Cortisol levels in scalp hair of patients with structural heart disease

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ABSTRACT

Background: Stress is considered a modifiable risk factor for cardiovascular disease. Scalp hair analysis is a tool to assess long-term exposure to the stress hormone cortisol. We aimed to determine the association between hair cortisol concentrations (HCC) and clinical characteristics in patients with structural heart disease. Additionally, we investigated potential predictors for longitudinal change in HCC.

Methods: The study consisted of 261 patients with structural heart disease from a randomized controlled trial of mindfulness training. One sample of scalp hair was used to determine HCC both at baseline and at 12-weeks follow-up. In 151 patients HCC was available (mean age: 41.3 years, range 18-65). We investigated the association between HCC at baseline and several physiological measures (BMI, blood pressure, heart rate, respiratory rate, 6-minute walk test), as well as psychological parameters (physical and mental component summary measure (SF-36), emotional distress (HADS), and perceived stress). Additionally, we used these clinical parameters to predict HCC change over time.

Results: The median HCC was 22.3 pg/mg hair (23.5 interquartile range). In multivariable linear regression analyses an association was observed between log-transformed HCC and BMI (β 0.171, p=0.037), respiratory rate (β 0.194, p=0.016), and the physical summary score (β -0.163, p=0.054). Independent predictors of log-transformed HCC change after 12 weeks were mental summary score (β -0.200, p=0.019) and diastolic blood pressure (β -0.171, p=0.049).

Conclusions: In patients with structural heart disease a positive association exists between HCC and BMI. Mental health status may predict a change in long-term cortisol over time.

INTRODUCTION

Chronic stressors such as anxiety, depression, and hopelessness are thought to be important risk factors for cardiovascular disease (CVD). While there is a large body of evidence linking psychological states to ischemic heart disease [1-5], little is known about the role of stressors and the human stress response in structural heart disease.

The human stress response is mainly mediated by two classes of hormones: glucocorticoids and catecholamines. Cortisol, the main glucocorticoid hormone in humans, is produced under influence of the hypothalamic-pituitary-adrenal axis (HPA-axis) [1] and affects virtually all aspects of physiology with mediating effects in inflammation, metabolism, and behavior. An extreme excess of cortisol (i.e., Cushing's syndrome) is associated with an increase of all features of the metabolic syndrome and cardiovascular disease risk [6]. In research, cortisol levels have mostly been measured in blood, saliva and urine, which reflect a time-point measurement of usually up to 24 hours of cortisol exposure. However, cortisol levels are highly variable due to acute stress, the diurnal rhythm, pulsatile secretion, and day-to-day fluctuations [7-9]. Therefore, measurements in body fluids are thought to be of limited value in evaluating long-term cortisol exposure.

In the past few years, scalp hair analysis has been validated as a method to evaluate long-term cortisol levels. Scalp hair grows at a rate of approximately 1 cm per month, therefore one cm hair represents cumulative cortisol exposure of one month. This technique can be used to create retrospective timelines of cortisol exposure by separating hair samples into segments and analyzing these separately [10, 11]. Hair cortisol concentrations (HCC) have been associated with a wide range of somatic and mental health outcomes [7, 12]. Of note, increased HCC has been associated with cardiovascular disease [13], metabolic syndrome [14] and obesity [15, 16] and may therefore provide a novel treatment target in cardiovascular risk management.

The aim of the current study is to investigate the cross-sectional association between cortisol levels, measured in scalp hair, and several clinically relevant physical and psychological characteristics, such as: exercise tolerance, blood pressure, body mass index(BMI), NT-proBNP, perceived stress, and subjective health status in patients with structural heart disease. Additionally, we investigated whether these characteristics act as predictors for cortisol change over a period of 12-weeks. We hypothesized that higher long-term cortisol levels are associated with an adverse clinical status and lower subjective health status.

METHODS

Setting

The current study was part of a single-center pragmatic randomized controlled trial on the effectiveness of an online mindfulness training compared to routine medical care at the outpatient cardiology clinic of the Erasmus MC, Rotterdam, the Netherlands. Ethical approval was obtained from the local Medical Ethics Committee and written consent was obtained from each participant before baseline measurements. Randomization was performed by dedicated computer software (2:1 ratio, blocks of 12 to receive mindfulness training or usual care (UC)). The study was registered at the Dutch trial register, NTR3453, http://www.trialregister.nl.

Briefly, adult patients with structural heart disease (including cardiomyopathies (CMP), congenital (ConHD), ischemic and valvular heart disease) visiting the outpatient clinic were eligible for inclusion. In the current sub-study, hair samples were collected if there was sufficient hair at the posterior vertex (≥4 centimeter (cm)).

Study variables

In order to document and adjust for baseline levels, traditional cardiovascular risk factors and demographics were determined: age, smoking, type of structural heart disease, and employment status.

Outcome measures

Outcome measures of all patients took place at pre- (T0) and post-intervention (12 weeks online mindfulness therapy, T1).

Blood sampling laboratory tests

Peripheral venous blood samples were obtained from all patients. Levels of N-terminal pro-brain natriuretic peptide (NT-proBNP) and creatinin were measured. We used the Elecsys system (Roche Diagnostics, Basel, Switzerland) to determine the NT-proBNP levels (normal values ≤ 14pmol/L).

Hair cortisol

At T1, hair samples were taken from the study participants. The samples consisted of approximately 100-150 hairs and were taken from the posterior vertex as close to the scalp as possible. The length of the hair had to be at least 4 cm, representing the 4 months since the start of the study. The samples were cut in 2 segments (see Figure 1): the proximal 1 cm (representing mean cortisol levels in the month before the T1 visit) and a section of 1 cm at the 4th cm from the scalp (representing the cortisol levels in the month before T0). The hair sample segments were prepared according to a standardized protocol that has been described in detail [11, 17]. Briefly, approximately 15 mg of hairs was weighed per hair segment, and put in a glass vial. Care was taken to make sure two hair segments did not differ in weight more than 1 mg within a subject.

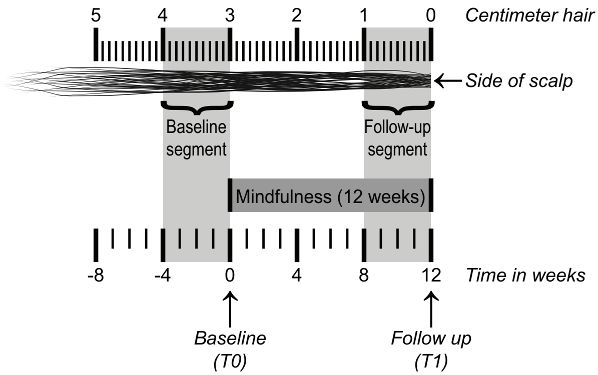

Figure 1. Hair sample and corresponding timeline.

After this, hair segments were finely minced using small scissors. Cortisol extraction took place in methanol, during 16 hours at 52 degrees Celsius while the samples were being gently shaken. After extraction, the methanol was transferred to clean glass tubes and evaporated under nitrogen stream. Samples were reconstituted in 250 μL of phosphate buffered saline (pH 8.0), and HCC were analyzed using a commercially available ELISA kit for cortisol in saliva (DRG Instruments GmbH, Marburg, Germany).

Clinical assessment

Physical examination included blood pressure, height, weight, respiratory rate, and heart frequency. The 6-minute walk test was used to measure overall physical fitness. The test was performed in a 20-meter-long corridor. We used colored pawns to indicate the 'start' and 'finish' marks.

Subjective health status

To evaluate subjective health status, we used the Short Form Health Survey-36 (SF-36) [18]. The SF-36 consist of 8 health status domains and within each domain the raw scores are summed and divided by the range of the scores. Subsequently transforming these raw scores will results in a score from 0 to 100 [18]. Higher scores indicate better functioning on each subdomain. We calculated the mental component summary measure (MCS), which consisted of the subdomains: vitality, social functioning, role-emotional functioning and mental health, and we calculated the physical component summary measure (PCS), which consisted of the subdomains physical functioning, role-physical functioning, bodily pain, general health [19].

Emotional distress

For emotional distress we used the Hospital Anxiety and Depression scale (HADS) to measure symptoms of anxiety and depression [20]. The questionnaire consist of 14-items and is divided in subscales on anxiety and depression. A 3 point Likert scale is used, with higher scores representing a greater level of emotional distress.

Stress

For the evaluation of perceived stress we used the Dutch version of the original 14 item Perceived Stress Scale (PSS) [21]. This scale assesses ''the degree to which individuals appraise situations in their lives as stressful" [21] on a 5-point Likert scale, with higher scores indicating higher levels of perceived stress (0=never, 4=very often). A total perceived stress score is generated by the sum of the individual items.

Statistical analysis

We present means ± SD for normally distributed characteristics. Non-normal distributed data were presented by median values (interquartile range (IQR)). HCC were not normally distributed and were therefore log-transformed for a normal-distribution for further statistical analyses. Associations between log-transformed HCC and patient characteristics were performed by analysis of variance and linear regression analysis.

First, we conducted linear regression analysis to evaluate the association between log-transformed HCC and age, sex, corticosteroid use, BMI, exercise tolerance, blood pressure, NT-proBNP levels, PCS, MCS, emotional distress and perceived stress. We performed multivariable linear regression analyses in which we adjusted for age, sex and BMI (model 1) and subsequently for age, sex, BMI and use of corticosteroids (model 2).

Second, we evaluated potential predictors of the change in HCC between the hair segments corresponding to baseline and 12 week follow-up. The delta of the log-transformed HCC was the dependent variable which was calculated by subtracting the cortisol level at T0 (baseline) from the T1 (post-intervention) value. We conducted univariable linear regression analyses with age, sex, corticosteroid use, BMI, exercise tolerance, blood pressure, NT-proBNP levels, PCS, MCS, emotional distress, perceived stress and mindfulness training as independent variables.. Multivariable linear regression analyses were performed in which we adjusted for age, gender and treatment allocation.

All statistical tests were two-sided and a p-value less than 0.05 was considered to indicate statistical significance. In order to challenge the stability of statistically significant results from the regression models, we used a bootstrapping approach (sample size 1000). Effect estimates of regression models are expressed as standardized regression coefficients. In all multivariable models, multicollinearity was low, as shown by a maximum variance influence factor (VIF) of 1.239, which is well below commonly used cut-off points for significant multicollinearity (VIF 4-10) [22]. All data were analyzed with IBM SPSS Statistics version 21.0 (IBM Corp., Somers, NY).

RESULTS

Characteristics of study participants

The main study comprised 324 participants. After 12 weeks, 261 (80%) returned for follow-up, during which 260 consented to provide a scalp hair sample (99.6%). We excluded 90 participants with a hair length of less than 4 cm. Of the 170 available scalp hair samples with sufficient length, a total of 151 cortisol levels could be measured above the detection limit in the distal segment, corresponding to the month before treatment, and these were included in the baseline analysis (Figure 2). In 141 hair samples, cortisol could be measured above the detection limit in both the distal and proximal hair segments. These samples were included in the analyses of change in HCC over time. The patient characteristics of the current study are shown in Table 1. The majority of the 151 included study participants were female (62.9%), and the mean (SD) age was 41.3(14.2) years. More than half of the patients were diagnosed with congenital heart disease (52.3%) whereas the others had cardiomyopathy (24.5%), valvular disease (17.2%), or IHD (6.0%).

Cortisol levels stratified by structural heart disease

The distribution of the cortisol levels among the types of structural heart disease can be seen in Figure 3. Patients with CMP had the highest median HCC (median 26.8, IQR: 23.6), and valve disease the lowest (median 21.5, IQR: 26.3). Between the four groups, no significant difference was found (p= 0.944).

Cortisol levels and physiological parameters (including exercise tolerance)

Table 2 shows the results of the univariable and multivariable linear regression analyses. The univariable analyses of HCC showed a positive association with BMI (estimate: 0.196, p=0.003), respiratory rate (estimate: 0.236, p=0.004) and use of corticosteroids (estimate: 0.241, p=0.003). After adjustment for age and sex, the correlation between HCC and BMI remained significant (estimate: 0.223, p=0.020). After adjustment for age, sex and BMI, the correlation between HCC and respiratory rate remained significant (estimate: 0.203, p=0.014). In the final adjusted model, when corticosteroid use was added, BMI (estimate: 0.171, p=0.037) and respiratory rate (estimate: 0.194, p=0.016) remained significant. All significant associations remained statistically significant (p<0.05) after bootstrapping. No significant correlations were found with other physiological parameters (Table 2).

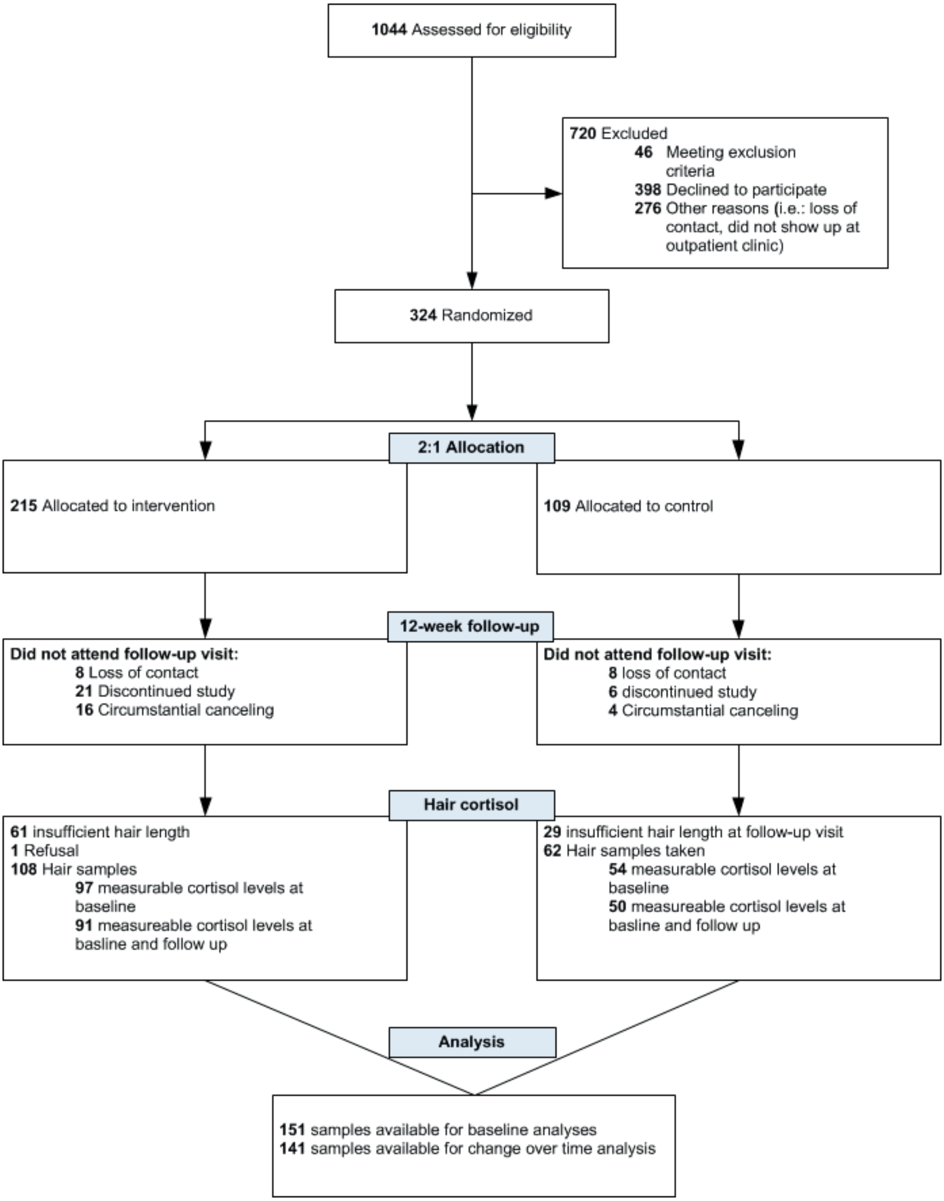

Figure 2. Flowchart.

Table 1. Baseline characteristics, for the total group and stratified by type of structural heart disease

	Total Sample N=151	CONG N= 79	CMP N=37	VALV N=26	IHD N=9
Demographics					
Age (years), mean (SD)	41.3 (14.2)	35.2 (11.9)	49.1 (13.6)	44.0 (14.0)	55.3 (7.8)
Female (%)	62.9	72.2	48.6	65.4	33.3
Physiological parameters					
Heart rate (beats/min), mean (SD)	68 (11)	70 (12)	65 (9)	67 (11)	70 (11)
Systolic blood pressure (mm Hg), mean (SD)	126 (15)	124 (15)	129 (16)	129 (16)	132 (18)
Diastolic blood pressure (mm Hg), mean (SD)	78 (10)	77 (12)	80 (8)	78(10)	79 (7)
Resting respiratory rate (breaths/min), mean (SD)	15 (2)	15 (2)	15 (2)	15 (2)	16 (3)
Body mass index (kg/m^2), mean (SD)	25.5 (4.9)	25.0 (5.2)	26.5 (4.4)	24.2 (3.6)	29.2 (6.2)
Exercise tolerance					
6 minute walk test distance (meters), mean (SD)	533 (82)	538 (84)	526 (90.3)	539 (59)	506 (88)
Laboratory measurements					
NT-proBNP, median (IQR), pmol/L	16.1 (26.7)	13.8 (16.3)	40.4 (46.9)	15.1 (24.8)	12.1 (16.8)
Creatinine, median (IQR), µmol/L	75 (19)	73 (17)	75 (17)	69.5 (24)	87 (17)
Cortisol at baseline, median, (IQR), pg/mg hair	22.3 (23.5)	23.2 (26.7)	26.6 (23.6)	21.5 (26.3)	19.5 (23.3)
Previous cardiac interventions, mean (SD)*	1.5 (1.2)	1.8 (1.3)	0.8 (0.9)	1.3 (0.9)	1.9 (1.2)
ICD (%)	9.9	3.8	27.0	0	22.2
PM (%)	6.0	10.1	0	3.8	0
Hair characteristics					
Hair coloring	37.1	36.7	43.2	30.8	33.3
Hair bleaching	7.9	12.7	0	7.7	0
Use of hair products	51.0	41.8	59.5	57.7	44.4
Frequency of hair washing (>2x/week)	60.1	67.9	60.0	34.6	66.7
Current medication (%)					
Beta-blocker	33.8	22.8	48.6	30.8	77.8
Statin	14.6	1.3	16.2	23.1	100
Aspirin	14.6	10.1	13.5	11.5	66.7
Ace-inhibitor	23.8	20.3	27.0	19.2	55.6
Angiotensin II antagonist	10.6	10.1	10.8	11.5	11.1
Calcium channel blocker	6.6	1.3	8.1	15.4	22.2
Nitrate	0.7	0	0	0	11.1
Cardiac glycoside	4.0	5.1	0	7.7	0
Diuretic	11.9	7.6	10.8	15.4	44.4
Anticoagulant	29.8	22.8	18.9	50	77.8
Antidepressant	4.0	3.8	5.4	0	11.1
Tranquilizer	2.0	2.5	2.7	0	0
Other	35.8	32.9	35.1	30.8	77.8
Intoxication					
Current smoking (%)	15.9	12.6	27.0	15.4	0

Abbreviations: SD, standard deviation; NT-proBNP, N-terminal pro-brain natriuretic peptide; IQR, interquartile range; ICD, implantable cardioverter-defibrillator; PM, pacemaker
*Surgical or percutaneous interventions

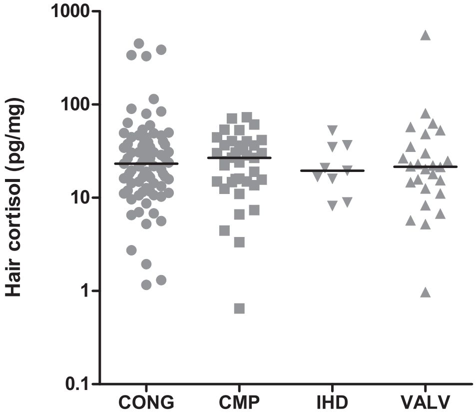

Figure 3. Individual hair cortisol levels at baseline, stratified by structural heart disease.
Note: logarithmic scale. Horizontal lines represent medians. Abbreviations: CONG, congenital heart disease; CMP, cardiomyopathy; IHD, ischemic heart disease; VALV, valvular heart disease.

Table 2. Correlations between patient characteristics and hair cortisol levels at baseline (n=151)

	Univariable analysis		**Multivariable model 1**[a]		**Multivariable model 2**[b]	
	Coefficient[c]	***P* value**	**Coefficient**[c]	***P* value**	**Coefficient**[c]	***P* value**
Age	0.038	0.645	-0.025	0.767	-0.044	0.600
Sex (female vs. male)	-0.074	0.366	-0.063	0.452	-0.105	0.202
Use of corticosteroids[d]	**0.241**	**0.003**	**0.241**	**0.003**	**0.241**	**0.003**
Physiological parameters						
BMI	**0.196**	**0.016**	**0.196**	**0.020**	**0.171**	**0.037**
6 minute walk test	-0.049	0.548	-0.022	0.817	0.019	0.839
Systolic blood pressure	0.120	0.144	0.065	0.458	0.076	0.374
Diastolic blood pressure	0.124	0.129	0.080	0.347	0.097	0.240
Respiratory rate	**0.236**	**0.004**	**0.203**	**0.014**	**0.194**	**0.016**
Heart frequency	0.132	0.105	0.117	0.157	0.089	0.269
NT-ProBNP	-0.046	0.574	-0.047	0.599	-0.040	0.646
Subjective parameters						
SF-36: PCS	**-0.209**	**0.010**	**-0.210**	**0.013**	-0.163	0.054
SF-36: MCS	-0.006	0.945	-0.009	0.915	-0.013	0.875
HADS: anxiety	-0.063	0.439	-0.066	0.419	-0.049	0.539
HADS: depression	0.145	0.076	0.130	0.112	0.120	0.133
Perceived stress scale	0.031	0.710	0.037	0.656	0.030	0.709

Abbreviations: BMI, body mass index; n/a, not applicable; NT-proBNP, N-terminal pro-brain natriuretic peptide; SF-36, Short-Form Health Survey 36; PCS, physical component summary measure; MCS, mental component summary measure; HADS, Hospital Anxiety and Depression Scale.
Statistically significant associations are formatted bold.
[a]adjusted for age, sex and BMI. When age, sex or BMI was the independent variable, the multivariable model was not adjusted for it. [b]adjusted for age, sex, BMI and corticosteroid use. When age, sex, BMI, or corticosteroids use was the independent variable, the multivariable model was not adjusted for it. [c]standardized regression coefficient. [d]includes inhalation, nasal, systemic and topical corticosteroids

Cortisol levels and subjective parameters (perceived/emotional (dis)stress)

In table 2, the correlations between HCC and subjective outcomes can be seen. In the unadjusted model, a negative correlation was found between HCC and the PCS of the SF-36 (estimate: -0.209, p=0.010), indicating a lower self-reported physical functioning in participants with higher HCC. After adjustment for age, sex and BMI, the correlation remained significant (estimate: -0.210, p=0.013). In the fully adjusted model when age, sex, BMI and corticosteroid use were entered, a borderline significant inverse association was found on PCS (estimate: -0.163, p= 0.054). All significant associations were also statistically significant (p<0.05) after bootstrapping. No significant associations were found between HCC and the other subjective outcomes (Table 2).

Predictors for change of cortisol levels

On average, HCC decreased in both study groups during follow-up from the hair segment corresponding to the month before treatment, to the hair segment in the last month of treatment (P<0.001, Figure 4).

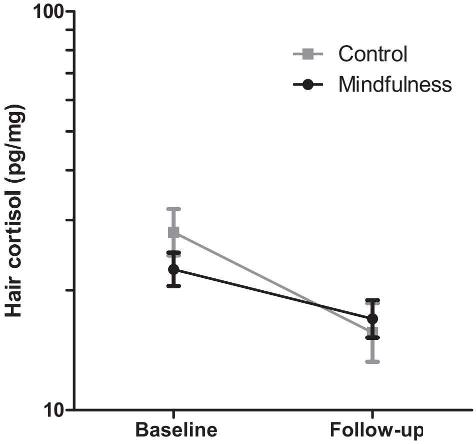

Figure 4. Change of hair cortisol levels between baseline and 12-week follow-up.
Note: logarithmic scale. Whiskers represent standard error of the mean.

Table 3 shows the results of the univariable and multivariable linear regression analyses of the association between baseline characteristics and change of HCC. Diastolic blood pressure was negatively associated with HCC (estimate: -0.181, p=0.032), indicating that HCC decreased more in individuals with higher diastolic blood pressure. After adjustment for age, sex and treatment allocation the association remained significant (estimate: -0.171, p=0.049).

Analyses in the subjective outcome parameters showed an independent association between the SF-36 MCS and HCC (estimate: -0.209, p=0.013), indicating that HCC decreased more in patients with a better MCS. The significant associations with change in cortisol remain significant in the bootstrapping approach ($p<0.05$), except for the association between cortisol and diastolic blood pressure in the multivariable model ($p=0.061$). No effect of mindfulness training was seen (Table 3, Figure 4).

Table 3. Correlations between patient characteristics and the change in hair cortisol levels over time (n=141)

	Univariable analysis		**Multivariable model 1[a]**	
	Coefficient[b]	***P* value**	**Coefficient[b]**	***P* value**
Age	-0.068	0.423	-0.075	0.388
Sex (female vs. male)	-0.020	0.817	-0.028	0.745
Use of corticosteroids	-0.075	0.376	-0.072	0.400
Treatment allocation (intervention vs control)	0.158	0.062	0.156	0.066
Physiological parameters				
Body mass index	-0.107	0.208	-0.100	0.250
6 minute walk test	0.080	0.347	0.758	0.450
Systolic blood pressure	-0.129	0.129	-0.135	0.125
Diastolic blood pressure	**-0.181**	**0.032**	**-0.171**	**0.049**
Respiratory rate	-0.063	0.460	-0.050	0.558
Heart frequency	-0.135	0.109	-0.137	0.108
NT-ProBNP	-0.057	0.507	-0.042	0.639
Subjective parameters				
SF-36: PCS	0.036	0.674	0.012	0.891
SF-36: MCS	**-0.209**	**0.013**	**-0.200**	**0.019**
HADS: anxiety	0.079	0.352	0.089	0.297
HADS: depression	0.041	0.627	0.042	0.620
Perceived stress scale	0.065	0.441	0.064	0.451

Abbreviations: BMI, body mass index; NT-proBNP, N-terminal pro-brain natriuretic peptide; SF-36, Short-Form Health Survey 36; PCS, physical component summary measure; MCS, mental component summary measure; HADS, Hospital Anxiety and Depression Scale

[a]adjusted for age, sex and group allocation. If age, sex, or group allocation was the independent variable, the multivariable model was not adjusted for it. [b]standardized regression coefficient

DISCUSSION

In this study we aimed to investigate the association between HCC and several clinical objective and subjective parameters and identify potential predictors for long-term change in cortisol levels in patients with structural heart disease. Our results showed that higher hair cortisol concentrations (HCC) were associated with higher BMI, higher respiratory rate and a lower score on the physical component summary score of the SF36. No association was found with perceived stress. After 12 weeks, lower HCC were found in the total study group. A more favorable baseline mental status (higher mental component summary score on the SF36) and higher baseline diastolic blood pressure predicted a stronger decrease in HCC after 12 weeks follow-up, whereas no significant effect of mindfulness training on the HCC was found.

In our study we used cortisol levels from hair samples, which are suitable to study effects of long-term cortisol exposure on clinical characteristics. Until now, only a few cross-sectional studies have been reported of HCC in individuals with CVD. In a recent report, Manenschijn et al.[13] showed that higher long-term cortisol levels measured in scalp hair are associated with a higher presence of CVD in an elderly population. Other studies have reported on the association between elevated cortisol levels and risk of ischemic heart disease and acute myocardial infarction [23, 24]. In the present study we did not investigate cardiovascular risk but these recent studies indicate that HCC may be used to determine whether individuals are at increased risk of developing a cardiovascular related event. We did not observe a difference in HCC between different categories of structural heart disease. This could be explained because in all categories, there is a similar disease burden, which therefore might impact the HPA axis and cortisol exposure in a similar way. However, this finding should be interpreted with caution, because our study was not powered to detect a difference in cortisol levels between categories of heart disease, and only contained a small number of patients with ischemic heart disease.

Our results show a positive association between HCC and BMI, which may represent a metabolically less favorable phenotype in individuals with a higher long-term cortisol exposure. The association between cortisol and adiposity has been debated for a long time, with most studies showing a relative increase in urinary cortisol, but a decrease or unaffected cortisol in time-point measures (blood or saliva) [25]. However, we and others have reported increased HCC in association with increased BMI and abdominal adiposity [14-16, 26-28], supporting the hypothesis that a slight increase in long-term cortisol exposure is associated with increased adiposity. Our results indicate that also in patients with structural heart disease long-term cortisol is associated with increased BMI, and could therefore modulate CVD risk.

In the present study no association was found between perceived stress and HCC. Interestingly, we found a borderline significant negative association on the physical component summary measure (PCS) of the SF36, whereas we did not find an association on actual physical functioning as measured with the 6MWT. This may suggest that long-term cortisol levels in the current population are affected more by physiological stress than psychological stress. Although we had no HCC levels for matched controls without structural heart disease in the present study, HCC does seem to be higher compared to our previously published healthy controls [15]. The PCS consists of several subjective components assessing physical functioning in daily life. A single objective measurement may be less sensitive in assessing the impact of physical limitation in daily life. There are limited reports that evaluate the relation between subjective physical functioning and HCC. Feller et al [29] found no association between HCC and the physical health score (measured with the SF-12). That study comprised of mainly older adults, whereas our population had a mean age of a little over 40 years. It is not unimaginable that limited physical functioning at younger age due to underlying structural heart disease causes more (physical or psychological) stress than it would do at an older age. In particular younger adults with complex congenital heart disease suffer from limited exercise capacity compared to their healthy counterparts [30].

We observed a decrease in HCC after 12 weeks in both study groups. In contrast to previous studies, we did not observe a wash-out effect in the hair analysis that could limit retrospective assessment of cortisol levels [31, 32]. Using the same hair sample to determine cortisol levels at T0 and T1, HCC turned out to be higher at baseline. This shows that a potential wash-out effect in the distal hair segments was not present in the current population. A decrease in HCC over time in both the mindfulness group and controls may be associated with participation in the study, or the one additional visit to the cardiologist that all study participants received. Participants in the control group did not receive any other additional attention beyond usual care. However, there may be other reasons for a decrease in HCC we are currently not aware of.

Interestingly, mental status was an independent predictor of lower cortisol levels after 12 weeks. Some reports suggest a positive relation between high cortisol levels and mental health status in individuals suffering from stressful conditions, such as depression and loneliness [7, 33, 34], thus we cannot rule out a potential modifying effect on stress. However, most of these studies did not use HCC and are therefore subject to the limitations of time-point and short-term cortisol measurements.

The decrease in HCC after 12 weeks could not be explained by allocation to mindfulness training. Until now, there is only one report that has described the effect of mindfulness training on HCC. In a pilot study on smoking cessation, mindfulness training was compared with cognitive-behavioral therapy [35]. In that study, Goldberg et al. reported lower HCC in the total sample after the interventions. The investigators suggest that participation in a mindfulness training may be independently associated with lower HCC. However, almost 75% of study participants quit smoking and this may have contributed to the decrease in HCC, since smoking has been found to be associated with higher cortisol levels [29, 36]. Further studies in this field have to be awaited.

Limitations of the current study must be addressed. First, the current study was a sub study of a larger randomized controlled trial and was therefore not powered to evaluate the effect of the online mindfulness training on cortisol levels. Secondly, we evaluated cortisol levels taken from hair samples at 12 weeks, which could have underestimated the full effect of the mindfulness training since the proximal hair fragment comprises the period from 8-12 weeks of treatment. Finally, all structural heart disease patients were included from the outpatient clinic of cardiology, not focusing on patients with psychological or psychiatric comorbidity. This may explain why we did not find any associations between HCC and psychological outcomes. However, we found evidence that a favorable mental status is associated with a stronger decrease in HCC. Conceivably, mental status affects the dynamic changes in long-term cortisol secretion.

The association between higher diastolic blood pressure and a stronger decrease in HCC over time is an intriguing finding. It is conceivable that a higher blood pressure, a known tissue effect of cortisol, represents a higher cortisol exposure. Furthermore, the higher diastolic blood pressure may represent a state of acute stress in part of the participants, analogous to the so-called white coat hypertension [37], which may be reflected in HCC. However, with both explanations, one would expect a positive correlation between diastolic blood pressure and HCC at baseline, which we did not find.

Notably, participants who used corticosteroids had higher HCC. We did not have sufficient data to perform a subgroup analysis into the different exogenous corticosteroids. Most administered corticosteroids may cause a slight suppression of the HPA-axis, and would therefore be expected to lower long-term cortisol secretion [38]. However, corticosteroids that are administered may cross-react in immunoassays such as used in the present study, and thereby erroneously increase HCC. Furthermore, patients using corticosteroids may present a subpopulation with a higher disease burden, which may in turn lead to higher endogenous cortisol production. Our results corroborate that exogenous corticosteroids are an important confounder in hair cortisol measurements, and should be taken into account in studies that use HCC.

In conclusion, our results show that long-term hair cortisol levels are associated with BMI and respiratory rate in patients with structural heart disease. Additionally, mental status was an independent predictor of change in hair cortisol levels over a period of 12 weeks.

The evaluation of HCC can provide a unique tool in the assessment of long-term cortisol levels and their changes over time in intervention studies. The level of HCC may provide additional information in the identification of individuals at risk of developing cardiovascular events, and help to unravel the interplay between psychological stress and cardiovascular disease. Therefore, long-term prospective studies are needed to evaluate the predictive value of chronically elevated long-term cortisol levels in patients with regard to cardiovascular risk. Further studies may show which lifestyle and medical interventions can modify long-term cortisol exposure, and thereby potentially improve cardiometabolic health.

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