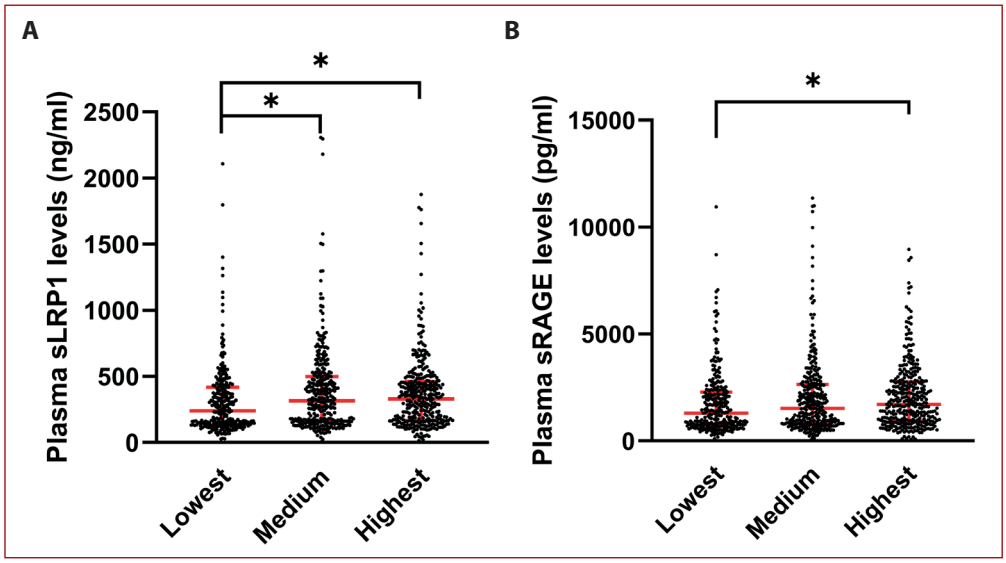

Figure 2. Comparison of plasma sLRP1 and sRAGE levels by RHR groups. Scatter plots for plasma sLRP1 levels (**A**) and sRAGE levels (**B**) in subjects with the lowest, medium, and highest RHR. The horizontal lines represent the median and interquartile range

Abbreviations: see Figure 1

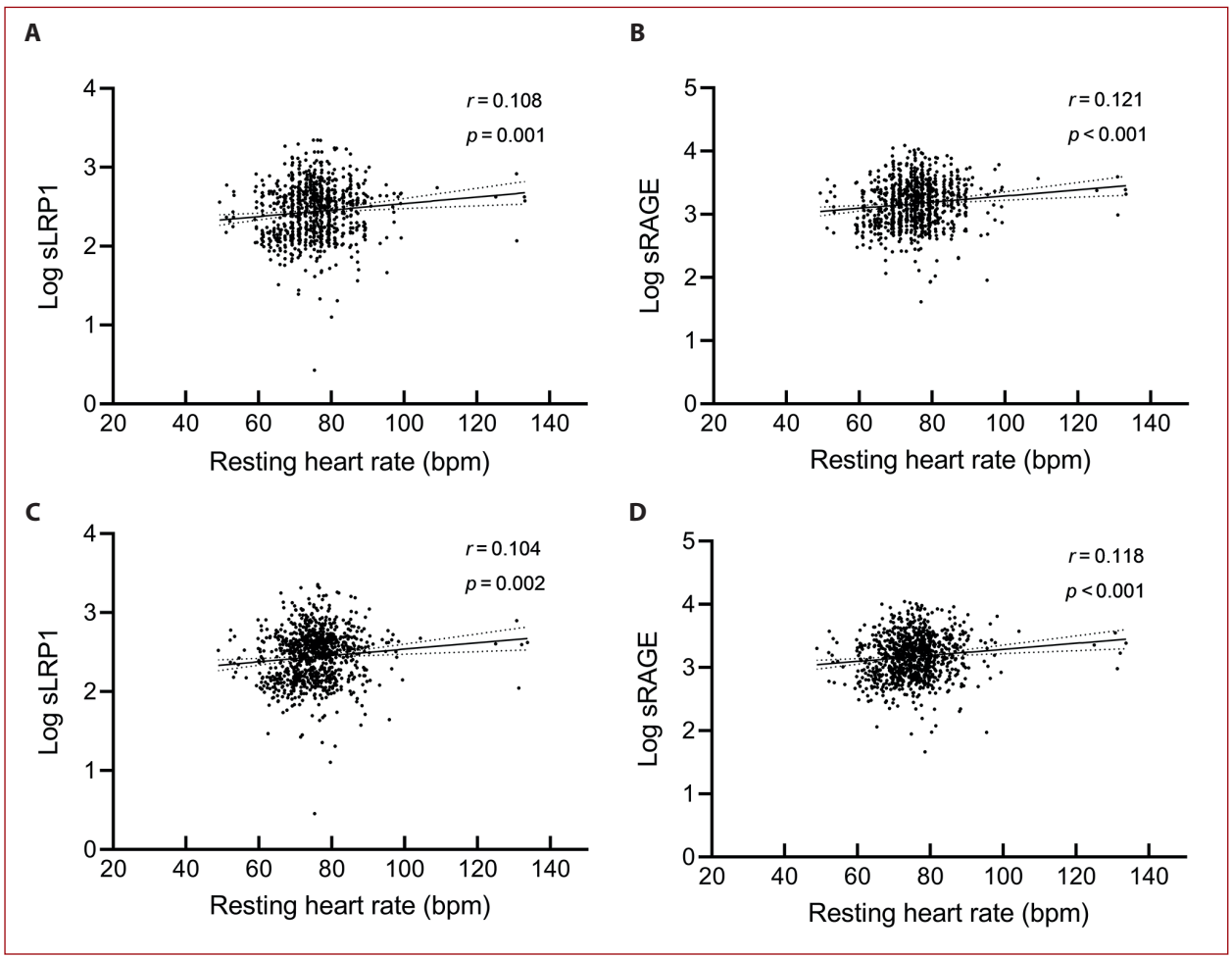

Figure 3. Partial correlations between plasma biomarkers and RHR. Partial correlations of RHR with plasma sLRP1 levels (**A, C**) and plasma sRAGE levels (**B, D**). Partial correlation coefficients and *p* values were obtained after the adjustment for age and sex (**A, B**), as well as for BMI, systolic blood pressure, diastolic blood pressure, log-transformed fasting blood glucose, log-transformed triglyceride level, total cholesterol level, HDL-C level, smoking, drinking, physical activity level, TIA or stroke, and CHD (**C, D**)

Abbreviations: see Figure 1 and Table 1

Table 4. Multiple linear regression models of RHR, *APOE* ε4 carrier status, log-transformed sLRP1, and log-transformed sRAGE in all 941 study participants

	Log sLRP1			Log sRAGE		
	β	95% CI	*P*-value	β	95% CI	*P*-value
Model 1						
RHR	0.004	0.002–0.007	0.001	0.005	0.002–0.007	<0.001
Model 1						
RHR	0.004	0.002–0.007	0.001	0.005	0.002–0.007	<0.001
APOE ε4 carrier status	–0.073	–0.130 to –0.016	0.01	–0.043	–0.103 to 0.016	0.15
Model 2						
RHR	0.004	0.002–0.006	0.002	0.005	0.002–0.007	<0.001
Model 2						
RHR	0.004	0.002–0.007	0.001	0.005	0.002–0.007	<0.001
APOE ε4 carrier status	–0.072	–0.130 to –0.015	0.01	–0.041	–0.102 to 0.019	0.18

β, unstandardized regression coefficient

Model 1: adjusted for age and sex

Model 2: adjusted for age, sex, BMI, systolic blood pressure, diastolic blood pressure, log-transformed fasting blood glucose, log-transformed triglyceride level, total cholesterol level, HDL-C level, smoking, drinking, physical activity level, TIA or stroke, and CHD

Abbreviations: see Figure 1, Table 1 and 2

Table 5. Comparison of demographic, clinical, and biological characteristics between *APOE* ε4 carriers and non-carriers

Characteristic	*APOE* ε4 non-carriers (n = 793)	*APOE* ε4 carriers (n = 148)	*P*-value
Age, years	54.7 (10.1)	54.7 (9.5)	0.98
Male, n (%)	328 (41.4)	51 (34.5)	0.12
Education, years	7 (4–9)	7 (5–8)	0.83
Hypertension, n (%)	311 (39.2)	58 (39.2)	1.00
Diabetes mellitus, n (%)	50 (6.3)	8 (5.4)	0.68
Dyslipidemia, n (%)	384 (48.4)	77 (52.0)	0.42
CHD, n (%)	27 (3.4)	3 (2.0)	0.61
TIA or stroke, n (%)	35 (4.4)	7 (4.7)	0.86
Active smoking, n (%)	243 (30.6)	35 (23.6)	0.09
Alcohol drinking, n (%)	123 (15.5)	20 (13.5)	0.53
Lack of physical activity, n (%)	126 (15.9)	20 (13.5)	0.46
BMI, kg/m^2	24.9 (3.1)	24.9 (3.0)	0.90
RHR, bpm	75.1 (8.3)	75.9 (9.7)	0.28
Systolic blood pressure, mm Hg	129.1 (16.9)	130.7 (18.8)	0.29
Diastolic blood pressure, mm Hg	80.6 (9.6)	80.9 (10.8)	0.68
Fasting blood glucose, mmol/l	5.35 (5.05–5.69)	5.35 (5.05–5.67)	0.75
Triglycerides, mmol/l	1.41 (1.01–1.97)	1.38 (1.06–1.89)	0.97
Total cholesterol, mmol/l	5.00 (0.96)	5.03 (0.99)	0.69
LDL-C, mmol/l	3.28 (0.91)	3.33 (0.87)	0.54
HDL-C, mmol/l	1.41 (0.31)	1.40 (0.31)	0.66
sLRP1, ng/ml	305.45 (158.16–465.50)	257.70 (131.96–425.99)	0.03
sRAGE, pg/mL	1605.71 (838.66–2607.51)	1351.97 (764.26–2435.87)	0.16

Data are shown as mean (standard deviation), median (interquartile range), or number (percentage)

Abbreviations: see Figure 1 and Table

Effect of APOE ε4 allele on RHR and plasma atherosclerosis biomarkers

The univariable analysis showed that plasma sLRP1 level (*APOE* ε4 non-carriers vs. *APOE* ε4 carriers, 305.45 [158.16–465.50] vs. 257.70 [131.96–425.99] ng/ml; $P = 0.03$) was significantly lower in *APOE* ε4 carriers than in non-carriers, but there were no significant intergroup differences in RHR (*APOE* ε4 non-carriers vs. *APOE* ε4 carriers, 75.1 [8.3] vs. 75.9 [9.7] bpm; $P = 0.28$) or plasma sRAGE level (*APOE* ε4 non-carriers vs. *APOE* ε4 carriers, 1605.71 [838.66–2607.51] vs. 1351.97 [764.26–2435.87] pg/ml; $P = 0.16$). The demographic, clinical, and other biological characteristics were similar between *APOE* ε4 carriers and non-carriers (Table 5).

Association between *APOE* ε4 allele and plasma atherosclerosis biomarkers

As shown in Table 4, when we included RHR and *APOE* ε4 carrier status simultaneously in every regression model, *APOE* ε4 carrier status was inversely associated with log-transformed plasma sLRP1 but not log-transformed plasma sRAGE. Meanwhile, the regression coefficients of

RHR were consistent with the previous results (Table 4). Furthermore, when the interaction term (RHR × *APOE* ε4 carrier status) was included in the multivariable linear regression models, it had a null effect in all models and the relationship between RHR, *APOE* ε4 carrier status, and log-transformed sLRP1 remained significant (data not shown).

DISCUSSION

This cross-sectional study demonstrated for the first time that RHR was positively associated with plasma sLRP1 and plasma sRAGE levels in rural Chinese adults aged 40 years and older. In addition, *APOE* ε4 carrier status was inversely associated with plasma sLRP1 level and did not modify the relationship between RHR and plasma sLRP1 level.

A large number of studies of healthy and asymptomatic subjects, as well as of patients with established CAD, demonstrated that RHR is a very important and major independent risk factor for cardiovascular morbidity and mortality [1]. Our results are in line with previous findings that demonstrated individuals with RHR of at least 78 bpm had nearly four times higher odds of developing CHD than those with the lowest RHR (≤71 bpm). Clinical observations and experimental evidence revealed that an elevated RHR enhances mechanical arterial wall stress and prolongs the exposure of the coronary endothelium to systolic low and oscillatory shear stress. Therefore, it can induce structural and functional changes in the endothelial cells that accumulate over time in atherosclerosis-prone regions, promoting atherosclerosis [15].

LRP1 is postulated to participate in numerous diverse physiological and pathological processes including vascular remodeling, foam cell biology, inflammation, and atherosclerosis [2]. Circulating sLRP1 levels indicate the presence of atherosclerosis-related conditions that lead to coronary events during follow-up [6]. De Gonzalo-Calvo et al. [5] found that patients with severe hypercholesterolemia had significantly higher sLRP1 concentrations than those with moderate hypercholesterolemia and normocholesterolemic controls, and sLRP1 levels were increased in the conditioned medium of coronary atherosclerotic plaque areas extracted from patients versus non-atherosclerotic areas of the same coronary arteries and patients. Furthermore, Chen et al. [16] demonstrated that sLRP1 was a novel biomarker for P_2Y_{12} receptor expression, which could aggravate atherosclerosis in atherosclerotic plaques. Thus, sLRP1 has been implicated as a novel biomarker for atherosclerosis [5]. The present study found that plasma concentrations of sLRP1 were significantly higher in the highest RHR group than in the medium and lowest RHR groups. Moreover, in partial rank correlation and multiple linear regression analyses, RHR was positively associated with log-transformed plasma sLRP1. All of these results strongly suggest that an elevated RHR was associated with increased plasma sLRP1 levels. Although the mechanism of how RHR affects plasma sLRP1 levels is unclear, many studies have shown that inflammation plays an important role in sLRP1 generation in plasma. In fact, sLRP1 is released when exposed to proinflammatory mediators and may promote inflammation by affecting macrophage physiology and activating microglia or other cells [4, 17]. Therefore, even though we did not measure inflammation biomarkers, it is possible to speculate that an elevated RHR stimulates plasma sLRP1 by increasing inflammation (Supplementary material, *Figure S1*). Moreover, an increased RHR reduces coronary perfusion and oxygen supply; this hypoxic condition upregulates LRP1 expression [18], thereby increasing plasma sLRP1 levels.

Mounting evidence strongly implicates the importance of RAGE in the initiation and progression of vascular atherosclerotic disease [7]. It is known that sRAGE acts as a decoy for RAGE ligands by sequestering RAGE ligands or competing with full RAGE for ligand binding and thus have cytoprotective effects against advanced glycation end products (AGEs)-RAGE interactions. Theoretically, low levels of sRAGE are markers of disease states. In a study of 2571 non-diabetic patients, Lindsey et al. [19] demonstrated using computed tomography that calcification in the coronary arteries is inversely related to plasma sRAGE levels, and similar results were obtained by Falcone et al. [20] in a study on 328 non-diabetic patients, in whom low sRAGE plasma levels were independently associated with the presence of CAD. However, several investigators reported contradictory findings that plasma levels of sRAGE were higher in patients with CAD and type 1 or type 2 diabetes [21, 22]. Moreover, sRAGE is directly positively associated with the extent of CAD, and elevated sRAGE is linked to a high risk of recurrent coronary events [8]. Circulating levels may be associated with both RAGE expression and proteolytic cleavage of RAGE mediated by matrix metalloproteinases (MMPs) and possibly other factors [23]. An elevated RHR could induce oxidative stress and thereby increase AGE production and accumulation [24]. Since AGEs upregulate both RAGE and MMP expression and production in various tissues [23], it is possible that elevated sRAGE levels in patients with a higher RHR may be due to a marked increase in serum AGE levels, which, in turn, would increase RAGE and MMPs. On the other hand, sRAGE levels might be elevated in higher RHR participants as a compensatory mechanism for increased AGE production. Moreover, the elevated RHR increases systemic inflammation [25], thereby stimulating MMP expression through AP-1 and nuclear factor-kappa beta activation [26], which would increase the cleavage of sRAGE from the cell surface. Conversely, it is also possible that the progression of atherosclerotic lesions in the coronary arteries may aggravate the release of atherosclerosis biomarkers [5], thereby increasing circulating sLRP1 and sRAGE concentrations (Supplementary material, *Figure S1*).

An interesting question arising from the present results is why *APOE* ε4 carrier status was associated with plasma sLRP1 levels rather than plasma sRAGE levels. The *APOE* ε4 allele impacts lipid and lipoprotein levels [10]. Studies of normolipidemic populations demonstrated an association

of *APOE* ε4 with increased total and low-density lipoprotein cholesterol levels, as well as increased apoB expression [10]. Although underlying mechanisms responsible for this phenomenon are less clear, LDL receptor family downregulation is perhaps the most commonly accepted explanation [27]. Such LDL receptor family downregulation in *APOE* ε4 carriers would thereby decrease their LRP1 levels and be associated with decreased sLRP1 level. In contrast, sRAGE changes in *APOE* ε4 carrier status are more complicated. Deo et al. [28] recently reported that sRAGE level was significantly (21%) higher in *APOE* ε4 carriers than in non-carriers while other researchers found a contrasting pattern for sRAGE, namely lower levels among *APOE* ε4 carriers than non-carriers [29]. Our study demonstrated no significant difference in plasma sRAGE levels between *APOE* ε4 carriers and non-carriers; this relationship remained nonsignificant after the adjustment for potential confounders. We speculate that this discrepancy might be due to the differences in characteristics of the study populations, which may have influenced the AGE-RAGE profile and the impact of *APOE* ε4.

Limitations

Our study has some limitations. First, only 30 patients with coronary heart disease met our inclusion and exclusion criteria; the small sample size would reduce the reliability of the conclusions. Second, many factors, such as congestive heart failure and hyperthyroidism, may affect RHR. Since we did not perform tests for brain natriuretic peptide (BNP), echocardiography, and thyroid function test, we cannot rule out that an elevated heart rate was a sign of mild heart failure or hyperthyroidism. Third, as heart rate is susceptible to different influencing factors [30], multiple recordings collected at shorter intervals could have shown a longitudinal course of RHR and may have increased its association with plasma atherosclerosis biomarkers. Fourth, although both sLRP1 and sRAGE participate in the pathogenesis of atherosclerosis, they do not fully reflect the extent of coronary atherosclerosis. Finally, although plasma atherosclerosis biomarkers were measured with RHR, they were measured only once, and pro-inflammatory mediators were not detected simultaneously. Therefore, our results need to be validated in additional longitudinal cohort studies.

CONCLUSIONS

In conclusion, we reported for the first time that RHR is positively associated with plasma levels of sLRP1 and sRAGE, which is not modified by *APOE* genotype. Since plasma sLRP1 and sRAGE levels are indicative of atherosclerosis, our findings suggest a potential link between RHR and the progression of coronary atherosclerosis, which may partially explain why the risk of CHD is associated with an increased RHR. Moreover, RHR monitoring may have clinical utility for identifying patients who are at risk of or in the early stages of CHD.

Article information

Conflict of interest: None declared.

Funding: This work was supported by the National Key Technology Research and Development Program of the Ministry of Science and Technology of China (grant no. 2015BAI13B01 [to QQ]) and the Youth Foundation of the Second Affiliated Hospital of Xi'an Jiaotong University.

REFERENCES

1. Böhm M, Reil JC, Deedwania P, et al. Resting heart rate: risk indicator and emerging risk factor in cardiovascular disease. Am J Med. 2015; 128(3): 219–228, doi: 10.1016/j.amjmed.2014.09.016, indexed in Pubmed: 25447617.
2. Actis DV, Chiabrando GA. The role of low-density lipoprotein receptor-related protein 1 in lipid metabolism, glucose homeostasis and inflammation. Int J Mol Sci. 2018; 19(6): 1780, doi: 10.3390/ijms19061780, indexed in Pubmed: 29914093.
3. Potere N, Del Buono MG, Mauro AG, et al. Low density lipoprotein receptor-related protein-1 in cardiac inflammation and infarct healing. Front Cardiovasc Med. 2019; 6: 51, doi: 10.3389/fcvm.2019.00051, indexed in Pubmed: 31080804.
4. Gorovoy M, Gaultier A, Campana WM, et al. Inflammatory mediators promote production of shed LRP1/CD91, which regulates cell signaling and cytokine expression by macrophages. J Leukoc Biol. 2010; 88(4): 769–778, doi: 10.1189/jlb.0410220, indexed in Pubmed: 20610799.
5. de Gonzalo-Calvo D, Cenarro A, Martínez-Bujidos M, et al. Circulating soluble low-density lipoprotein receptor-related protein 1 (sLRP1) concentration is associated with hypercholesterolemia: A new potential biomarker for atherosclerosis. Int J Cardiol. 2015; 201: 20–29, doi: 10.1016/j.ijcard.2015.07.085, indexed in Pubmed: 26285183.
6. de Gonzalo-Calvo D, Elosua R, Vea A, et al. Soluble low-density lipoprotein receptor-related protein 1 as a biomarker of coronary risk: Predictive capacity and association with clinical events. Atherosclerosis. 2019; 287: 93–99, doi: 10.1016/j.atherosclerosis.2019.06.904, indexed in Pubmed: 31247347.
7. Lindsey JB, Cipollone F, Abdullah SM, et al. Receptor for advanced glycation end-products (RAGE) and soluble RAGE (sRAGE): cardiovascular implications. Diab Vasc Dis Res. 2009; 6(1): 7–14, doi: 10.3132/dvdr.2009.002, indexed in Pubmed: 19156622.
8. Wang X, Xu T, Mungun D, et al. The relationship between plasma soluble receptor for advanced glycation end products and coronary artery disease. Dis Markers. 2019; 2019: 4528382, doi: 10.1155/2019/4528382, indexed in Pubmed: 31275446.
9. Fujisawa K, Katakami N, Kaneto H, et al. Circulating soluble RAGE as a predictive biomarker of cardiovascular event risk in patients with type 2 diabetes. Atherosclerosis. 2013; 227(2): 425–428, doi: 10.1016/j.atherosclerosis.2013.01.016, indexed in Pubmed: 23384720.
10. Bennet AM, Di Angelantonio E, Ye Z, et al. Association of apolipoprotein E genotypes with lipid levels and coronary risk. JAMA. 2007; 298(11): 1300–1311, doi: 10.1001/jama.298.11.1300, indexed in Pubmed: 17878422.
11. Song Y, Stampfer MJ, Liu S. Meta-analysis: apolipoprotein E genotypes and risk for coronary heart disease. Ann Intern Med. 2004; 141(2): 137–147, doi: 10.7326/0003-4819-141-2-200407200-00013, indexed in Pubmed: 15262670.
12. Stengård JH, Zerba KE, Pekkanen J, et al. Apolipoprotein E polymorphism predicts death from coronary heart disease in a longitudinal study of elderly Finnish men. Circulation. 1995; 91(2): 265–269, doi: 10.1161/01.cir.91.2.265, indexed in Pubmed: 7805227.
13. Inbal A, Freimark D, Modan B, et al. Synergistic effects of prothrombotic polymorphisms and atherogenic factors on the risk of myocardial

infarction in young males. Blood. 1999; 93(7): 2186–2190, indexed in Pubmed: 10090925.
14. Jiang Yu, Shang S, Li P, et al. Pulse pressure is associated with plasma amyloid-β transport dysfunction. J Hypertens. 2018; 36(3): 569–579, doi: 10.1097/HJH.0000000000001565, indexed in Pubmed: 28938337.
15. Giannoglou GD, Chatzizisis YS, Zamboulis C, et al. Elevated heart rate and atherosclerosis: an overview of the pathogenetic mechanisms. Int J Cardiol. 2008; 126(3): 302–312, doi: 10.1016/j.ijcard.2007.08.077, indexed in Pubmed: 18068835.
16. Chen J, Pi S, Yu C, et al. sLRP1 (soluble low-density lipoprotein receptor-related protein 1): A novel biomarker for P2Y12 (P2Y purinoceptor 12) receptor expression in atherosclerotic plaques. Arterioscler Thromb Vasc Biol. 2020; 40(6): e166–e179, doi: 10.1161/ATVBAHA.120.314350, indexed in Pubmed: 32349534.
17. Brifault C, Gilder AS, Laudati E, et al. Shedding of membrane-associated LDL receptor-related protein-1 from microglia amplifies and sustains neuroinflammation. J Biol Chem. 2017; 292(45): 18699–18712, doi: 10.1074/jbc.M117.798413, indexed in Pubmed: 28972143.
18. Castellano J, Aledo R, Sendra J, et al. Hypoxia stimulates low-density lipoprotein receptor-related protein-1 expression through hypoxia-inducible factor-1α in human vascular smooth muscle cells. Arterioscler Thromb Vasc Biol. 2011; 31(6): 1411–1420, doi: 10.1161/ATVBAHA.111.225490, indexed in Pubmed: 21454812.
19. Lindsey JB, de Lemos JA, Cipollone F, et al. Association between circulating soluble receptor for advanced glycation end products and atherosclerosis: observations from the Dallas Heart Study. Diabetes Care. 2009; 32(7): 1218–1220, doi: 10.2337/dc09-0053, indexed in Pubmed: 19366975.
20. Falcone C, Emanuele E, D'Angelo A, et al. Plasma levels of soluble receptor for advanced glycation end products and coronary artery disease in non-diabetic men. Arterioscler Thromb Vasc Biol. 2005; 25(5): 1032–1037, doi: 10.1161/01.ATV.0000160342.20342.00, indexed in Pubmed: 15731496.
21. Nakamura K, Yamagishi Si, Adachi H, et al. Elevation of soluble form of receptor for advanced glycation end products (sRAGE) in diabetic subjects with coronary artery disease. Diabetes Metab Res Rev. 2007; 23(5): 368–371, doi: 10.1002/dmrr.690, indexed in Pubmed: 17024691.
22. Challier M, Jacqueminet S, Benabdesselam O, et al. Increased serum concentrations of soluble receptor for advanced glycation endproducts in patients with type 1 diabetes. Clin Chem. 2005; 51(9): 1749–1750, doi: 10.1373/clinchem.2005.051961, indexed in Pubmed: 16120960.
23. Prasad K. Low levels of serum soluble receptors for advanced glycation end products, biomarkers for disease state: myth or reality. Int J Angiol. 2014; 23(1): 11–16, doi: 10.1055/s-0033-1363423, indexed in Pubmed: 24627612.
24. Moldogazieva NT, Mokhosoev IM, Mel'nikova TI, et al. Oxidative stress and advanced lipoxidation and glycation end products (ALEs and AGEs) in aging and age-related diseases. Oxid Med Cell Longev. 2019; 2019: 3085756, doi: 10.1155/2019/3085756, indexed in Pubmed: 31485289.
25. Nanchen D, Stott DJ, Gussekloo J, et al. Resting heart rate and incident heart failure and cardiovascular mortality in older adults: role of inflammation and endothelial dysfunction: the PROSPER study. Eur J Heart Fail. 2013; 15(5): 581–588, doi: 10.1093/eurjhf/hfs195, indexed in Pubmed: 23250912.
26. Reddy VS, Prabhu SD, Mummidi S, et al. Interleukin-18 induces EMMPRIN expression in primary cardiomyocytes via JNK/Sp1 signaling and MMP-9 in part via EMMPRIN and through AP-1 and NF-kappaB activation. Am J Physiol Heart Circ Physiol. 2010; 299(4): H1242–H1254, doi: 10.1152/ajpheart.00451.2010, indexed in Pubmed: 20693392.
27. Martínez-Martínez AB, Torres-Perez E, Devanney N, et al. Beyond the CNS: The many peripheral roles of APOE. Neurobiol Dis. 2020; 138: 104809, doi: 10.1016/j.nbd.2020.104809, indexed in Pubmed: 32087284.
28. Deo P, Dhillon VS, Chua A, et al. APOE ε4 carriers have a greater propensity to glycation and sRAGE which is further influenced by RAGE G82S polymorphism. J Gerontol A Biol Sci Med Sci. 2020; 75(10): 1899–1905, doi: 10.1093/gerona/glz259, indexed in Pubmed: 31677348.
29. Dhillon VS, Deo P, Chua A, et al. Sleep duration, health promotion index, sRAGE, and APOE-ε4 genotype are associated with telomere length in healthy Australians. J Gerontol A Biol Sci Med Sci. 2022; 77(2): 243–249, doi: 10.1093/gerona/glab264, indexed in Pubmed: 34508574.
30. Plaza-Florido A, Sacha J, Alcantara J. Short-term heart rate variability in resting conditions: methodological considerations. Kardiol Pol. 2021; 79(7-8): 745–755, doi: 10.33963/KP.a2021.0054, indexed in Pubmed: 34227676.