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Hair and fingernail cortisol and the onset of acute coronary syndrome in the middle-aged and elderly men

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ABSTRACT

Cortisol levels in hair and fingernail samples could represent hormone levels that have accumulated over the past weeks and months. In this study, by using retrospective indexes, the associations between cortisol and the onset of acute coronary syndrome (ACS) were investigated among middle-aged and elderly men. We measured hair/fingernail cortisol levels in 73 ACS patients and 93 healthy controls; hair and fingernail samples for ACS patients were collected within a few weeks after the onset of ACS. The results indicated the patients exhibited significantly higher cortisol levels in their hair and fingernails compared with the healthy controls. In multivariate logistic regression analyses, adjusting for the traditional cardiovascular risk factors for ACS, high levels of hair or fingernail cortisol were associated with two- to three-fold increased risk of ACS, compared with low levels. We demonstrated that cortisol exposure over a relatively long period, assessed by hair and fingernail samples, was associated with the onset of ACS.

1. Introduction

Acute psychosocial stress triggers an activation of the hypothalamic-pituitary-adrenal axis, which causes the adrenal cortex to secrete cortisol. Traditionally, cortisol has been measured in blood and saliva samples, which revealed hormone levels for a short time period. Recently, cortisol has been reportedly measured in hair samples (Russell et al., 2012). Scalp hair grows at an average rate of 1.0 cm/month; 1.0 cm of scalp hair may be used to determine the level of the hormone secreted during a period of 1 month. Previous studies reported that psychosocial stress was associated with hair cortisol. Elevated hair cortisol levels were observed in long-term unemployed individuals (Dettenborn et al., 2010), dementia caregivers (Stalder et al., 2014), and people who had been recently exposed to major life events (Staufenbiel et al., 2014). Furthermore, hair cortisol was also associated with health outcomes such as depression (Dettenborn et al., 2012), posttraumatic stress disorder (Stuedte-Schmiedgen, et al., 2015), and cognitive decline after stroke (Ben Assayag et al., 2017).

Furthermore, more recent focus has been given to cortisol measured in fingernail samples. Fingernails grow at an average rate of 1.0 mm/10 days (Gupta et al., 2005); therefore, 1.0 mm of fingernail may retrospectively reflect hormone levels over 10 days. Several months are required for nails to fully extend from the nail matrix (de Berker et al.,

2007; Gupta et al., 2005). Therefore, fingernail samples may reflect cortisol levels several months prior to clipping. Previously, cortisol levels in fingernail samples were associated with those in saliva samples collected 4 or 5 months before (Izawa et al., 2015). Another study reported that psychosocial stress in the past, but not the present, was associated with elevated cortisol levels in fingernails (Izawa et al., 2017). These findings supported the notions that fingernails retrospectively represent past hormone levels. However, evidence concerning fingernail cortisol and health outcomes has been lacking. Only one study reported elevated fingernail cortisol levels in subjects experiencing a major depressive episode (Herane-Vives et al., 2018). Fingernail samples can easily be collected by the participant, and only small amounts are required. Therefore, further evidence is needed regarding fingernail cortisol.

In this study, we focused on coronary heart disease (CHD), one of the well-known stress-related diseases. Chronic psychosocial factors such as socioeconomic status (e.g., lower income and educational level), as well as episodic psychosocial distress, accelerate the development of arteriosclerosis in the coronary artery and contribute to the onset of acute coronary syndrome (Kop, 1997). One cross-sectional study using blood samples (Reynolds et al., 2010) and one prospective study using saliva samples (Schoorlemmer et al., 2009) supported the associations between cortisol and coronary heart disease; however,

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other prospective studies using blood samples did not (Phillips et al., 2010; Rod et al., 2010; Smith et al., 2005). Cortisol levels in blood and saliva represent hormone levels for a short time period with large diurnal rhythms, which might produce inconsistent results. Hair and fingernail cortisol represent hormone levels accumulated over the past weeks and months, which could show some advantages for investigating this association. Previously, only a few studies have investigated the association between hair cortisol and CHD (Abell et al., 2016; Manenschijn et al., 2013; Pereg et al., 2011), and two of them (Abell et al., 2016; Manenschijn et al., 2013) only investigated the cross-sectional associations of hair cortisol and a self-reported history of CHD. To our knowledge, no studies have investigated the association between fingernail cortisol and CHD.

The purpose of this study was to investigate the associations between the onset of acute coronary syndrome (ACS) and cortisol levels in hair and fingernail samples. We measured hair/fingernail cortisol levels in ACS patients and healthy controls. Hair and fingernail samples for ACS patients were collected within a few weeks after the onset of ACS to retrospectively assess cortisol levels before the onset. We expected higher cortisol levels in hair and fingernails to be found in ACS patients, because psychosocial stress could contribute to the onset of ACS (Kop, 1997). We further investigated the associations between hair/fingernail cortisol and ACS, adjusting for ACS risk factors (e.g., dyslipidemia, hypertension) and psychosocial factors (e.g., stressful life events), since these risk factors may have confounded the associations. Furthermore, only one study previously reported the association between hair and fingernail cortisol (Izawa et al., 2015); therefore, this study investigated this association.

2. Methods

2.1. Participants

A case-control study was designed to investigate the relationships between hair/fingernail cortisol and ACS. The patient group comprised 73 men with ACS (acute myocardial infarction and unstable angina pectoris) who were admitted to a hospital located in Fuchu-city in Tokyo from September 2012 to October 2015. A medical doctor and psychologists approached the patients and asked them to participate in this study a few weeks after the onset of ACS (mean (SD) = 20.6 (8.9) days). The definite diagnosis of ACS was based on the patients' clinical histories as taken by a cardiologist, standard ECG readings, and plasma enzyme elevations.

The control group consisted of healthy 93 men living in Tokyo and its surrounding area (Kanagawa, Saitama, and Chiba). They were recruited via an internet-based survey during from October to November 2013. A portion of the data was identical to that used in a previous study (Izawa et al., 2015).

The inclusion criteria of patients and controls were as follows: (1) men; (2) no recurrent ACS (patients), or no previous history of cardiovascular diseases (controls); (3) aged 35–79 years; (4) no adrenal dysfunction (e.g., Cushing syndrome); and (5) not having taken steroid medications in the previous six months.

We were unable to measure cortisol levels in 9 hair samples and 2 fingernail samples due to technical reasons (e.g., insufficient sample volume, technical errors, measured absorbance being over the standard range of the kit). Further, some hair cortisol values (1 control and 1 patient) and fingernail cortisol values (2 controls) were over 3 SD above the mean, and we excluded these data from the analyses. Therefore, 155 hair cortisol samples (92 controls and 63 patients) and 162 fingernail cortisol samples (90 controls and 72 patients) were ultimately analyzed.

Written informed consent was obtained from each participant, and the study was approved by the institutional review board of the Sakakibara Heart Institute and the National Institute of Occupational Safety and Health, Japan.

2.2. Questionnaires

Participants were asked about their marriage status, job status, smoking status, educational background (e.g., high school, university), frequency of hair washing and manicures, and use of hair dye.

Additionally, to determine whether the participants had recently experienced a stressful life event, they were asked to complete the Japanese version of the Social Readjustment Rating Scale (Nomura, 1994), which included 28 stressful life events (e.g., death of spouse, unemployment). Participants were asked whether they had experienced any of 28 life events during the previous year using a yes/no format. If they had, they were asked when the event had occurred. In this study, we counted the total number of stressful life events in the previous six months, because the hair/fingernail samples could reflect hormone levels at any point within the previous weeks or months, up to six.

2.3. Biological risk factors

Data concerning low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, hemoglobin A1c, height, weight, and use of medications for dyslipidemia, hypertension, and diabetes mellitus were also obtained from both groups.

2.4. Procedure

Hair strands were collected by carefully cutting with fine scissors as close as possible to the scalp from a posterior vertex region. Samples of up to 6 cm long were obtained to determine the cortisol levels over the previous 6 months. However, for patients who had longer periods of time between the ACS onset and hair collection, hair strands between 0 and 1 cm from the scalp were not obtained, because these portions could reflect cortisol levels after hospitalization.

For the collection of fingernail samples, participants were asked to grow their fingernails for 2 weeks and provide samples from every digit by clipping the nail directly into a reclosable poly bag to avoid losing any part of the sample.

2.5. Hair and fingernail hormone extraction and enzyme immunoassay

Our hair and fingernail hormone extraction method was identical to that used in a previous study (Izawa et al., 2015). Hair samples were washed 3 times in 2.5 ml of isopropanol, the nail samples were washed twice in 5 ml of isopropanol, and these samples were dried overnight. Hair and nail samples were ground using a mixer mill (Retsch MM300, Germany) at 30 Hz for 15 min and 40 min, respectively. Fifteen milligrams of hair and nail powder were weighted out, and 1.5 ml of pure methanol was added for cortisol extraction over a period of 24 h under slow rotation. Following this, the samples were spun in a microcentrifuge at 10,000 rpm for 2 min, and 1.0 ml of the clear supernatant was evaporated at 60 °C until completely dry.

Cortisol level was determined by an enzyme immunoassay method using the EIA Kit (Salimetrics LLC, USA). The evaporated samples were re-suspended in 100 µl of the assay diluent included in the EIA Kit, and the levels of cortisol in the diluent were analyzed according to the manufacturer's instructions. For hair cortisol, the intra-assay and inter-assay variations were 4.2% and 4.7%, respectively. For nail cortisol, the intra-assay and inter-assay variations were 3.6% and 4.7%, respectively. The findings are presented as pg cortisol /mg hair or fingernail (pg/mg).

2.6. Statistical analyses

Independent t-tests and chi-squared tests were conducted to compare biological factors, lifestyle factors, and psychosocial factors between the controls and patients. Mann–Whitney U-tests were conducted to compare the cortisol levels between the controls and patients.

Multivariate logistic regression analyses were performed to estimate the odds ratios (ORs) of the cortisol levels for ACS, with ACS status (patient = 1, control = 0) as a dependent variable and hair or fingernail cortisol as an independent variable. Hair/fingernail cortisol levels were categorized into three groups (low, medium, or high) based on tertile values because hair and fingernail cortisol values were not normally distributed even after conducting a statistical transformation (e.g., a logarithmic transformation), and it was previously reported that the associations between cortisol and health outcomes are not always linear (e.g., cortisol awakening response and depression, as reported by Chida and Steptoe, 2009). High and medium groups were compared against the low group. We estimated crude ORs, as well as ORs adjusted for the ACS traditional risk factors (age, obesity, dyslipidemia, diabetes mellitus, hypertension, and current smoking status). Obesity was defined as body mass index ≥ 25 kg/m². Dyslipidemia was defined as LDL ≥ 140 mg/dl and/or HDL < 40 mg/dL, and/or medical treatment for dyslipidemia. Diabetes mellitus was defined as hemoglobin A1c $\geq 6.5\%$, and/or medical treatment for diabetes mellitus. Hypertension was defined as medical treatment for hypertension. Furthermore, multivariate logistic regression analyses were performed to estimate ORs adjusting for psychosocial factors as a means to test whether psychosocial factors could be confounding the associations between hair/fingernail cortisol and ACS. In these analyses, we also estimated P values for trends from the logistic regression analyses, including an ordinal variable for cortisol levels (low = 1, medium = 2, high = 3) in the model.

In the preliminary analyses, we further investigated the logistic regression analyses while excluding the participants who reported use of hair dye (N = 39) or the use of manicure (N = 2). However, the results were not largely different; therefore, we reported the results of logistic regression analyses including those participants.

Pearson and Spearman rank-order correlations were also conducted to investigate the relationships between hair cortisol, fingernail cortisol, traditional risk factors, and psychosocial factors.

3. Results

3.1. Characteristics of the controls and patients

As shown in Table 1, the patients exhibited lower levels of HDL cholesterol, and the percentage of persons with dyslipidemia was higher among the patients. Current smokers were more prevalent among the patients than the controls. For the psychosocial factors, more highly educated persons were more prevalent among the controls, and the patients reported higher frequency of stressful life events within the previous six months.

3.2. Hair/fingernail cortisol levels in the controls and patients

Medians (ranges) of hair cortisol were 9.2 (3.1–45.9) pg/mg and 11.9 (1.3–67.2) pg/mg for controls and patients, respectively. Medians (ranges) of fingernail cortisol were 7.0 (2.2–56.7) pg/mg and 8.2 (3.9–85.6) pg/mg for controls and patients, respectively. Patients significantly exhibited higher cortisol levels in both hair (U = 2339.5, $p = 0.042$) and fingernails (U = 2493.5, $p = 0.012$, Fig. 1).

3.3. Hair/fingernail cortisol, traditional risk factors, and ACS risk

In multivariate logistic regression analyses, high levels of hair cortisol were associated with an elevated risk of ACS in the crude (OR = 2.36 [95% CI 1.07–5.19], P trend = 0.032) as well as the traditional risk factor-adjusted models (OR = 3.27 [95% CI 1.28–8.33], P trend = 0.012, Table 2). Further, we also found that high levels of fingernail cortisol were associated with an elevated risk of ACS in the crude (OR = 2.23 [95% CI 1.02–4.88], P trend = 0.046) as well as the traditional risk factor-adjusted models (OR = 2.48 [95% CI 1.02–6.06],

P trend = 0.049, Table 3).

The proportion of traditional risk factors did not differ among the low, medium, and high hair cortisol groups or among the low, medium, and high fingernail cortisol groups. We also computed rank-order correlations between hair/fingernail cortisol and traditional risk factors, and we found a significant correlation between hair cortisol and the diabetes mellitus status of the patient ($r_s = .18$, $p = 0.023$).

3.4. Hair/fingernail cortisol, psychosocial factors, and ACS risk

We found significant differences in educational background and number of stressful life events between patients and controls. Therefore, in the subsequent multivariate logistic regression analyses, we estimated ORs additionally adjusting for psychosocial factors (specifically, stressful life events and educational level) to test whether or not inclusion of these psychosocial factors in the model altered the associations between hair/fingernail cortisol and ACS. High levels of hair cortisol were still associated with an elevated risk of ACS (OR = 2.90 [95% CI 1.12–7.46], P trend = 0.026, Table 2). However, the association between high fingernail cortisol and ACS was attenuated and not significant (OR = 2.14 [95% CI 0.85–5.38], P trend = 0.105, Table 3).

The proportion of marriage status, job status, and educational background as well as number of stressful life events did not differ among the hair cortisol groups or the fingernail cortisol groups. We also computed rank-order correlations between hair/fingernail cortisol and psychosocial factors, and we found significant correlations between hair and fingernail cortisol and the number of stressful life events ($r_s = .17$, $p = 0.036$ and $r_s = .15$, $p = 0.052$, respectively). Further, men with a higher educational levels ($r_s = -.16$, $p = 0.044$) and married men ($r_s = -.15$, $p = 0.061$) exhibited lower fingernail cortisol.

3.5. Correlations between hair and fingernail cortisol

We found a significant rank-order, but not a Pearson correlation, between hair and fingernail cortisol level ($r_s = 0.23$, $p = 0.004$; $r = .04$, $p = .619$). However, when excluding two data with higher fingernail cortisol levels (56.7 and 85.6 pg/mg), we found both significant rank-order and Pearson correlations ($r_s = 0.24$, $p = 0.003$; $r = 0.22$, $p = 0.006$, respectively; Fig. 2).

4. Discussion

The purpose of this study was to investigate the associations between the onset of acute coronary syndrome and cortisol levels in hair and fingernail samples. We found higher cortisol levels in hair and fingernail samples among the ACS patients compared with healthy controls. Additionally, less educated persons were more prevalent among the patients, and they reported higher frequency of stressful life events over the previous six months, implying that the patients had been under unfavorable psychosocial conditions before the onset, as we expected. The findings on the psychosocial conditions were also consistent with the previous prospective findings (e.g., Hirokawa et al., 2006; Iso et al., 2002). The persons with traditional risk factors such as dyslipidemia and smoking habits were more prevalent among the patients, but the associations between hair/fingernail cortisol and ACS remained after adjusting for the effects of the risk factors in the logistic regression analyses. Further, inclusion of psychosocial factors in the logistic regression analyses attenuated the association between fingernail cortisol, but not hair cortisol, and ACS.

We found higher hair cortisol levels among the patients, indicating higher cortisol levels for several months before ACS onset, because hair samples can retrospectively reveal accumulated hormone levels (Russell et al., 2012). Additionally, the logistic regression analyses revealed that high level of hair cortisol was associated with a three-fold increase in the risk of ACS. Previously, the reported associations between cortisol in saliva and blood and CHD were inconsistent (Reynolds

Table 1
Characteristics of the controls and patients.

	Controls (N = 93)	ACS patients (N = 73)	t scores or χ^2 scores ^a	p
Biological factors				
Age, mean \pm SD	59.6 \pm 9.2	60.5 \pm 9.4	0.57	0.567
Gender (men), n (%)	93 (100.0)	73 (100.0)		
Body mass index, mean \pm SD	23.7 \pm 3.2	24.4 \pm 2.8	1.50	0.137
Obesity, n (%) ^b	23 (31.5)	29 (31.2)	0.00	0.964
LDL (mg/dL), mean \pm SD	121.2 \pm 27.8	126.1 \pm 37.6	1.05	0.313
HDL (mg/dL), mean \pm SD	58.6 \pm 14.8	46.5 \pm 12.8	5.53	< 0.001
Dyslipidemia, n (%) ^c	30 (32.3)	53 (72.6)	26.62	< 0.001
Diabetes mellitus, n (%) ^d	10 (10.8)	13 (17.8)	1.71	0.192
Hypertension, n (%) ^e	24 (25.8)	29 (39.7)	3.65	0.056
Lifestyle factors				
Smoking status (current smoker), n (%)	18 (19.4)	26 (35.6)	5.55	0.011
Hair dye use, n (%) ^f	24 (26.1)	14 (22.2)	0.30	0.583
Hair wash (frequency per week), mean \pm SD ^f	5.1 \pm 2.3	5.2 \pm 2.2	0.24	0.810
Manicure use, n (%) ^g	1 (1.1)	1 (1.4)	0.03	0.874
Psychosocial factors				
Marriage status (married men), n (%)	76 (81.7)	59 (80.8)	0.02	0.883
Education (university graduates or those with higher education), n (%)	72 (77.4)	43 (58.9)	6.59	0.010
Job status (working men), n (%)	67 (72.0)	58 (80.0)	1.21	0.272
Number of stressful life events in the previous six months, mean \pm SD	0.37 \pm 0.64	0.74 \pm 1.30	2.25	0.027

^a independent t-tests or chi-squared tests were conducted.

^b defined as body mass index \geq 25.

^c defined as LDL \geq 140 mg/dl and/or HDL < 40 mg/dL, and/or medical treatment for dyslipidemia.

^d defined as hemoglobin A1c \geq 6.5%, and/or medical treatment for diabetes mellitus.

^e defined as medical treatment for hypertension.

^f n = 92 and 63 for controls and ACS patients, respectively.

^g n = 90 and 72 for controls and ACS patients, respectively.

et al., 2010; Phillips et al., 2010; Rod et al., 2010; Schoorlemmer et al., 2009; Smith et al., 2005), perhaps because saliva and blood samples represented hormone levels for a short time period with a large diurnal rhythm. Pereg et al. (2011) reported that hair cortisol was associated with an increased risk of acute myocardial infarction when hair samples were collected a few days after hospitalization. It is worth noting that both their study (Pereg et al., 2011) and this study reported that CHD patients exhibited higher cortisol levels in hair by approximately 30% compared with controls, although reported cortisol levels differed substantially, which may be due to the fact that in the previous study (Pereg et al., 2011) hair strands were not washed with alcohol solvent before the extraction. Furthermore, two previous studies cross-

sectionally investigated the associations between hair cortisol and CHD, and one supported the association in elderly participant samples (Manenschijn et al., 2013), while the other did not in a large observational cohort (Abell et al., 2016). However, these studies only investigated the associations of hair cortisol with a self-reported history of CHD, not with onset of CHD.

We also found a higher fingernail cortisol level among the patients. A previous study found that 3–4 months were required for nails to fully extend from the nail matrix (Buzalaf et al., 2006). Our previous study (Izawa et al., 2015) also indicated that cortisol levels in fingernail samples were associated with those from saliva samples collected 4 or 5 months previously. Therefore, the findings of this study could indicate

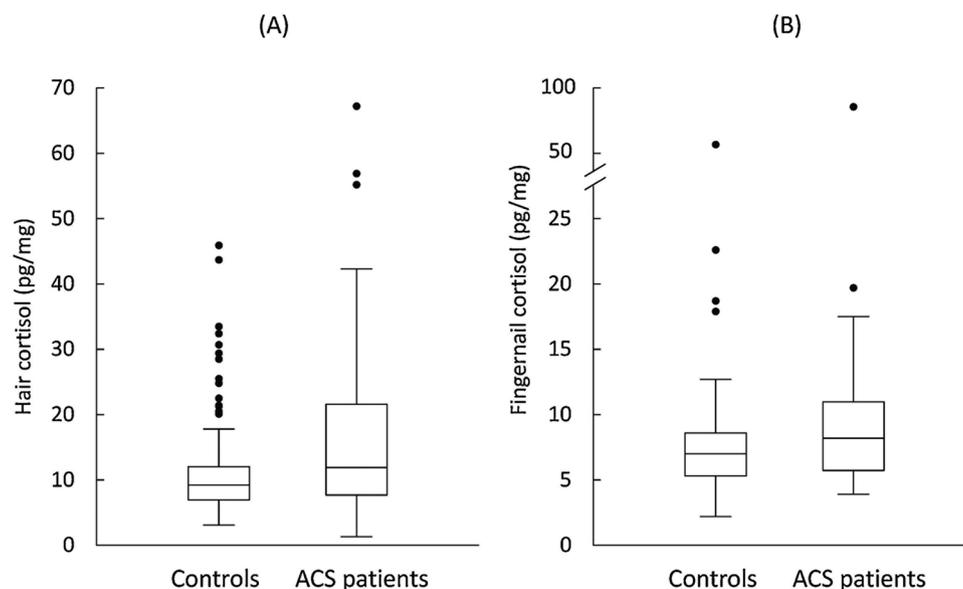


Fig. 1. Boxplots of hair (A) and fingernail (B) cortisol levels in the controls and ACS patients. Patients significantly exhibited higher cortisol levels in hair and fingernails ($p < 0.05$, Mann–Whitney U-test).

Table 2
Risk of ACS and hair cortisol (N = 155)^a.

Risk factors	Crude OR (95% CI)	OR (95% CI) adjusting for traditional risk factors	OR (95% CI) adjusting for psychosocial factors
Hair cortisol			
Low	1.00	1.00	1.00
Medium	0.75 (0.33-1.70)	0.91 (0.35-2.35)	0.85 (0.32-2.27)
High	2.36 (1.07-5.19)	3.27 (1.28-8.40)	2.90 (1.12-7.46)
Age (per yr)		1.01 (0.97-1.06)	1.01 (0.97-1.06)
Current smoking		2.42 (1.01-5.80)	2.28 (0.92-5.67)
Obesity		0.92 (0.40-2.11)	0.86 (0.36-2.07)
Dyslipidemia		7.65 (3.44-17.00)	7.35 (3.26-16.59)
Diabetes mellitus		0.92 (0.29-2.88)	0.91 (0.28-2.94)
Hypertension		1.52 (0.65-3.52)	1.68 (0.70-4.03)
Stressful life events (per event)			1.44 (0.84-2.46)
Higher education ^b			0.52 (0.22-1.24)

^a Logistic regression analyses were conducted to estimate the crude and adjusted odds ratios (ORs) and 95% confidence intervals (CI) of ACS. Significant ORs ($p < .05$) are indicated in bold.

^b University graduates or those with higher education.

Table 3
Risk of ACS and fingernail cortisol (N = 162)^a.

Risk factors	Crude OR (95% CI)	OR (95% CI) adjusting for traditional risk factors	OR (95% CI) adjusting for psychosocial factors
Fingernail cortisol			
Low	1.00	1.00	1.00
Medium	1.27 (0.60-2.72)	1.06 (0.45-2.50)	0.93 (0.38-2.27)
High	2.23 (1.02-4.88)	2.48 (1.02-6.07)	2.14 (0.85-5.38)
Age (per yr)		1.01 (0.97-1.05)	1.01 (0.97-1.05)
Current smoking		2.14 (0.96-4.80)	1.98 (0.86-4.56)
Obesity		0.87 (0.39-1.90)	0.80 (0.35-1.83)
Dyslipidemia		5.52 (2.68-11.37)	5.31 (2.53-11.13)
Diabetes mellitus		1.01 (0.36-2.82)	1.02 (0.35-2.97)
Hypertension		1.81 (0.82-3.98)	1.91 (0.83-4.35)
Stressful life events (per event)			1.47 (0.90-2.40)
Higher education ^b			0.50 (0.22-1.13)

^a Logistic regression analyses were conducted to estimate the crude and adjusted odds ratios (ORs) and 95% confidence intervals (CI) of ACS. Significant ORs ($p < .05$) are indicated in bold.

^b University graduates or those with higher education.

higher cortisol levels among the patients several months before ACS onset. Further, the logistic regression analyses revealed that high levels of fingernail cortisol were associated with a two-fold increase in the risk of ACS. To our knowledge, this is the first study demonstrating the association between fingernail cortisol and ACS. Compared with hair samples, fingernail samples have some advantages: the samples can be self-collected, and only small amounts are required. Considering that fingernail cortisol was significantly correlated with hair cortisol in this study, future studies could investigate the value of fingernail samples for predicting health outcomes.

The pathophysiological mechanisms underlying the relationship between ACS and chronically elevated cortisol need to be considered. Psychosocial factors could contribute to the development of arteriosclerosis in the coronary artery and the onset of ACS by a number of different biological pathways (Kop, 1997). In a previous study, for example, heightened cortisol reactivity to mental stress was associated with coronary artery calcification (Hamer et al., 2012). Other studies also reported that higher cortisol levels contributed to hypercoagulable states such as increased concentration of fibrinogen (Lippi et al., 2008; von Känel et al., 2007). These pathophysiological states could link

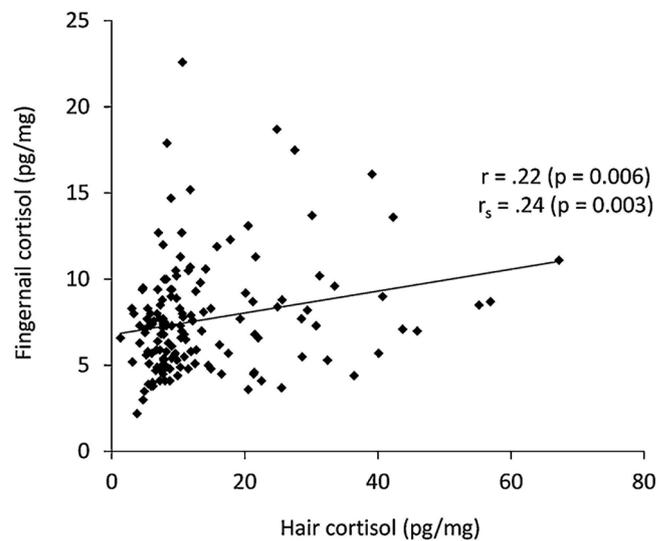


Fig. 2. Scatter plots illustrating the relationship between the cortisol levels in hair and fingernail samples (n = 149). r: Pearson product-moment correlation, r_s : Spearman rank-order correlation.

cortisol and ACS onset.

In this study, we observed associations between hair/fingernail cortisol and the onset of ACS, but these associations should be interpreted carefully. First, in the logistic regression analyses, inclusion of psychosocial factors (stressful life events and educational level) attenuated the association between fingernail cortisol and ACS, which could imply that psychosocial factors confounded the association between fingernail cortisol and ACS and that fingernail cortisol might not be directly associated with the onset of ACS. There is a possibility that, in this study, fingernail cortisol could reflect the stress level of the participants before the onset of ACS, but its physiological effect on ACS was relatively small, which could be related to the fact that fingernails reflect hormone levels for shorter periods of time compared with hair samples. Second, although cortisol measured in the hair and fingernails reflect cortisol levels prior to experiencing the ACS event, it is still possible that subclinical disease (e.g., chest pain) before the onset of ACS may have increased cortisol levels. Third, we only compared the ACS patients with healthy controls, but not with patients hospitalized for the other diseases. Therefore, it is not possible to say that the association is specific to ACS, and cortisol levels could possibly be associated with other diseases. However, a previous study (Pereg et al., 2011) reported the association between CHD and hair cortisol, in which CHD patients were compared with patients hospitalized for other diseases. Their results suggested that cortisol could be specific to ACS. Fourth, we also found a significant correlation between hair cortisol and diabetes mellitus, which was consistent with previous findings (e.g., Abell et al., 2016; Manenschijn et al., 2013; Stalder et al., 2013). Careful attention should be paid to the role of traditional cardiovascular risk factors, including diabetes mellitus, when considering the relationship between cortisol and CHD. Although we found that hair/fingernail cortisol levels were independently associated with ACS after controlling for cardiovascular risk factors, it is still possible that high cortisol levels additionally contributed to ACS through more severe cardiovascular risk factors.

We found a statistically significant association between hair and fingernail cortisol, but the association was relatively weak. In this study, the fingernail samples could show cortisol levels for anywhere from two weeks to several months prior to the ACS onset, because we collected fingernail samples that had grown for two weeks, and several months are required for nails to fully extend from the nail matrix (de Berker et al., 2007; Gupta et al., 2005). The hair samples, however,

could show cortisol levels for as long as the previous six months. The time gap between the two specimens may contribute to the weak correlation. For example, cortisol elevation by stressful experience one month before the onset of ACS could be reflected in the hair but not the fingernail samples. Although fingernail cortisol was previously investigated in several studies, the validity of the measurement was not fully established. Two studies (Frugé et al., 2017; Izawa et al., 2015) confirmed the associations between cortisol levels in the fingernail samples and other specimens. Future studies need to systematically investigate the validity of fingernail cortisol.

This study has several other limitations as well. The patients were from one hospital, and the controls were recruited via an internet-based survey. This may have caused sampling bias, although we carefully checked the backgrounds of the participants, as demonstrated in Table 1. Furthermore, the patients and controls consisted of only male participants. This was because among the Japanese, CHD was more common in males than in females. Further, considering the obvious hormonal differences between males and females, we only included male participants in this study. Future studies could investigate the associations among psychosocial stress, cortisol, and onset of ACS in a larger, less-biased sample.

In conclusion, we retrospectively investigated the associations between onset of ACS and cortisol levels using hair and fingernail samples from middle-aged and elderly men. We found higher cortisol levels in hair and fingernail samples among the ACS patients compared with the healthy controls, indicating higher cortisol levels among the patients for several weeks or months before the onset of ACS. The associations remained after the adjustments for the other ACS risk factors. High levels of hair or fingernail cortisol were associated with a two- to three-fold increased risk of ACS. The association between fingernail cortisol and ACS was, however, attenuated after the adjustments for psychosocial factors. We demonstrated that cortisol exposure over a relatively long period, as assessed by hair and fingernail samples, was associated with the onset of ACS.

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Conflict of interest

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