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Association of Hair Cortisol Concentration with Prevalence of Major Cardiovascular Risk Factors and Allostatic Load

Authors' Contribution:

Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

BCDEF 1 **Eglė Mazgelytė**
ABCDEF 1 **Dovilė Karčiauskaitė**
BD 1 **Aušra Linkevičiūtė**
ABD 1 **Asta Mažeikienė**
ABD 2 **Neringa Burokienė**
ABD 1 **Rėda Matuzevičienė**
BD 1 **Mantas Radzevičius**
CD 3 **Aušra Janiulionienė**
CD 4 **Audronė Jakaitienė**
CD 5 **Lina Dindienė**
ABDG 1 **Zita Aušrelė Kučinskienė**

1 Department of Physiology, Biochemistry, Microbiology, and Laboratory Medicine, Institute of Biomedical Sciences, Faculty of Medicine, Vilnius University, Vilnius, Lithuania
2 Clinics of Internal Diseases, Family Medicine and Oncology, Institute of Clinical Medicine, Faculty of Medicine, Vilnius University, Vilnius, Lithuania
3 Centre of Laboratory Medicine, Vilnius University Hospital Santaros Klinikos, Vilnius, Lithuania
4 Department of Human and Medical Genetics, Institute of Biomedical Sciences, Faculty of Medicine, Vilnius University, Vilnius, Lithuania
5 Faculty of Mathematics and Natural Sciences, Kaunas University of Technology, Kaunas, Lithuania

Corresponding Author: Eglė Mazgelytė, e-mail: egle.mazgelyte@mf.vu.lt
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Background: The high prevalence of cardiovascular diseases cannot be explained completely by conventional risk factors such as older age, smoking, diabetes mellitus, hypertension, obesity, and dyslipidemia. Results of recent studies indicate that chronic stress may be an independent risk factor for cardiovascular morbidity and mortality. Thus, the aim of our study was to investigate the associations between the hair cortisol concentration (HCC), which is considered as a potential biomarker of long-term psychosocial stress, and traditional cardiovascular risk factors, including smoking, dyslipidemia, hypertension, and obesity.





Material/Methods: Fasting blood samples and anthropometric and lifestyle data were collected from 163 apparently healthy men. HCC was determined using high-performance liquid chromatography. Allostatic load (AL) index, defined as an integrated score of multiple interacting systems involved in the adaptation to adverse physical or psychosocial situations, was also calculated.

Results: We found that many prevalent cardiovascular risk factors, including hypertension, smoking, higher than recommended waist circumference (WC), and low-density lipoprotein cholesterol (LDL-C) median values, are associated with higher HCC. Hair cortisol level was also positively associated with the manifestation of individual cardiovascular risk factors such as higher-than-recommended total cholesterol, LDL-C, non-high-density lipoprotein cholesterol, body mass index, and WC median values. Moreover, a significant positive relationship between HCC and AL index was observed.

Conclusions: The results of this study suggest that increased prevalence of traditional cardiovascular risk factors is associated with higher HCC. Also, both HCC and AL index might be appropriate markers for the evaluation of chronic stress level.

MeSH Keywords: **Allostasis • Risk Factors • Cardiovascular Diseases • Hair • Hydrocortisone • Stress, Psychological**

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Background

Cardiovascular diseases (CVDs) such as ischemic heart disease and stroke are the major causes of death in many countries [1,2]. In addition to conventional cardiovascular risk factors such as obesity, dyslipidemia, hypertension, and smoking, there is growing evidence that chronic psychosocial stress plays a role in the pathogenesis of CVD [3]. Previous studies [4,5] demonstrated that different psychosocial factors, including job stress, anxiety, depression, social isolation, and some personality traits are associated with increased CVD risk.

The major challenge of studies investigating the effect of chronic stress on the prevalence of CVD is the selection of method for the evaluation of psychosocial stress level. Stress can be assessed using subjective and objective methods, including self-reported questionnaires and stress biomarkers measured in different matrices [6–10]. Since a response to the perceived psychosocial stress results in activation of the hypothalamic-pituitary-adrenal axis (HPA), the high level of its end-product, cortisol, is frequently used as a biomarker of psychological stress. Investigation of saliva, serum, and urine provides information about the cortisol production for up to 24 h, while long-term integrated hormone secretion can be assessed by measuring cortisol concentration in scalp hair, as each centimeter of scalp hair represents 1 month of past cortisol exposure [7,10]. Another approach to evaluating chronic stress level is the calculation of allostatic load (AL) index, which is defined as a measure of “wear and tear” on the body and represents a dysfunction of different physiological systems involved in response to the perturbations caused by a stressor [11].

Since prior studies [12] showed that chronic stress is associated with 40–60% higher risk of CVD, it is hypothesized that glucocorticoids (cortisol in humans) serve as mediators of relations between stress and CVD. Cortisol exerts its biological effects by modulating lipid metabolism, as it promotes lipolysis in the peripheral fat depots and increases lipogenesis in visceral fat tissue [13,14]. In addition, cortisol regulates blood pressure by acting on renal mineralocorticoid receptors, which results in salt and water retention and consequently increased plasma volume [13,15]. Also, sustained HPA axis activation and cortisol exposure lead to glucocorticoid-resistance, which is associated with decreased capability of cortisol to suppress proinflammatory cytokine (IL-1 β , IL-6, TNF- α , INF- γ) production and induce antiinflammatory cytokine (IL-4, IL-10, IL-13) secretion [16]. Moreover, it has been shown that chronic psychosocial stress induced activation of sympathetic nervous system results in endothelial dysfunction due to indirect activation of NADPH oxidase, which catalyzes the generation of reactive oxygen species [17,18]. Additionally, it is speculated that psychosocial stress is associated with increased platelet activation and reactivity. Matsuhisa et al. [19] reported

a positive association between chronic, but not acute, stress and agonist-induced (thrombin and adenosine diphosphate) platelet aggregation biomarkers. Nonetheless, the majority of studies [19,20] were conducted using animal models or focused mainly on the effect of acute stress on platelet activation biomarkers.

Since there is growing evidence that chronic stress-induced long-term cortisol exposure concentration is related to obesity, metabolic syndrome, and CVD, the objective of our study was to analyze the association between HCC and the prevalence of traditional cardiovascular risk factors, including dyslipidemia, smoking, hypertension, and obesity. Due to the importance of inflammation in the pathogenesis of CVD, we investigated the relationship between HCC and systemic inflammation biomarkers, including white blood cell (WBC) count, neutrophil percentage, and C-reactive protein (CRP), as well as a relatively new biomarker, cyclophilin A (CypA), which presumably contributes to cardiovascular inflammation. Finally, we evaluated chronic stress level by calculating the allostatic load (AL) index, which represents dysregulation of multiple systems likely involved in the pathogenesis of cardiovascular disease, and we investigated the association between AL index and HCC values.

Material and Methods

Study population

The study included 163 apparently healthy men (age, 24–55 years), who were recruited consecutively from the Outpatient Department of Vilnius University Hospital Santaros Klinikos. Participants completed a self-reported questionnaire on sociodemographic and lifestyle characteristics, including age, marital status, monthly income, smoking status, and physical activity. All anthropometric parameters (height, weight, waist circumference), resting arterial blood pressure (systolic and diastolic), and heart rate values were assessed by trained personnel. The study protocol was approved by the Lithuanian Bioethics Committee (No. 15820-15-807-319). All participants provided written informed consent before entering the study.

Biochemical analyses

All blood samples were collected under fasting conditions and were analyzed in the Centre of Laboratory Medicine of Vilnius University Hospital Santaros Klinikos. Specifically, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triacylglycerol (TAG), glucose, and high-sensitivity CRP levels in blood serum were determined using routine methods (Architect ci8200, Abbott, USA). The quantitative determination of CypA in blood serum was made using enzyme-linked immunoassay (Gemini, Strattec

Biomedical, Germany) with reagents from LDN® (Nordhorn, Germany). Non-high-density lipoprotein cholesterol (non-HDL-C) concentration was calculated by subtracting the HDL-C value from a TC.

Hair sampling, preparation, and analysis

HCC was determined from the most proximal segment of the outer 3 cm of scalp hair, representing the 3 months prior to sampling grown hair. The hair samples were stored at room temperature in envelopes until analysis.

Samples were prepared and analyzed using the modified Raul et al. [21] and De Palo et al. [22] methods. Firstly, 20–50 mg of hair sample was weighed and finely cut with scissors, then washed twice in isopropanol and put into polypropylene tubes. Later, hair samples were incubated in 2 ml Sorensen buffer for 16 h at 40°C in the presence of 10 ng 6 α -methylprednisolone as an internal standard. Each sample then was transferred to the solid-phase extraction column, which was rinsed with the sequence of solvents [22]. The eluates were evaporated under a stream of nitrogen gas and resuspended with 100 μ l high-performance liquid chromatography (HPLC) in mobile phase.

Chromatographic separation was performed on a HPLC system (Shimadzu Nexera X2). A 10- μ l sample was injected on the HPLC column (Zorbax Eclipse XDB – C8, 4.6 \times 150 mm, 5 μ m). The chromatographic isocratic separation was carried out with a binary mobile phase of acetonitrile and water (2: 3, v/v), and UV light absorbance was measured at 245 nm wavelength. The average retention time of the cortisol was 4.12 min.

Determination of malondialdehyde (MDA) concentration in blood serum

Samples were prepared and analyzed according to the methodology of Khoschrosur et al. [23] using the HPLC method with fluorescence detection. Chromatographic separation was performed on the HPLC system (Shimadzu Nexera X2). A 20- μ l sample was injected on the HPLC column (Agilent Poroshell 120 EC–C18, 3 \times 100 mm, 2.7 μ m). The chromatographic isocratic separation was carried out with a binary mobile phase of methanol and 50 mM phosphate buffer, pH 6.8 (2: 3, v/v). Fluorescence detection was performed at 230 nm excitation and 430 nm emission wavelengths. The average retention time of the malondialdehyde-thiobarbituric acid adduct was 1.63 min.

Determination of blood serum total antioxidant activity by ferric-reducing antioxidant power (FRAP) assay

Total antioxidant capacity was assessed by using FRAP assay using a slightly modified method by Benzie et al. [24]. The FRAP reagent was prepared by mixing acetate buffer (pH 3.6), TPTZ

(2,4,6-tripyridyl-s-triazine) and FeCl₃·6H₂O solutions in the ratio 10: 1: 1. 100 μ l of the blood serum, 2 ml of the FRAP reagent, and 900 μ l of distilled water, incubated for 30 min at room temperature, and the absorbance was measured at 593 nm wavelength (Shimadzu UV – 1601, Japan). All serum samples were analyzed in triplicate. FRAP value was determined from the obtained calibration curves using standard FeSO₄ solutions. Results are expressed in μ mol Fe²⁺/l.

Evaluation of platelet function

Platelet function was measured within 10 min after blood collection, using flow cytometry (BD FACSCanto, BD Biosciences, USA). Data were analyzed using BD FACS Diva software (version 6.1.2). Two tubes were stained with CD42a-PerCP-Cy5.5 (glycoprotein IX) and CD61-APC (integrin beta-3), which are both platelet-specific markers. A combination of forward and side scatter characteristics, together with positive expression CD42a and CD61, was used to identify platelets. One tube was additionally stained with CD63-PE (a platelet alpha granule integrin), which is a marker of platelet degranulation. Another tube was additionally stained with PAC-1-FITC (platelet activation complex-1), which reacts with activated glycoprotein IIb/IIIa (integrin α 2iib3) and represents platelet activation/aggregation status. Platelet agonists were added to the tubes to elicit platelet activation: TRAP (thrombin receptor-activating peptide) in the tube stained with CD63, and ADP (adenosine diphosphate) in the tube with PAC-1. The results are presented as the percentage of platelets expressing the activation marker (CD63 and PAC-1) after agonist stimulation.

Calculation of allostatic load (AL) indices

Although there is a general agreement to include cardiovascular, metabolic, and immune systems in the calculation of allostatic load index, there is a huge variation in the selection of biomarkers and determination of high-risk cut-off point values for individual biomarker [27]. In this study, AL indices were calculated as a sum of 5 separate physiological system (cardiovascular, metabolic, immune, oxidative stress, and platelet activation) risk scores (SRS). In total, 18 biomarkers were used for the calculation of AL indices. Contrary to other studies, we included oxidative stress and platelet activation biomarkers in the estimation of the AL index as the potential contributors to chronic stress induced allostatic load. Moreover, a relatively new inflammatory biomarker, CypA, was incorporated along with traditional biomarkers (CRP, WBC count, and neutrophil percentage) in the calculation of immune system risk score. However, sympathetic nervous system parameters such as urine epinephrine or norepinephrine levels, commonly used in previous studies, were not available in our study.

Each biomarker was dichotomized into high- and low-risk values: 1 point was given if the biomarker was in the high-risk range and 0 if not. Thus, the higher AL index value indicates the greater effect of chronic stress exposure on physiologic dysregulation [25–27]. To determine the high-risk cut-off point values, the study sample was divided into quartiles and high risk was defined as greater than the 75th percentile for all variables except for HDL-C and FRAP measures. For these variables, high risk was defined as a value less than the 25th percentile [27]. The SRS was in the range from 0 to 7, depending on the number of biomarkers in each system, while the overall AL index ranged from 0 to 18.

Statistical analysis

Statistical analysis was performed with R version 3.4.1.2. Quantitative variables are presented as mean \pm standard deviation (SD) for normally distributed or median (interquartile range) (IQR) for non-normally distributed variables. For categorical variables, absolute and relative frequencies were calculated. As not all variables were distributed along the normal distribution, Spearman's rank coefficient was used to quantify the strength of the correlation between HCC and inflammation, oxidative stress, or platelet activation biomarkers. The comparisons of HCC among the 4 different groups based on the number of cardiovascular risk factors were performed using nonparametric Kruskal-Wallis analysis followed by the Mann-Whitney U test with Bonferroni correction. The latter test was also used for the comparison of allostatic load index between 2 hair cortisol concentration groups. The level of statistical significance was set at 0.05 for two-tailed testing.

Results

Sample characteristics

Sociodemographic and lifestyle characteristics of the study sample are shown in Table 1. Overall, the median (IQR) age was 39 (16.5) years. Self-reported data showed that most subjects were non-smokers (83.1%), engaged in sedentary work (69.4%), and were physically active outside working hours (81.8%). Descriptives of anthropometric and cardiometabolic indicators, neuroendocrine, inflammation, oxidative stress, and platelet activation biomarkers of the study sample are shown in Table 2. The median values of lipid and glucose metabolism biomarkers (TC, HDL-C, TAG, and fasting glucose), except for LDL-C, were within the current reference range of a general population.

The median (IQR) hair cortisol concentration was 46.2 (119.3) ng/g.

Table 1. Sociodemographic and lifestyle characteristics of the study sample.

Variables	Median (IQR) or n (%)
Age (years)	39 (16.5)
Marital status*	
Married	115 (71.9%)
Single/divorced/widowed	45 (28.1%)
Income*	
Lower than national average monthly wage	25 (15.6%)
Equal to or higher than national average monthly wage	135 (84.4%)
Additional job*	
Yes	39 (24.4%)
No	121 (75.6%)
Night job*	
Yes	20 (12.5%)
No	140 (87.5%)
Smoking status*	
Smoker	27 (16.9%)
Non-smoker	133 (83.1%)
Physical activity at work*	
Inactive	111 (69.4%)
Active	49 (30.6%)
Leisure-time physical activity*	
Inactive	29 (18.2%)
Active	130 (81.8%)

* Variable has missing data.

Hair cortisol concentration and cardiovascular risk factor

To determine the association between the hair cortisol concentration and cardiovascular risk factors, we divided the study participants into 2 groups according to the prevalence of the major risk factors, including obesity, smoking, hypertension, and dyslipidemia. The manifestation of these risk factors was evaluated using the latest update of the European Guidelines on cardiovascular disease prevention in clinical practice prepared by the European Society of Cardiology [28].

The results of this study showed that participants with higher-than-recommended TC (≥ 5.20 mmol/l), LDL-C (≥ 3.00 mmol/l), and non-HDL-C (≥ 3.80 mmol/l) values had a significantly

Table 2. Anthropometric, cardiometabolic indicators, neuroendocrine, inflammation, oxidative stress, and platelet activation biomarkers of the study sample.

Variables	Mean ±SD or median (IQR)		Range
Anthropometric and cardiometabolic indicators			
Weight (kg)	84.20	(17.05)	54.30–178.00
Height (cm)	183.06±6.33		166.00–198.50
Body mass index (kg/m ²)	25.04	(4.75)	16.95–45.40
Waist circumference (cm)	89	(15)	69–141
Resting systolic blood pressure (mmHg)	129	(16.5)	93–175
Resting diastolic blood pressure (mmHg)	76	(13)	57–104
Resting heart rate (bpm)	63	(14)	40–94
Fasting glucose (mmol/l)	5.24	(0.52)	4.15–7.61
Total cholesterol (mmol/l)	5.02	(1.39)	3.04–8.73
High-density lipoprotein cholesterol (mmol/l)	1.19	(0.33)	0.58–2.21
Low-density lipoprotein cholesterol (mmol/l)	3.11	(1.17)	1.56–5.95
Non-high-density lipoprotein cholesterol (mmol/l)	3.78	(1.49)	1.99–7.61
Triacylglycerols (mmol/l)	1.17	(0.86)	0.41–9.94
Neuroendocrine biomarker			
Hair cortisol (ng/g)	46.18	(119.29)	0.63–858.31
Inflammation biomarkers			
C-reactive protein (mg/l)	0.64	(0.96)	0.10–31.00
Cyclophilin A (ng/ml)	43.52	(60.20)	0.33–81.00
WBC count (10 ⁹ /l)	5.47	(1.52)	2.82–9.45
Neutrophil percentage (%)	47.96±8.33		29.18–73.31
Oxidative stress biomarkers			
Malondialdehyde (µg/l)	97.48	(35.62)	56.03–251.42
Ferric reducing ability of plasma (µmol Fe ²⁺ /l)	47.67	(11.41)	28.50–79.98
Platelet activation biomarkers			
CD63+ platelets stimulated with TRAP (%)	28.85	(21.05)	0.40–69.20
PAC-1 + platelets stimulated with ADP (%)	49.40	(37.20)	0.10–90.20

higher HCC median (IQR) compared to those with normal lipid profile (34.28(114.01) ng/g vs. 64.69(131.80) ng/g, $p=0.012$, 38.14(77.22) ng/g vs. 55.12(141.72) ng/g, $p=0.010$, and 36.82 (123.82) ng/g vs. 54.68 (113.28) ng/g, $p=0.041$, respectively). However, there were no significant differences of HCC between normal and higher-than-recommended TAG (<1.70 mmol/l vs. ≥ 1.70 mmol/l) or lower-than-recommended (>1.00 mmol/l vs. ≤ 1.00 mmol/l) HDL-C level groups (44.38(117.32) ng/g vs. 54.68(119.88) ng/g, $p=0.306$, 41.04 (117.77) ng/g vs. 69.79 (155.05) ng/g, $p=0.351$, respectively). Also, participants with greater BMI (>25.00 kg/m²) and WC (94 cm) values showed significantly higher hair cortisol concentration median (IQR)

values than subjects with normal BMI (≤ 25 kg/m²) and WC (<94 cm) results (32.13(83.25) ng/g vs. 55.12(141.72) ng/g, $p=0.0189$, 34.98(88.41) ng/g vs. 77.16(152.14) ng/g, $p=0.007$, respectively). Although the difference in HCC between hypertensive ($\geq 140/90$ mmHg) and non-hypertensive (<140/90 mmHg) participants did not reach statistical significance, the tendency of higher HCC median (IQR) among the hypertensive participants was observed (67.24(120.50) ng/g vs. 37.78(111.52) ng/g, $p=0.052$). Similarly, there was no significant difference in HCC median (IQR) between the smokers and non-smokers (64.69(130.74) ng/g vs. 39.82(102.50) ng/g, $p=0.113$).

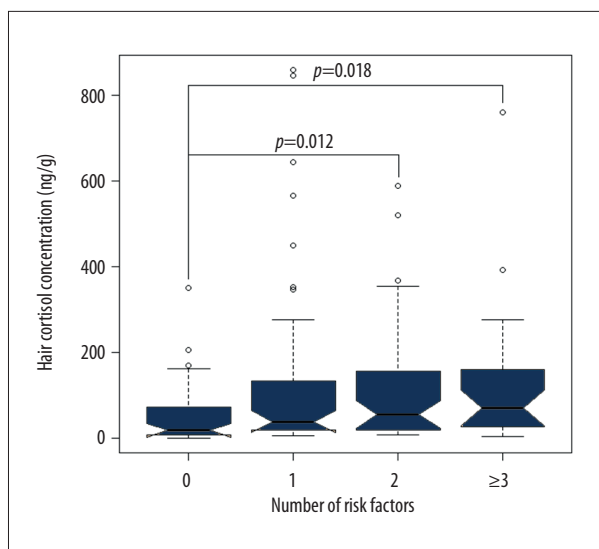


Figure 1. Box plot graph of hair cortisol concentration according to the number of prevalent cardiovascular risk factors.

Due to the well-known synergistic effect of multiple cardiovascular risk factors, we divided the entire study sample into 4 groups according to the prevalence of cardiovascular risk factors: higher-than-recommended LDL-C concentration and WC value, smoking, and hypertension. The results indicated that 30.4% of participants had 1 risk factor, 27.2% had 2 risk factors, 15.8% had 3 or 4 risk factors, and 26.6% of subjects showed no evidence of increased cardiovascular risk. The comparisons among the 4 groups showed a statistically significant trend of increase in HCC with increasing cardiovascular risk, with a statistically significant difference between the first and the third (no risk factors vs. 2 risk factors), and between the first and the fourth (no risk factors vs. ≥ 3 risk factors) groups (Figure 1).

Hair cortisol concentration and inflammatory, oxidative stress, and platelet activation biomarkers

Our results demonstrated a weak positive correlation between HCC and neutrophil percentage, but no associations were

found between HCC and other inflammatory biomarkers such as CRP, CyPA, and WBC count. In addition, no relationship was observed between the HCC and oxidative stress (MDA, FRAP) or platelet activation (CD63+ and PAC-1+ platelets stimulated with TRAP and ADP, respectively) biomarkers. Correlations between the HCC and inflammation, oxidative stress, and platelet activation biomarkers are shown in Table 3.

Allostatic load and hair cortisol concentration

To investigate the association between the HCC and 5 distinct systems (cardiovascular, metabolic, immune, oxidative stress, and platelet activation) risk scores or allostatic load index values, we divided the entire study sample into 2 groups according to HCC median (HCC ≤ 46.182 ng/g vs. HCC >46.182 ng/g). The median values of AL indices were low in both higher and lower HCC groups, as the possible ranges were 0–18. Nevertheless, we found significant differences in cardiovascular and metabolic systems risk scores and multiple systems AL index median (IQR) values between 2 HCC groups. No associations were found between the immune, oxidative stress, and platelet activation systems risk scores median (IQR) values and HCC. The high-risk cut-off point values for individual biomarkers used for AL index calculation are provided in Table 4. Differences in individual systems risk scores and allostatic load index median (IQR) values according to HCC groups are shown in Table 5.

Discussion

Although a number of studies have addressed the association between the HCC and individual cardiovascular risk factors, there are limited data describing the relationship between HCC and the number of prevalent cardiovascular risk factors. Thus, the main discovery of our work is the association of higher HCC and the increased number of cardiovascular risk factors, which indicates elevated risk of CVD. These results are in accordance with the evidence of the association of chronic stress (evaluated subjectively using self-reported questionnaires or

Table 3. Correlations between hair cortisol concentration and inflammation, oxidative stress, and platelet activation biomarkers.

Variables	Spearman's r	p-value
C-reactive protein (mg/l)	0.108	0.171
Cyclophilin A (ng/ml)	0.090	0.260
WBC count ($10^9/l$)	0.089	0.262
Neutrophil percentage (%)	0.197	0.012
Malondialdehyde ($\mu g/l$)	0.087	0.301
Ferric reducing ability of plasma ($\mu mol Fe^{2+}/l$)	0.102	0.203
CD63+ platelets stimulated with TRAP (%)	0.066	0.416
PAC-1 + platelets stimulated with ADP (%)	-0.018	0.840

Table 4. High-risk cut-off point values of individual biomarkers used in calculating the allostatic load index.

System	Biomarker	n	High-risk cut-off point
Cardiovascular	Resting SBP (mmHg)	163	≥136
	Resting DBP (mmHg)	163	≥83
	Resting heart rate (bpm)	163	≥71
Metabolic	BMI(kg/m ²)	163	≥27.91
	WC (cm)*	162	≥98
	Fasting glucose (mmol/l)	163	≥5.50
	Triacylglycerols (mmol/l)	163	≥1.74
	HDL cholesterol (mmol/l)	163	≤1.055
	LDL cholesterol (mmol/l)	163	≥3.79
	Total cholesterol (mmol/l)	163	≥5.83
Immune	CRP (mg/l)*	162	≥1.30
	Neutrophil percentage (%)*	161	≥52.81
	WBC(10 ⁹ /l)*	161	≥6.28
	CypA (ng/ml)*	160	≥81.00
Oxidative stress	MDA (µg/l)*	142	≥119.30
	FRAP (µmol Fe ²⁺ /l)*	158	≤42.92
Platelet activation	CD63+ platelets stimulated with TRAP (%)*	152	≥40.35
	PAC-1 + platelets stimulated with ADP (%)*	129	≥66.40

* Variable has missing data.

Table 5. Differences in individual systems' risk scores and allostatic load index values according to hair cortisol concentration groups.

Variable	Median (IQR)		p-value
	HCC ≤46.182 ng/g	HCC >46.182 ng/g	
SRS (cardiovascular)	0 (1)	1 (2)	0.028
SRS (metabolic)	1 (2)	2 (3)	0.009
SRS (immune)	1 (1)	1 (2)	0.135
SRS (oxidative stress)	0 (1)	1 (1)	0.243
SRS (platelet activity)	0.5 (1)	0.5 (1)	0.138
AL index	3.5 (3)	5 (3)	0.004

objectively measuring HCC) and increased risk of CVD [7,29,30]. A previous cross-sectional study Manenschijs et al. [7] showed that high hair cortisol concentration (fourth HCC quartile) is associated with a 2.7 times increased CVD risk, including coronary heart disease, stroke, and peripheral arterial disease in community-dwelling elderly participants. Furthermore, the results of a prospective case-control study conducted by Pereg and coworkers [29] showed significantly higher HCC in patients with acute myocardial infarction compared with a control group. Similarly, the large case-control INTERHEART [30]

study reported that presence of subjectively evaluated psychosocial stress, including stress at home or work, financial stress, stressful life events, and depression, is associated with increased risk of myocardial infarction.

The present study also examined the relationship between hair cortisol level and the manifestation of traditional cardiovascular risk factors. We found a positive association of higher HCC with greater-than-recommended BMI and WC values. These findings are in line with a study [31] in which obese (BMI ≥30 kg/m²)

participants had significantly higher mean hair cortisol level than normal-weight (BMI 18.5–25 kg/m²) or overweight (BMI 25–29.9 kg/m²) participants and subjects with raised WC (≥ 102 cm in men and ≥ 88 cm in women), indicating central obesity was associated with elevated HCC compared with participants whose waist circumference was in normal range. In other similar studies [32,33], the waist-to-hip ratio was determined as an additional anthropometric measure and a positive correlation was found between HCC and waist-to-hip ratio values. Since the biological effect of cortisol is manifested in the accumulation of abdominal fat, the increase in parameters related to body fat distribution, such as waist circumference and waist-to-hip ratio, may be the consequence of chronic cortisol exposure. In addition, a previous study [34] examined the association of HCC with the prevalence of metabolic syndrome diagnosed by the increase of 3 or more risk factors, including elevated waist circumference, blood pressure, triacylglycerol, long-term glucose levels, and reduced HDL cholesterol concentration. The authors found that individuals with metabolic syndrome had significantly higher HCC as compared to those without metabolic syndrome. Also, we found a positive association between increased HCC and higher-than-recommended TC and LDL-C concentration values. The existing data on the relationship between dyslipidemia and HCC is controversial. A previous study [35], in which healthy subjects and patients with major depression were recruited, showed significant positive correlations between the hair cortisol or cortisone (the inactive form of cortisol) and triacylglycerol levels in blood serum in both study groups, but in our study found no significant differences in HCC between the participants with higher-than-recommended and normal TAG concentrations. In contrast to our study, Stalder et al. [34] found no relationship between HCC and lipid metabolism biomarkers such as LDL-C and TAG levels, but discovered a significant negative correlation between HCC and HDL-C level in a large occupational cohort. These inconsistencies may arise from the fact that both genders and participants with a wider range of age were involved in previous studies [34,35], while the present study was limited to only young and middle-aged men. However, since dyslipidemia is one of the main cardiovascular risk factors, further research is clearly needed for the examination of chronic cortisol exposure and its effect on lipid metabolism.

The results of the present study demonstrate the tendency toward higher HCC in hypertensive subjects compared with non-hypertensive individuals. However, the data on the relationship between arterial blood pressure and hair cortisol level is inconsistent. While some previous research [34,36] reported a positive association between mean arterial blood pressure or systolic blood pressure and HCC, other studies [37,38] failed to demonstrate the link between arterial blood pressure and hair cortisol level. In addition, Feller et al. [32] found no association of prevalent hypertension (evaluated subjectively by

study participants) and HCC. Moreover, they found a negative relationship between objectively measured diastolic blood pressure and HCC. These inconsistent results could be explained by differences in characteristics of the study samples, as some previous studies [37,38] involved patients with structural heart disease or coronary artery disease, while the present and other similar studies [34,36] assessed subjects with no current diagnosis of cardiovascular disease.

Our findings also revealed no significant association between smoking status and HCC. Similar results have been reported by a previous study [34], but different findings were obtained in another study [32] in which higher HCC was observed in current smokers compared with non-smokers. These ambiguous results may arise from the fact that the relationship between cigarette smoking and HCC may be dose- and time-dependent, but these 2 aspects were not yet been assessed.

It is hypothesized that chronic stress induces long-term cortisol exposure, resulting in glucocorticoid receptor resistance that leads to diminished downregulation of inflammatory processes. The risk of chronic inflammatory diseases such as atherosclerosis is increased when inflammation duration is longer and intensity is elevated [39]. Our results indicate a weak but statistically significant correlation between HCC and neutrophil percentage value. This finding may be important, as there is growing evidence that neutrophils take part in the progression of atherosclerosis by forming neutrophil extracellular traps that enhance cytokine production in macrophages and activate T helper 17 cells responsible for maintaining the inflammatory process [40,41]. Additionally, results in a very recent paper by Penz et al. [42] show that the increase in HCC is associated with changes in distribution of leukocytes, resulting in expansion of neutrophils and decrease in lymphocytes and monocytes. Moreover, inflammation-induced synthesis of proinflammatory cytokines derived from monocytes and macrophages enhances the release of CRP, which itself promotes inflammation and atherogenesis [39,43]. However, we found no correlation between HCC and CRP level in blood serum. Similarly, the association between HCC and a relatively new cardiovascular inflammation biomarker, CypA, was also non-significant.

In this work, chronic stress level was measured using 2 different techniques: HCC measurement and AL index calculation. While determination of HCC represents the activity of the hypothalamic-pituitary-adrenal axis, AL index is a measure of cumulative “wear and tear” on the body caused by the dysregulation of multiple physiological systems (e.g., metabolic, cardiovascular, immune) in response to stressors. The very first technique used for the calculation of allostatic load index containing 10 biomarkers (TC, HDL-C, glycosylated hemoglobin, waist-to-hip ratio, dehydroepiandrosterone sulfate, urinary epinephrine, norepinephrine, and cortisol) was introduced and

validated in the MacArthur Study of Successful Aging [44]. The ability of allostatic load index to predict negative health outcomes was evaluated over a 2.5-year follow-up in which significantly elevated mortality risk was observed. Also, a more recent study [25] showed a high test-retest reliability (intra-class correlation coefficient 0.88) between 2 consecutive days of measurement of 6 different physiological systems representing the AL index. We found significantly increased cardiovascular and metabolic systems risk scores and allostatic load index values in the higher HCC group. This is in line with a study [25] in which 18 biomarkers were included in the estimation of AL index, and a moderate correlation between HCC and AL index was observed. We suggest that both techniques could be applied in the assessment of physiological response to long-term stress, since our findings showed a positive association between HCC and AL index values. On the other hand, there are many challenges in using these 2 techniques in chronic stress research. Firstly, different methods are used for the determination of HCC, including enzyme-linked immunoassay, liquid chromatography with fluorescence detection or gas chromatography, and liquid chromatography coupled with mass spectrometry. Secondly, there are many methodological differences regarding the calculation of AL index, including variation in the number and combination of biomarkers and definition of high-risk cut-off point values [11,25–27].

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Conclusions

In conclusion, our findings indicate that higher HCC is significantly associated with the prevalence of some cardiovascular risk factors, such as higher-than-recommended BMI, WC, TC, LDL-C, and non-HDL-C values. Furthermore, greater CVD risk assessed by the number of prevalent cardiovascular risk factors, including smoking, hypertension, and greater-than-recommended WC and LDL-C values, is associated with higher HCC. Finally, our results suggest that both HCC measurement and AL index calculation techniques are valuable for the assessment of chronic stress level. However, there are some methodological considerations (e.g., differences in HCC measurement analytical techniques or AL index calculation algorithms) regarding the use of these methods in chronic stress research. Thus, future studies in this field should focus on the standardization and validation of objective chronic stress evaluation techniques.

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Conflicting of interests

None.

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