JIM Review

doi: 10.1111/joim.13739

Long-term glucocorticoids in relation to the metabolic syndrome and cardiovascular disease: A systematic review and meta-analysis

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Abstract. Kuckuck S, Lengton R, Boon MR, Boersma E, Penninx BWJH, Kavousi M, et al. Long-term glucocorticoids in relation to the metabolic syndrome and cardiovascular disease: A systematic review and meta-analysis. *J Intern Med.* 2023;**00**:1–18.

The striking link of Cushing's syndrome with the metabolic syndrome (MetS) and cardiovascular disease (CVD) suggests that long-term exposure to extremely high cortisol levels catalyzes cardiometabolic deterioration. However, it remained unclear whether the findings from the extreme glucocorticoid overabundance observed in Cushing's syndrome could be translated into more subtle variations in long-term glucocorticoid levels among the general population, for example, due to chronic stress. Here, we performed a systematic review (PROSPERO: CRD42023425541) of evidence regarding the role of subtle variations in long-term biological stress, measured as levels of scalp hair cortisol (HairF) and cortisone (HairE), in the context of MetS and CVD in adults. We also performed a meta-analysis on the cross-sectional difference in HairF levels between individuals with versus without CVD. Seven studies were included regarding MetS, sixteen regarding CVD, and one regarding both. Most articles indicated a strong, consistent cross-sectional association of higher HairF and HairE levels with CVD, which was confirmed by our meta-analysis for HairF (eight studies, SMD = 0.48, 95% confidence intervals [CIs]: 0.16–0.79, $p = 0.0095$). Moreover, these relationships appear largely independent of standard risk factors. Age seems relevant as the effect seems stronger in younger individuals. Results regarding the associations of HairF and HairE with MetS were inconsistent. Altogether, long-term biological stress, measured as HairF and HairE, is associated with the presence of CVD, and less consistently with MetS. Prospective studies need to evaluate the directionality of this relationship and determine whether HairF and HairE can be used in addition to standard risk factors in predicting future cardiometabolic deterioration.

Keywords: cardiovascular disease, cardiovascular risk factors, hair glucocorticoids, HPA-axis, metabolic syndrome

Introduction

The worldwide prevalence of obesity and related cardiometabolic diseases is increasing at an alarming rate [1], indicating an urgent need for action to understand and target risk factors for cardiovascular and metabolic deterioration. The metabolic syndrome (MetS) is a clustering of metabolic complications that includes central obesity, hyperglycemia, hypertension, and dyslipidemia, which increase the risk of cardiovascular disease (CVD) and all-cause mortality [2]. Depending on the diagnostic criteria, 12.5%–31.4% of the world's adult population was estimated to be affected by MetS in 2021, whereas CVD is the leading cause of death worldwide [3, 4]. Thus, gaining a better understanding of the mechanisms underlying the development of MetS and/or CVD is of great importance for global public health.

A potential vulnerability factor in the pathogenesis of MetS and CVD is the exposure to stress and stress-related hormones—including the glucocorticoid hormone cortisol and its inactive form, cortisone [5]. Both are produced by the adrenal glands in response to the activation of one of the body's major stress systems: the hypothalamic–pituitary–adrenal (HPA)-axis [5, 6]. Support for a role of glucocorticoids in the development of MetS can be found in the striking resemblance between features of MetS and a rare condition called Cushing's disease. The latter is characterized by an adrenocorticotropic hormone-releasing adenoma leading to long-term extreme glucocorticoid excess. Overlap includes the presence of central obesity, hypertension, dyslipidemia, and disturbed glucose metabolism as well as pathophysiological sequelae, such as chronic inflammation, adipokine dysregulation, and oxidative stress [7–9]. Although these observations indicate that long-term exposure to supraphysiological levels of glucocorticoids contributes to the development of MetS features and CVD risk, the importance of more subtle variations within a common physiological range remains less clear.

Since about one decade, subtle variations of long-term glucocorticoid exposure can be quantified by measuring concentrations of cortisol and cortisone in scalp hair (hair glucocorticoid [HairGC]), posing a reliable and noninvasive biomarker of chronic biological stress (see Fig. 1 [10–12]). Here, we provide an overview of current knowledge regarding the relationship of long-term HairGC with MetS and CVD. In contrast to previous overviews [13, 14], we do not only describe the role of hair cortisol (HairF), but also hair cortisone (HairE) in relation to cardiometabolic health. In addition, we investigate the impact of age and sex in the relationship among HairGC, MetS, and CVD.

Methods

We report this systematic review and meta-analysis in concordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [15]. This systematic review was registered at the PROSPERO database (registration number CRD42023425541, June 1, 2023). Included were studies with $\geq$15 participants, investigating the role of HairGC in the context of established MetS and/or CVD in adults (mean age >18 years, the presence of MetS/CVD according to the definition of authors, CVD also comprising stroke and intracranial aneurysms). This included (1) cross-sectional studies investigating HairGC levels in individuals with versus without MetS, (2) cross-sectional studies investigating HairGC levels in individuals with versus without CVD, (3) cross-sectional studies investigating HairGC levels among individuals with CVD according to different disease severities and characteristics, and finally (4) longitudinal studies investigating the role of HairGC levels in the disease course of individuals with CVD.

Search strategy and selection criteria

A comprehensive search strategy was designed by a university health sciences librarian in order to identify studies regarding the role of HairF and/or HairE in the context of established MetS and/or CVD. The search strategy included the elements "hair," "cortisol/cortisone," and "MetS" or "CVD," including their synonyms without any exclusion criteria other than "studies in humans" and "English full-text." The complete search strategy can be found in Material S1. The search was conducted in the following databases in April 2023: Medline (ALL), Embase, Web of Science, and Cochrane. Search results were exported to EndNote20 (Clarivate Analytics). References were imported into Endnote and duplicates removed prior to screening by the medical librarian [16].

Afterwards, all identified studies were assessed independently by two authors (SK and RL) in the title/abstract screening stage. Studies were included for full-text screening if HairGC (or any of their synonyms) were mentioned alongside any term regarding metabolism and/or cardiovascular health in the title, abstract, or keywords. Subsequently, full-texts were assessed for eligibility by SK. Uncertainties were resolved by discussion between SK and RL. Articles were included if they reported any cross-sectional or longitudinal data regarding the association of HairGC with established MetS and/or CVD in adults. Case reports, project proposals, and study protocols were excluded, as well as animal studies, reviews, and studies in patients undergoing glucocorticoid replacement therapy due to primary adrenal disease. We also excluded studies looking exclusively at subcategories of MetS or subclinical CVD. However, in the case of studies in which individuals with subcategories of MetS/CVD or subclinical CVD were considered a part of the group "CVD," next to clinical cases, we included the study in the

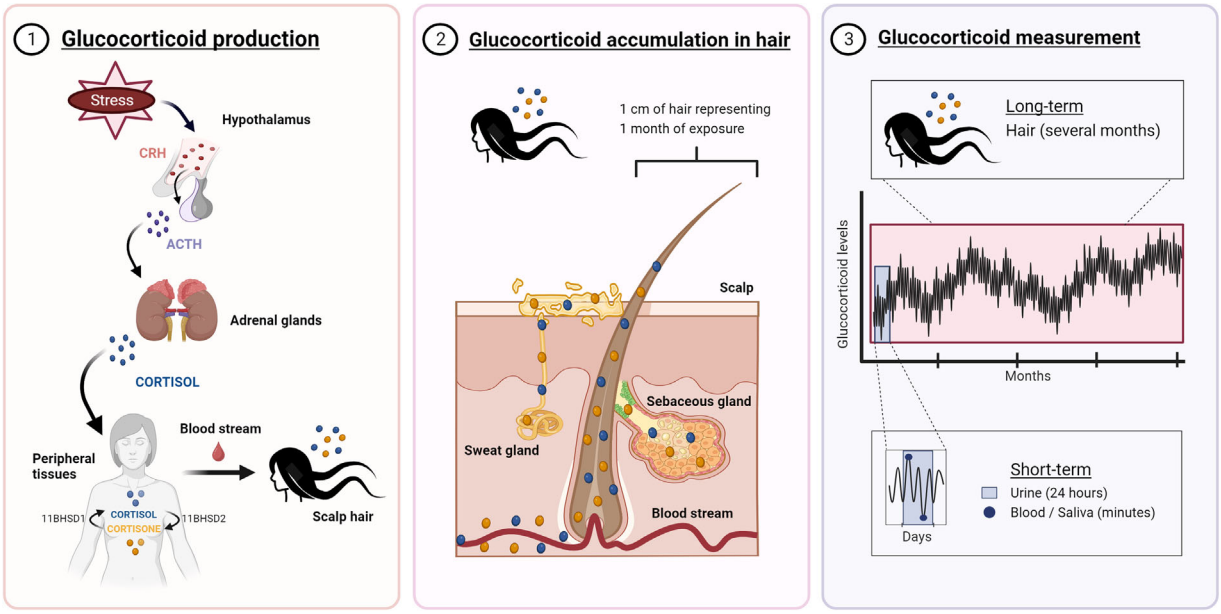

Fig. 1 *Schematic illustration of (1) glucocorticoid production in response to stress (hypothalamic–pituitary–adrenal [HPA]-axis), (2) proposed mechanisms of glucocorticoid storage in hair, and (3) glucocorticoid measurements in the context of time. (1) In response to stress, the hypothalamus produces corticotrophin-releasing hormone (CRH), which causes the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary. Subsequently, ACTH is transported to the adrenal glands, where it induces the production of glucocorticoids such as cortisol. Via the blood stream, cortisol is transported into peripheral tissues, where the 11β-hydroxysteroid dehydrogenase (11BHSD) proteins locally convert cortisol into its inactive form cortisone, and back, in a tissue-specific manner. Cortisone and cortisol are transported through the body and, among others, stored in hair [6,9]. (2) Although the precise mechanisms by which glucocorticoids are incorporated into hair are still not completely understood, the prevailing hypothesis proposes diffusion from blood capillaries into growing hair cells. Additional mechanisms include the incorporation from sweat and/or sebum. As hair grows approximately 1 cm/month, 1 cm of hair is assumed to reflect 1 month glucocorticoid exposure (for details, see Refs. [10–12]). (3) Glucocorticoid measurements in traditional matrices reflect acute/short-term glucocorticoid exposure (ranging from minutes up to 24 h), which are subject to considerable within-day pulsations as well as day-to-day variations and are therefore prone to confounding. In contrast, hair glucocorticoid measurements allow for quantifying the average long-term exposure to glucocorticoid levels over months, which reduces the likelihood of confounding, for example, due to acute physiological and/or psychological stress or lack of patient adherence to sampling instructions [10]. Source: Created with BioRender.com.*

review. In one case in which data of the same participants were reported in two studies, we included the study with the most recent data and the larger dataset [17, 18]. An overview of all exclusions is provided in Supplementary Fig. S2.

Data extraction and risk of bias assessment

Data regarding participants, methodology, and outcomes (including reported statistics) were extracted from all included studies by two authors (SK and RL) independently using a standardized data extraction sheet. Risk of bias (RoB) was also assessed by these two authors independently using the Quality in Prognostic Studies (QUIPS) tool [19]. Briefly, the QUIPS tool provides guidance in assessing potential sources of bias regarding the following study domains: study participation (1), study attrition (2), prognostic factor measurement (3), outcome measurement (4), confounding measurement (5), and statistical analysis (6). In cases in which longitudinal data were not available or were only present in a very small sample ($n < 15$), we omitted the domain of study attrition (2). Any discrepancies were resolved by discussion among the authors.

Meta-analysis

A meta-analysis regarding the difference in HairGC levels between individuals with versus without CVD was conducted in R version 4.3.1 [20]. Sample

size, as well as raw and log-transformed means with standard deviations (SDs) and/or medians with quartiles (q1, q3, and/or IQR and/or minimum–maximum range) per group were extracted from the articles. If essential data ([log-]means, [log-]SD, medians, IQR, minimum–maximum, and/or sample size per group) were not reported, we contacted the corresponding authors to ask if they could provide us with the respective information. One article was based on our own dataset, which enabled us to extract the data directly ourselves [21].

If mean (SD) was not available, medians (IQR/range) were converted to means (SD) prior to analyses following the method of Wan et al. [22]. In one case in which only median (IQR)—but not exactly the first and third quartiles—were reported, we used the formula mean ≈ median and SD ≈ (q3–q1)/1.35, as described by the Cochrane Handbook and later shown to be appropriate for large sample sizes by Wan et al. [22, 23].

To identify between-group differences of participants with versus without CVD across studies, we used a random-effects model on the standardized mean differences ("SMD," reflected as Hedges "g") of the log-transformed mean (SD) of HairF based on inverse variance weighting for pooling as described in the Cochrane Handbook [23]. In cases in which log-transformed means (SD) were not available, those were estimated following the method of Higgins et al. [24]. Group differences were reported as SMD, including 95% CIs. SMD analyses were performed using the R metafor package [25]. Publication bias was assessed visually through a funnel plot (see Fig. S3). Due to a high probability of large heterogeneity among studies, a random-effects model was applied. Causes of heterogeneity were explored using subgroup analyses. Specifically, subgroup analyses included (1) separate analyses by age group, divided into either only middle-aged individuals (mean/median age <65 years) or older adults (mean/median age >65 years), as well as (2) only studies comprising coronary artery disease (CAD) patients as CVD cases, (3) only studies using enzyme-linked immunoassay ("ELISA") for HairF measurements, (4) only studies with an RoB <2 in Dimension 5 of the QUIPS tool (Confounding), (5) only studies performed in locations suggesting a predominantly Caucasian population, or (6) only studies predominantly including men (>75% of total sample, approximately equally divided over the CVD as well as non-CVD group). For women, such an analysis was not possible due to limited data.

As HairF is often not normally distributed and may sometimes still not be after logarithmic transformations, we additionally performed a sensitivity analysis on the "median of the differences of medians" (MDM) using the R metamedian package to account for potential influences of outliers on the SMD analyses [26]. This was only possible for a subset of studies due to missing data regarding median and/or IQR. As the MDM method assumes that the quantitative outcome (HairF) is measured on a comparable scale across studies [26], one study was not included in the MDM analysis even though median and IQR were known because the measurement range differed considerably from the other studies [27].

For all analyses, heterogeneity was assessed through I^2. Significance was set at $\alpha < 0.05$.

We refrained from applying a meta-analysis to studies regarding MetS as the majority of available articles comprise high proportions of individuals with non-cardiovascular chronic conditions, which we do not deem representative for the general population (see Table 1). Only two studies in otherwise healthy populations were available for MetS, which we regard as too small to derive meaningful conclusions (a minimum of ≥3 studies was considered necessary for the main analysis). Similarly, we refrained from performing a meta-analysis of HairE in relation to CVD, as only two studies were available in this context (see Table 2). One study was not included in the meta-analysis of HairF in relation to CVD due to the very small number of CVD cases ($n = 3$, a minimum of ≥5 cases was considered necessary for statistical analyses) [28]. Large heterogeneity of research questions and study designs also precluded any meta-analyses with respect to cross-sectional studies relating HairGC to CVD severity, as well as longitudinal outcomes (see Tables 3 and 4).

Results

Study selection

A total of 325 records were collected for the title/abstract screening, of which 1 was identified through the full-text of another article. A total of $n = 83$ (25.5%) studies were eligible for full-text screening. Of those, we excluded studies in children ($n = 6$), studies without original hair data

Table 1. *Cross-sectional studies investigating hair glucocorticoid (HairGC) levels in individuals with versus without metabolic syndrome (MetS).*

Author [refs.]	Diagnostic criteria for MetS	Significant association	Sample size, no MetS	Sample size, MetS	Health status of study population	Geographic location	Age (years)	Users of GC medication	HairGC measure-ment	HairGC reported	Risk of bias
Stalder et al. [29]	JIS[a]	Yes	964 (163 women)	294 (24 women)	Healthy	Germany (city unknown)	Median = 39, range = 16–64	Excluded	LC/MS	HairF and HairE	⓪⓪⓪⓪⓪
Mazgelyte et al. [34]	IDF[b]	Yes	125 (only men)	38 (only men)	Healthy	Vilnius, Lithuania	No MetS: median = 35, IQR = 18; MetS: median = 42.5, IQR = 10	Excluded	LC/MS	Only HairF	⓪①⓪⓪⓪
van den Heuvel, et al. [31]	JIS[a]	No	140 (only women)	76 (only women)	Trauma-exposed individuals of whom $n = 110$ with PTSD	Cape Town, South Africa	Mean = 43.8; range = 20–79	Excluded	LC/MS	Only HairF	⓪①⓪⓪⓪
Langerak et al. [33]	NCEP ATP III[c]	Yes	68 (unknown sex distribution)	23 (unknown sex distribution)	Only HIV patients	Rotterdam, the Netherlands	Mean = 47.3 ± 11.5 SD	Excluded	ELISA	Only HairF	②①⓪⓪①
Kuehl et al. [32]	IDF[d]	Yes	18 (unknown sex distribution)	50 (unknown sex distribution)	Healthy, major depression	Hamburg, Germany	Mean = 41.3	Excluded	LC/MS	HairF and HairE	②⓪⓪①①
van den Heuvel et al. [28]	JIS[a]	No	34 (only women)	22 (only women)	Healthy, Parkinson's disease	Cape Town, South Africa	Healthy: mean = 55.7 ± 6.9 SD; Parkinson's disease: mean = 64.5 ± 8.4 SD	Excluded	LC/MS	HairF and HairE	⓪⓪⓪①①
van den Heuvel et al. [30]	JIS[a]	No	31 (unknown sex distribution)	6 (unknown sex distribution)	Healthy, schizophrenia	Cape Town, South Africa	Healthy: mean = 26.4 ± 5.8 SD; schizophrenia: mean = 30.1 ± 6.9 SD	Excluded	LC/MS	Only HairF	②⓪⓪①①
Staufenbiel et al. [35]	Not described	No	54 (unknown sex distribution)	17 (unknown sex distribution)	Only individuals with bipolar disorder	The Hague, the Netherlands	Median = 52; IQR = 43–62	Not described	ELISA	Only HairF	②①②②①

Abbreviations: ELISA, enzyme-linked immunosorbent assay; HairE, hair cortisone; HairF, hair cortisol; HIV, human immunodeficiency virus; IDF, International Diabetes Federation; JIS, Joint Interim Statement; LC/MS, liquid chromatography-mass spectrometry; NCEP ATP III, National Cholesterol Education Program Adult Treatment Panel III; PTSD, post-traumatic stress disorder.

[a]Alberti et al. [49].

[b]Alberti et al., [51] (IDF Commun).

[c]National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III), [51].

[d]Alberti et al., 2005 (Lancet).

[e]But opposite of what expected: (d) rating of QUIPS dimensions in the following order: 1 (study participation), 3 (prognostic factor), 4 (outcome measurement), 5 (study confounding), and 6 (statistical analysis and reporting). Dimension 2 (study attrition) does not apply in the case of cross-sectional studies and was therefore left out. A rating of "0" indicates "low", "1" indicates "moderate", and "2" indicates "high" risk of bias.

Table 2. *Cross-sectional studies investigating hair glucocorticoid (HairGC) levels in individuals with versus without cardiovascular disease (CVD).*

Author [refs.]	CVD definition	Significant association	Sample size no CVD (total *N*, *n* women)	Sample size CVD (total *N*, *n* women)	Geographic location	Age (years)	Users of GC medication	HairGC measure-ment	HairGC reported	Risk of bias[c]
Abell et al. [36]	Coronary heart disease	No	2664 (unknown sex distribution)	1011 (unknown sex distribution)	London, UK	Mean = 69.8, range = 59–85	Included, reported	LC/MS	Only HairF	②⓪①①⓪
Bergquist et al. [37]	History of CAD (subclinical/clinical)	Yes[a]	15 (4 women)	9 (0 women)	Emory, United States	Mean CAD = 60.8; mean CAD = 51.1	Included, not further described	ELISA	Only HairF	①①①②①
Feeney et al. [39]	1 or ≥2 of the following CVDs: high blood pressure, angina, heart attack, heart failure, stroke or transient ischemic attack, high cholesterol, heart murmur, and irregular heart rhythm	Yes	716 (unknown sex distribution)	688 (1 CVD) 472 (≥2 CVD) (unknown sex distribution)	Dublin, Ireland	Mean = 66.5, range = 54–94	Excluded	LC/MS	HairF and HairE	②⓪⓪②⓪
Izawa et al. [40]	ACS	Yes	92 (only men)	63 only men)	Tokyo, Japan	Range = 35–79, ACS: mean = 60.5 ± 9.4 SD; no ACS: mean = 59.6 ± 9.2 SD	Excluded	ELISA	Only HairF	①②⓪①⓪
Manenschijn et al. [21]	Stroke, coronary heart disease or peripheral artery disease	Yes	167 (116 women)	114 (70 women)	Amsterdam, the Netherlands	Median = 74.8, 70.1–79.6 IQR, range = 65–85	Excluded	ELISA	Only HairF	⓪①⓪①⓪
Nafisa et al. [27]	CA	Yes	500 (110 women)	500 (110 women)	Rawalpindi, Pakistan	CA: median = 52.76, range = 28–70; no CA: median = 50.61, range = 25–69	Not described	ELISA	Only HairF	⓪①⓪①⓪
Pereg et al. [43]	Acute myocardial infarction	Yes	56 (only men)	56 (only men)	Kfar Saba, Israel	Mean = 61 ± 10.9 SD	Excluded	ELISA	Only HairF	⓪①⓪⓪⓪
Stomby et al. [17]	CAD	Yes	3.134 (1.994 women)	203 (56 women)	Sweden (nationwide)	Range = 50–65	Not described	RIA	Only HairF	⓪①①①⓪
Bossé et al. [48]	CAD	Yes	336 (unknown sex distribution)	262 (unknown sex distribution)	Montréal, Canada	Mean = 65.23 ± 6.89 SD	Included, not further described	CLIA	Only HairF	②①①②⓪
van den Heuvel et al. [28]	Previous angina, myocardial infarct, cerebrovascular accident	Yes[b]	53 (unknown sex distribution)	3 (unknown sex distribution)	Cape Town, South Africa	Mean = 39, range = 45–78	Excluded	LC/MS	HairF and HairE	⓪⓪⓪①①

Abbreviations: ACS, acute coronary syndrome; CA, coronary atherosclerosis; CAD, coronary artery disease; CLIA, chemiluminescent immunoassay; ELISA, enzyme-linked immunosorbent assay; GC, glucocorticoids; HairE, hair cortisone; HairF, hair cortisol; LC/MS, liquid chromatography–mass spectrometry; RIA, radioimmunoassay.

[a]But opposite of what expected.

[b]But only for HairE.

[c]Rating of QUIPS dimensions in the following order: 1 (study participation), 3 (prognostic factor), 4 (outcome measurement), 5 (study confounding), and 6 (statistical analysis and reporting). Dimension 2 (study attrition) does not apply in the case of cross-sectional studies and was therefore left out. A rating of "0" indicates "low", "1" indicates "moderate", and "2" indicates "high" risk of bias.

Table 3. Cross-sectional studies investigating hair glucocorticoid (HairGC) levels among individuals with different cardiovascular disease (CVD) diagnoses and severities.

Author [refs.]	CVD definition	Objective	Significant association	Sample size (*N*, *n* women)	Geographic location	Age (years)	Users of GC medication	HairGC measurement	HairGC reported	Risk of bias[c]
Dowlati et al. [38]	Patients with CAD	Compared HairF among those with and without CABG	Yes, higher HairF in CABG	121 (29 women)	Toronto, Canada	Mean = 11.6	Not described	ELISA	Only HairF	①①⓪②⓪
Dowlati et al. [38]	Patients with CAD	Compared HairF among those with and without angina	No	121 (29 women)	Toronto, Canada	Mean = 11.6	Not described	ELISA	Only HairF	①①⓪②⓪
Kim et al. [41]	Patients with intracranial aneurysms	Compared HairF among those with and without ruptures	Yes, higher HairF in ruptured group	68 (17 women)	Gwangju, Korea	Ruptured: mean = 55.2 ± 15.2 SD; non-ruptured: mean = 55.9 ± 9.8 SD	Excluded	ELISA	Only HairF	①②⓪①①
Nafisa et al. [27]	Patients with abnormal coronary angiograms	Compared HairF in individuals with single-, double-, and triple-vessel diseases	Yes, higher HairF with more vessel disease	500 (220 women)	Rawalpindi, Pakistan	Mean = 51.69 ± 9.51 SD	Not described	ELISA	Only HairF	⓪①⓪①⓪
Pereg et al. [42]	Patients with stable chronic systolic heart failure	Related HairF to severity (NYHA score, exercise capacity, left ventricular ejection fraction)	Yes, positive correlation of HairF with NYHA score and negative correlation with exercise capacity	44 (only men)	Kfar Saba, Israel	Mean = 69.8 ± 11.2 SD	Excluded	ELISA	Only HairF	⓪①⓪②⓪
Saleem et al. [44]	Patients with CAD (based on previous AMI, CABG[a], or PCI[b])	Compared HairF between groups (AMI, CABG[a], and PCI[b])	Yes, more CABG[a] patients in higher HairF group	56 (8 women)	Toronto, Canada	Mean = 66 ± 11 SD	Not described	ELISA	Only HairF	⓪①⓪②①

(*Continued*)

Table 3. *Continued*

Author [refs.]	CVD definition	Objective	Significant association	Sample size (*N*, *n* women)	Geographic location	Age (years)	Users of GC medication	HairGC measurement	HairGC reported	Risk of bias[c]
Younge et al. [46]	Patients with structural heart disease	Compared HairF among congenital heart disease, cardiomyopathy, ischemic heart disease, and valvular heart disease	No significant difference among the different groups of structural heart disease	151 (95 women)	Rotterdam, the Netherlands	Mean = 41.3 ± 14.2 SD	Included, corrected for in analyses	ELISA	Only HairF	①①⓪⓪⓪
Younge et al. [46]	Patients with structural heart disease	Also related HairF to respiratory rate and physical component score	Yes, positive association of HairF with respiratory rate; negative association with self-reported physical functioning	151 (95 women)	Rotterdam, the Netherlands	Mean = 41.3 ± 14.2 SD	Included, corrected for	ELISA	Only HairF	①①⓪⓪⓪
Ben Assayag et al. [47]	Patients with ischemic stroke or transient ischemic attack	Related HairF to MRI measurements	Yes, positive correlation between HairF and lesion volume	65 (37 women)	Tel Aviv, Israel	Mean = 66.7 ± 6.6 SD	Excluded	CLIA	Only HairF	①①⓪⓪⓪
Ben Assayag et al. [47]	Patients with ischemic stroke or transient ischemic attack	Compared HairF of patients with ischemic stroke vs. transient ischemic attack	No difference between groups	65 (37 women)	Tel Aviv, Israel	Mean = 66.7 ± 6.6 SD	Excluded	CLIA	Only HairF	①①⓪⓪⓪

Abbreviations: AMI, acute myocardial infarction; CABG, coronary artery bypass graft surgery; CAD, coronary artery disease; CLIA, chemiluminescent immunoassay; ELISA, enzyme-linked immunosorbent assay; GC, glucocorticoids; HairF, hair cortisol; MRI, magnetic resonance imaging; NYHA, New York Heart Association; PCI, percutaneous coronary intervention.

[a]Minimum 6 weeks ago.

[b]Minimum 3 weeks ago.

[c]Rating of QUIPS dimensions in the following order: 1 (study participation), 3 (prognostic factor), 4 (outcome measurement), 5 (study confounding), and 6 (statistical analysis and reporting). Dimension 2 (study attrition) does not apply in the case of cross-sectional studies and was therefore left out. A rating of "0" indicates "low", "1" indicates "moderate", and "2" indicates "high" risk of bias.

Table 4. Longitudinal studies investigating the role of hair glucocorticoid (HairGC) levels among cardiovascular disease (CVD) patients.

Author [refs.]	CVD definition	Objective	Follow-up period	Significant association	Sample size (*N*, *n* women)	Geographic location	Age (years)	Users of GC medication	HairGC measure-ment	HairGC reported	Risk of bias[c]
Pereg et al. [42]	Patients with stable chronic systolic heart failure	Baseline HairF as predictor for future heart failure–related hospitalization	1 year	Trend toward higher baseline HairF in hospitalized vs. non-hospitalized patients	44 (only men)	Kfar Saba, Israel	Mean = 69.8 ± 11.2 SD	Excluded	ELISA	Only HairF	0 0 1 0 2 0
Saleem et al. [44]	Patients with CAD (based on previous AMI, CABG[a], or PCI[b])	Tested HairF as predictor for improvements in verbal memory performance after cardiac rehabilitation	1 year	Yes, higher HairF at baseline predicts less improvement at follow-up	56 (8 women)	Toronto, Canada	Mean = 66 ± 11 SD	Not described	ELISA	Only HairF	0 0 1 0 2 1
Wagner-Skacel et al. [45]	Patients with CVD (CAD, CABG[a], PCI[b], MI, or ACS) undergoing rehabilitation (standard care, yoga, or meditation)	Compared HairF before and after rehabilitation	4 weeks	Yes, HairF decreased after rehabilitation (no differences among treatment groups)	30 (7 women)	St. Radegund, Austria	Mean = 58.8 ± 9.8 SD	Not described	ELISA	Only HairF	0 0 1 0 2 0
Younge et al. [46]	Patients with structural heart disease receiving either standard cardiac treatment at outpatient clinic or online mindfulness training	Compared HairF before and after treatment	12 weeks	Yes, HairF decreased after treatment (no difference between groups)	151 (95 women)	Rotterdam, the Netherlands	Mean = 41.3 ± 14.2 SD	Included, corrected for in regressions	ELISA	Only HairF	1 1 1 0 0 0
Younge et al. [46]	Patients with structural heart disease receiving either standard cardiac treatment at outpatient clinic or online mindfulness training	Also investigated predictors of change in HairF over time	12 weeks	Yes, more favorable mental status and higher blood pressure at baseline predicted stronger decreases in HairF	151 (95 women)	Rotterdam, the Netherlands	Mean = 41.3 ± 14.2 SD	Included, corrected for in regressions	ELISA	Only HairF	1 1 1 0 0 0
Ben Assayag et al. [47]	Patients with acute ischemic stroke or transient ischemic attack	Tested HairF as predictor for cognitive decline after stroke	6, 12, and 24 months	Yes, higher baseline HairF in people with worse cognitive decline	65 (37 women)	Tel Aviv, Israel	Mean = 66.7 ± 6.6 SD	Excluded	CLIA	Only HairF	1 2 1 0 0 0

Abbreviations: ACS, acute coronary syndrome; AMI, acute myocardial infarction; CABG, coronary artery bypass graft surgery; CAD, coronary artery disease; CLIA, chemiluminescent immunoassay; ELISA, enzyme-linked immunosorbent assay; GC, glucocorticoids; HairF, hair cortisol; NYHA, New York Heart Association; PCI, percutaneous coronary intervention.

[a]Minimum 6 weeks ago.

[b]Minimum 3 weeks ago.

[c]Rating of QUIPS dimensions in the following order: 1 (study participation), 2 (study attrition), 3 (prognostic factor), 4 (outcome measurement), 5 (study confounding), and 6 (statistical analysis and reporting). To be read from left to right; first the top line and then the bottom line. A rating of "0" indicates "low", "1" indicates "moderate", and "2" indicates "high" risk of bias.

($n = 2$), studies investigating the role of HairF and HairE exclusively in the context of subclinical cardiometabolic deterioration ($n = 49$), one out of two studies that were performed in the same population ($n = 1$), and a study in patients undergoing glucocorticoid replacement therapy ($n = 1$). Finally, a total of $n = 24$ articles were included, out of which $n = 7$ investigated the role of HairGC in relation to MetS [29–35], $n = 16$ examined the relation of HairGC with CVD [17, 21, 27, 36–48] and $n = 1$ reported results regarding both [28] (see Fig. S2). For an overview of study designs, see Table S4.

Systematic review

Metabolic syndrome. The eight studies with cross-sectional data regarding the relationship between HairGC and MetS included data of $n = 1960$ participants in total ($n = 526$ individuals with MetS, $n = 1434$ without MetS). Of these eight articles, only one reported longitudinal analyses with a very small sample size at follow-up ($n = 11$) [30]. Therefore, we focus on describing cross-sectional data in the context of MetS. The proportion of women across all individuals with HairGC data was known in $n = 6$ of the $N = 8$ included articles, comprising $n = 532$ women (29.9%) in a total of 1779 participants (Table S5). Five of the eight studies (62.5%) were performed in Europe. All remaining studies were performed by the same research group in Cape Town, South Africa. The corresponding author of those three studies confirmed that there was no overlap between the study populations. Diagnostic criteria for MetS varied, with those of the Joint Interim Statement being applied most frequently (four articles, 50%) [49]. The most commonly used laboratory method to quantify HairGC levels was liquid chromatography–mass spectrometry ("LC/MS," used in $n = 6$ studies [75%]). A detailed description of study characteristics regarding HairGC and MetS is provided in Table 1.

Three studies reported the cross-sectional finding of higher HairGC levels in individuals with MetS [29, 32, 34]. One study in patients with human immunodeficiency virus (HIV) found an association opposing the expected result, in the sense that patients with lower HairGC had a higher risk of having MetS [33]. The other four studies did not find any association between HairGC and MetS [28, 30, 31, 35]. Notably, all of the five studies that did not find the expected result of higher HairGC levels in people with MetS were performed in samples in which at least a substantial part of the included individuals were suffering from non-metabolic chronic afflictions, including HIV, post-traumatic stress disorder, schizophrenia, major depression, and bipolar disorder.

Of note, two out of the three studies that did find a significant positive association between HairGC levels and MetS were completely or predominantly (84.8%) composed of men [29, 34], whereas for the third study, this was not clearly described [32]. In two of the studies in which a positive association was described, analyses were adjusted for risk factors associated with cardiometabolic deterioration. Specifically, in one study, the authors corrected for sex; age; hair-washing frequency; hair treatments; smoking; gamma-GT levels; daily fruit, salad, and vegetable consumption; and physical activity level [29], which attenuated—but did not fully dissolve—the association of HairGC with MetS observed in the unadjusted analyses. In the other study, analyses were corrected for age, recreational physical activity, and social support [34], resulting in a trend toward an association of HairF and MetS ($p = 0.078$). Intriguingly, all of the studies reported significantly higher MetS odds only in the highest tertile [32, 34] or, respectively, the highest one/two quartiles [29] of HairGC levels, but not in the middle compared to the lowest HairGC group. Of the three studies, which reported both HairF and HairE, one reported a stronger association of HairE with MetS [29], one reported very similar associations for HairE and HairF [32], and one reported no association for either [28].

Cross-sectional studies comparing HairGC levels between individuals with CVD and individuals without CVD. We identified 10 cross-sectional studies comparing participants with versus without CVD regarding their HairGC levels, including data of in total $N = 11{,}114$ individuals ($n = 3381$ with CVD, $n = 5556$ women [50%], see Table S6). Although the proportion of women within the total sample was known for all 10 studies, the sex distribution per CVD versus non-CVD group was not clearly described in four of these studies [28, 36, 39, 48]. Outcome definitions of CVD diagnoses varied among studies, mostly including CAD [17, 27, 36, 37, 40, 43, 48] but also multiple different diseases being combined as "CVD" [21, 28, 39]. Two studies included cerebrovascular outcomes [21, 28]. Most studies were performed in Europe ($n = 4$; 40%), followed by the United States, Japan, Pakistan, Israel, Canada, and South Africa (each $n = 1$). The most commonly used laboratory method to

quantify HairGC levels was ELISA, used in six studies (60%). A detailed overview of the study characteristics is depicted in Table 2.

In individuals with CVD, 8 out of 10 cross-sectional studies found higher HairGC levels compared to individuals without CVD [17, 21, 27, 28, 39, 40, 43, 48]. One very small study reported an association opposing the expectations, with non-CAD participants having higher HairGC levels than people with CAD [37], whereas one study did not find any association [36]. Many studies adjusted their analyses for classic CVD risk factors (most often including age, hypertension, lipid levels, diabetes, and smoking) [17, 21, 27, 36, 40, 43], and except for one [36], all reported significant associations, both before and after adjustment. One study even reported the strongest association between HairF and CVD risk, when compared to all other classic CVD risk factors entered into the model [27]. Out of the two cross-sectional studies that reported both HairF and HairE in the context of CVD, one reported an association of CVD with both [39], whereas the other one showed such a relationship only for HairE [28]—suggesting a potential added value of measuring HairE, besides HairF, in this context.

Cross-sectional studies comparing HairGC levels among individuals with different CVD diagnoses/severities. A total of seven cross-sectional studies were included, which compared HairGC levels among CVD patients with different conditions and disease severities, including data of $n = 1626$ individuals, of which $n = 435$ were women (26.8%). Diagnoses varied from CAD [27, 38, 44] to systolic heart failure, structural heart disease, stroke/transient ischemic attack, and intracranial aneurysms. Studies were carried out in geographically diverse populations, including Canada and Israel (each $n = 2$, 28.6%), as well as Korea, Pakistan, and the Netherlands (each $n = 1$, 14.3%). In all studies except one, ELISAs were used to assess HairGC levels. A detailed description of the study characteristics is provided in Table 3.

All included studies reported an association between CVD characteristics and HairF within individuals with CVD. Two studies included patients with CAD attending cardiac rehabilitation and compared HairF levels between those who had undergone coronary artery bypass graft surgery (minimum 6 weeks ago) and those who had not [38, 44]. Both studies reported higher levels of HairF among patients who had undergone surgery (although both analyses were not adjusted for sex, age, or any other risk factor). Similarly, another study found higher HairF levels among patients who recently had a intracranial aneurysm rupture compared to patients with an unruptured aneurysm [41]. The association persisted after adjustment for smoking, hypertension, aneurysm size, and previous CVD. On the other hand, Saleem et al. [44] did not find any association between HairF levels and weeks since an acute coronary event in patients with acute myocardial infarction, coronary bypass graft surgery (minimum 6 weeks ago), or percutaneous coronary intervention (minimum 3 weeks ago).

Three studies investigated HairF in relation to different measures of physical functioning among CVD patients [42, 46, 47]. Of those, one described a negative correlation of HairF with exercise capacity measured as performance in a treadmill test among patients with systolic heart failure, and a positive association with disease severity assessed via the New York Heart Association (NYHA) class [42]. However, no associations were observed with two other measures of physical functioning—including left ventricular ejection fraction and serum levels of the N-terminal prohormone of brain natriuretic peptide. The analysis was not adjusted for any risk factors. Another study found higher HairF levels to be associated with a higher respiratory rate and lower self-reported physical functioning in patients with structural heart disease [46], which was still observed after adjusting for age, sex, body-mass-index (BMI), and use of corticosteroids. The authors did, however, not find any difference in HairF between forms of structural heart disease (including congenital heart disease, cardiomyopathy, ischemic heart disease, and valvular heart disease). A study in patients with acute stroke or transient ischemic attack reported that higher HairF was associated with larger lesion volume, although no association was found with stroke severity assessed as NIH stroke scale (no statistical adjustments for risk factors) [47]. Interestingly, HairF, but not lesion volume, subsequently predicted poststroke cognitive impairments in these patients (see later). Finally, a study among patients with coronary atherosclerosis showed higher HairF levels in patients with triple-vessel disease compared to those with double- or single-vessel disease [27]. Again, the analysis was not adjusted. HairE was not reported in any of these studies.

Longitudinal studies regarding HairGC levels among CVD patients. We found five studies

investigating the longitudinal relation of HairGC with health outcomes in the context of CVD. Diagnoses included CAD [44, 45], stable chronic systolic heart failure [42], structural heart disease [46], and stroke [47]. In total, data of $n = 346$ individuals were included (42.50% women, see Table S8). Two studies (40%) were carried out in Israel; the others were performed in Canada, Austria, and the Netherlands (each $n = 1$, 20%). ELISA measurements were used in all studies to assess HairGC levels except one. HairE was not measured. A detailed description of the study characteristics is provided in Table 4.

Two studies compared HairF levels before and after CVD treatment [45, 46]. Younge et al. [46] compared HairF levels in $n = 151$ patients with structural heart disease ($n = 95$ women) before and after a 12-week CVD treatment (standard care or mindfulness training) and found decreased levels at the end of treatment, which was independent of whether someone received mindfulness training or not. The authors also investigated predictors for the change in HairF levels in response to treatment and found that people with a more favorable mental health status were more likely to exhibit a decrease in HairF [46]. Wagner-Skacel et al. [45] measured HairF levels in a group of $n = 30$ CVD patients ($n = 7$ women) undergoing a 4-week rehabilitation program aimed to reduce stress (either using standard care, yoga, or meditation). The authors report that HairF levels decreased at the end of rehabilitation, compared to the 2 months before the start of rehabilitation, irrespective of the treatment group. Nevertheless, these findings somewhat oppose the results of another study that did not show a relationship between HairF and time since the last acute coronary event [44].

The other three longitudinal studies investigated the role of HairF levels in predicting future health outcomes among CVD patients. One showed that higher baseline HairF levels tended to predict subsequent 1-year hospital admission due to heart failure ($p = 0.08$) [42]. However, major limitations of this study are the small sample size ($n = 44$ men, 25 admissions) and the fact that analyses were not adjusted for classic risk factors. Another study reported higher baseline HairF levels among individuals with stronger cognitive decline after stroke (including global cognitive score as well as executive and verbal functioning, memory, and motor skills) after correcting for age, sex, BMI, and genetic risk for dementia [47]. Finally, the fifth study showed that higher HairF levels predict less improvement in verbal memory performance following cardiac rehabilitation even after controlling for age, sex, BMI, and maximal oxygen uptake [44]. No prospective studies were available predicting CVD onset in healthy populations.

Meta-analysis

Sufficient data were available from 8 studies (7383 individuals in total, 2367 with CVD, 4338 women [58.8%]) to analyze the pooled SMD between individuals with CVD versus non-CVD regarding their HairF levels [17, 21, 27, 37, 39, 40, 43, 48]. As expected, the analysis revealed significantly higher HairF levels among individuals with CVD versus non-CVD (SMD = 0.4755, 95% CI: 0.1576–0.7934, $p = 0.0095$, see Fig. 2). However, the model also showed very high heterogeneity ($I^2 = 96\%$, $p < 0.001$). A subgroup analysis, including only studies in older adults (mean/median age >65 years), slightly attenuated heterogeneity ($I^2 = 80\%$, $p = 0.0061$) and, in trend, confirmed higher HairF levels among patients with CVD compared to the non-CVD group as were seen in the main analysis, although the effect size was smaller (SMD = 0.3139, 95% CI: −0.0839 to 0.7116, $p = 0.0769$) [21, 39, 48]. In the subgroup of middle-aged individuals (mean/median age <65 years), heterogeneity remained high ($I^2 = 90\%$, $p < 0.001$), but we saw a larger effect size regarding higher HairF levels in individuals with CVD versus non-CVD (SMD = 0.5659, 95% CI: −0.0201 to 1.1519, $p = 0.0552$) [17, 27, 37, 40, 43]. There were no major decreases in heterogeneity when pooling studies, which (1) predominantly included men [27, 40, 43], (2) included only CAD as CVD [17, 27, 37, 40, 43, 48], (3) used ELISA to measure HairF [21, 27, 37, 40, 43], (4) were performed in a predominantly Caucasian population [17, 21, 37, 39, 40], or (5) had an RoB <2 for confounding [17, 21, 27, 40, 43] (all $I^2 > 90\%$, $p < 0.001$). An overview regarding the results of the subgroup analyses is provided in Table S11.

The MDM sensitivity analysis was performed in a subset of four studies ($n = 2336$ individuals, 1346 with CVD, 1586 women [67.9%]) [21, 37, 39, 40], due to lacking data for the other studies and, in one case [27], a considerably different measurement range compared to the other studies. Again, we saw significantly higher HairF levels among individuals with CVD compared to non-CVD (MDM = 1.4179, 95% CI: 0.1693–2.6665, $p = 0.0260$).

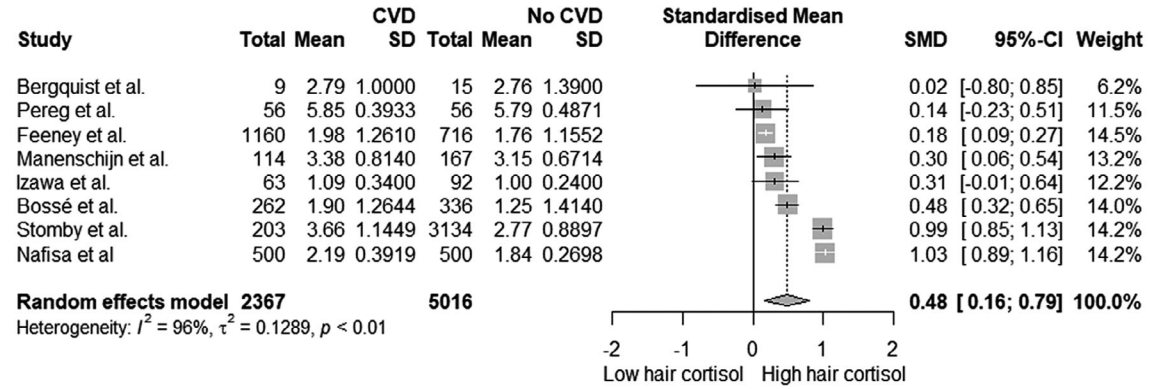

Fig. 2 *Forest plot of the meta-analysis comparing hair cortisol levels between individuals with versus without cardiovascular disease (CVD). Depicted are the log-transformed mean (SD) of hair cortisol levels per group (either received from authors of the original studies or estimated following the method of Higgins et al. [24]).*

Heterogeneity was acceptable (I^2 = 17%, p = 0.4765). Subgroup analyses are described in Material S12.

Discussion

To our knowledge, this is the first systematic review and meta-analysis regarding the association of long-term biological stress measured via HairGC levels with the MetS and CVD. In contrast to previous reviews in this context which have focused on the role of HairF, we also described evidence regarding hair cortisone (HairE) and explored the impact of age and sex on the relationship of HairGC with MetS and CVD. In short, we found that, despite large heterogeneity between studies, a vast majority of articles indicate a strong and consistent cross-sectional association between HairGC and CVD, which was confirmed by our meta-analysis for HairF (n = 8 studies, SMD = 0.48, 95% CI: 0.16–0.79, p = 0.0095). The results of the meta-analysis also suggest a potential impact of age on this relationship—with a stronger effect seen among middle-aged, compared to older, subjects. Results regarding the relationship between HairGC and MetS were less conclusive.

Metabolic syndrome

Only three out of eight included articles reported higher levels of HairGC among individuals with MetS [29, 32, 34]. A closer look at those studies suggests that sex might play a role in this context: Remarkably, out of the three studies, which found the expected effect, one included no women at all and one included a very large proportion of men (84.8%) [29, 34]. For the third study, this was not described. Meanwhile, the populations in which no association was seen between the presence of MetS and HairGC mostly comprised women (>50%) [28, 30, 31, 35]. This is especially intriguing in the view of the fact that HairGC levels have consistently been found to be higher in men than in women [17, 21, 36, 39, 48, 50], whereas men have an almost twofold higher risk of CVD-mortality than women [51]. Of course, the number of included studies here is very small, and conclusions must be drawn very carefully in this context. However, this observation suggests that the association between HairGC and MetS might be stronger in men than in women, which should be investigated in future research.

Another relevant factor influencing the relationship between HairGC levels and MetS may be an individual's health status. All five studies that did not find the expected result of higher HairGC levels among individuals with MetS [28, 30, 31, 35]—or in one case even an inverse association [33]—were performed in populations, which predominantly comprised individuals with non-metabolic chronic health impairments, some of which have previously been shown to potentially affect activity of the HPA-axis and its interactions with other body systems [52, 53]. Another possible reason for the heterogeneous results may be the difference of ethnicities between study populations. Indeed, three out of the four study populations in which no association was found exclusively included people who self-identified as colored [28, 30, 31], whereas the three studies that did report the expected association between HairGC and MetS were performed in populations, which were presumably comprised

predominantly of Caucasians [29, 32, 34]. Partially in line with this, higher HairF levels have been reported in colored versus white individuals, indicating potential differences in HPA-axis activation and/or functioning [36]. Nevertheless, the reasons underlying the different results cannot be derived at the current time, due to the large heterogeneity between the currently available studies.

Importantly, out of the three studies that reported a positive relationship between HairGC and MetS, two indicated that the observed difference was (at least partially) independent of standard risk factors related to health behavior and age [29, 34]. Intriguingly, the relationship between HairGC and MetS may not be linear, as indicated by studies reporting significantly higher odds of having MetS only in the highest, but not middle, ranges of HairGC levels when compared to the group with the lowest levels [29, 32, 34]. Moreover, two of these studies suggest an equally strong, or even stronger, association of HairE with MetS than was seen for HairF [29, 32], which is in line with studies regarding BMI and waist circumference [54, 55], as well as studies regarding CVD [18, 21]. Mechanistically, it has been speculated that cortisone may represent a more stable marker for the long-term systemic reservoir of available glucocorticoids in the body. That is because locally, a specific fraction of the systemic cortisol may be converted into the inactive form cortisone via the 11β-hydroxysteroid dehydrogenase (11β-HSD) enzyme type 2 at the scalp, before being stored in hair [29, 56]. This would also serve as a conceivable explanation for the observation that HairE values are consistently found to be higher than HairF levels [29, 57]. In addition, the laboratory measurement of HairE is often more precise and reliable than HairF [57]. Altogether, these data indicate the relevance of measuring HairE in addition to HairF whenever possible. Currently, however, HairF still remains the more frequently measured biomarker in the context of long-term HairGC in relation to cardiometabolic health.

Cardiovascular disease

The presented evidence regarding the association of HairGC with CVD from cross-sectional and longitudinal studies and our meta-analysis indicates that HairGC levels are associated with the presence, severity, and prognosis of CVD. Eighty percent of the included cross-sectional studies comparing CVD with non-CVD groups found higher HairGC levels in people with CVD—a result that persisted across various diagnoses [17, 21, 27, 28, 39, 40, 43, 48] and was confirmed by the quantitative data from our meta-analysis of HairF. Importantly, these findings differ from previous evidence regarding short-term matrices of cortisol measurements assessed in saliva, urine, and blood, which have yielded much more inconsistent results [58–61].

In line with findings regarding MetS, literature indicates that the relationship of HairGC levels with CVD may not be linear, as the odds of having CVD are especially increased among patients in the highest, but not middle, range of HairGC levels [18, 21]. Moreover, the association seems to be largely independent of standard CVD risk factors, such as age, hypertension, lipid levels, diabetes, and smoking [21, 27, 40, 43]—many of which, notably, are implied in the definition of MetS [49, 66, 67]. On the other hand, Stomby et al. [17] reported that hypertension, hyperlipidemia, and diabetes do, in fact, mediate the relation between HairGC levels and CVD. Altogether, evidence suggests that the association of HairGC levels with CVD is partially, though not completely, mediated by effects of HairGC excess on MetS but both relationships also seem to encompass independent components. Indeed, previous evidence has linked chronic HPA-axis hyperactivity (e.g., due to chronic stress) not only to dysregulations in lipid and glucose metabolism but also suggested direct associations with atherosclerotic processes, including platelet activation, endothelial dysfunction, and inflammation [55, 62, 63]. The exact extent of the (in-)dependency of both pathways remains to be unraveled in future studies. Some studies—although they were cross-sectional in the sense of a one-time HairGC measurement—showed higher HairGC levels in the months preceding an acute myocardial event among people with CVD compared to either (1) patients who were hospitalized for another reason or (2) healthy controls, suggesting a potential causative association [40, 43]. Partly in-line with this, longitudinal data suggest that recovery from CVD has a positive effect on HairGC levels [45, 46] but also point out the predictive value of HairF for future disease recovery and deterioration [42, 44, 47], indicating a potentially bidirectional relationship. It is unfortunate that HairE was not reported in these longitudinal studies.

Finally, our SMD subgroup analysis suggested a potential impact of age on this relationship. Studies among older individuals (mean/median age >65 years) show a smaller difference in HairGC levels between the CVD and non-CVD groups. Meanwhile, the effect regarding the association of HairGC levels with the presence of CVD was stronger within the subgroup of middle-aged adults (mean/median age <65 years), although higher heterogeneity was observed in the subgroup of middle-aged, compared to older, subjects. Notably, recent studies suggest that the impact of traditional cardiovascular risk factors generally declines with aging [64, 65]. This could imply that other pathways and markers could have a larger contribution to cardiovascular events among the elderly.

Including measurements of HairE in addition to HairF may pose a very valuable contribution to our understanding of the relationship between HPA-axis (hyper-) activity and cardiometabolic health. Future studies should measure both and explore potential differences. In addition, sex- and age-stratified analyses would be useful to clarify the role of these two potential moderators in the relationship between HairGC and cardiometabolic health outcomes. Moreover, longitudinal studies will be needed to assess the potential of HairGC to predict the onset of MetS and CVD. Finally, it would also be very interesting to investigate the utility of HairGC in clinical trials and to explore whether levels of baseline HairGC can be used to (1) predict an individual's response to treatment, as well as (2) how HairGC levels change in response to different medications and whether they might pose a marker for treatment success evaluation.

Limitations and strengths

There are some limitations to be considered with respect to this study. First, it must be pointed out that, with regard to MetS, currently only cross-sectional studies are available (except a longitudinal study in $n = 11$ individuals, which we regard as negligible). Although previous evidence indicates that higher HairGC levels can predict future cardiometabolic outcomes such as increases in BMI and waist circumference [55], the cross-sectional nature of the current evidence precludes conclusions regarding the potential of HairGC levels to predict the future onset of MetS. Similarly, much more data are needed in this context with regard to the longitudinal association between HairGC and CVD. Second, the sex distribution per MetS versus non-MetS/CVD versus non-CVD group was not always clearly described in the articles, whereas, as described before, sex may be a relevant moderator in the relationship between HairGC and cardiometabolic outcomes. Third, although our broad approach regarding the inclusion of articles enabled us to display a comprehensive picture of the current evidence, the heterogeneity of the included articles must be kept in mind as varying study characteristics may have impacted the results. Study results should therefore be assessed individually in the context of the respective report.

Finally, even though the SMD meta-analysis is based on logarithmic mean values (or their estimates), we cannot completely rule out effects of outliers as we did not have the original data of some articles, and HairF data are often not normally distributed. Meanwhile, the MDM analysis relies on data measured via a comparable scale, whereas small differences between different HairGC lab measurement techniques cannot be completely ruled out, even though we included studies using comparable ranges. In addition, the number of available studies was rather small, which limited our abilities for subgroup and sensitivity analyses, and in some cases, we had to apply estimates of logarithmic means (SD) in order to include as many studies as possible. The results therefore have to be interpreted with caution. Nevertheless, we still consider the results of the meta-analysis informative for the following reasons: (1) Consistency: The median-based MDM sensitivity analysis supported the results regarding the SMD and is also in-line with the qualitative observation that 80% of included studies regarding CVD have detected the same result as was found in the quantitative analysis, and (2) Subgroup analyses: Due to subgroup analyses, we were able to quantitatively investigate potential reasons for differences in study results, which suggested a role of age in the relationship between HairGC and CVD. Altogether, the use of qualitative descriptions—along with metric and nonparametric meta-analytic approaches—enabled us to depict a comprehensive picture of the available evidence and investigate potential factors that may influence the relationship between HairGC levels and CVD.

Conclusion

Long-term glucocorticoid exposure—measured as levels of the stress hormones cortisol and

cortisone in hair—is rather consistently associated with the presence of MetS and CVD, independently of known risk factors. We were able to confirm this in a meta-analysis for HairF and CVD. Associations seem most pronounced in individuals within the highest ranges of HairGC levels but not the more subtle increases, indicating that the relationship between HairGC levels and cardiometabolic health outcomes may not be linear. Prospective longitudinal studies are needed to evaluate the directionality of this association and determine whether HairGC can be used as an addition to currently known risk factors for future risk to develop cardiometabolic disease. In this context, the additional measurement of HairE seems highly valuable as it may relate more closely to cardiovascular health outcomes than HairF.

Author contributions

Susanne Kuckuck was mainly responsible for the conceptualization, data curation, analysis, investigation, methodological decisions, project administration, validation, visualization, and writing of the manuscript. Robin Lengton played a supporting role in data curation, investigation, methodological decisions, project administration, visualization, and editing of the manuscript. Mariëtte Boon was involved in the conceptualization, methodological decisions, validation, and editing of the manuscript. Eric Boersma supervised the methodological decision-making, especially with regard to data analysis, and was involved in the validation as well as editing of the manuscript and funding acquisition. Brenda Penninx was involved in the funding and resource acquisition, methodological decisions, supervision, data visualization, as well as editing of the manuscript. Maryam Kavousi was involved in the conceptualization, data curation, supervision of methodological decision-making, funding acquisition, and editing of the manuscript. Elisabeth van Rossum was leading the supervision and was involved in the conceptualization, funding and resource acquisition, methodological decision-making, and editing of the manuscript.

Acknowledgments

The authors wish to thank Wichor Bramer from the Erasmus MC Medical Library for developing the search strategies and to Priscilla Vlaming for statistical advice. We also wish to thank all the authors who provided us with additional information regarding their articles. In addition, we would like to thank Annelieke Roest and Layal Chaker for their conceptual advice regarding meta-analyses. Finally, we acknowledge that Fig. 1 was created with BioRender.com.

Conflict of interest statement

The authors declare no conflicts of interest.

Funding information

This article was written as part of the research project “Stress in Action”: www.stress-in-action.com. Stress in Action is financially supported by the Dutch Research Council and the Dutch Ministry of Education, Culture and Science (NWO gravitation grant number 024.005.010).

Data availability statement

The data and codes underlying this article will be shared upon reasonable request to the corresponding author. Data from individual studies included in this systematic review and meta-analysis can be found in the respective references.

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