Hair analysis reveals subtle HPA axis suppression associated with use of local corticosteroids: the Lifelines Cohort Study

Wester VL, Noppe G, Savas M, van den Akker ELT, de Rijke YB, van Rossum EFC.

Psychoneuroendocrinology, 2017, 80:1-6

ABSTRACT

Background and aims: Scalp hair is increasingly used to measure the long-term exposure to endogenous glucocorticoids hormones. Long-term cortisone (HairE) and cortisol (HairF) have been associated with obesity, metabolic syndrome, cardiovascular disease and psychopathology. However, little is known about the influence of the use of local corticosteroids and major stressful life events on hair glucocorticoids.

Materials and methods: We determined HairE and HairF using liquid chromatography - tandem mass spectrometry in 295 adult participants of the population-based Lifelines cohort study (75% females, median age 42). We collected anthropometry and fasting metabolic laboratory values, questionnaires on hair characteristics, recent use of corticosteroids, and recent major stressful life events.

Results: After adjustment for covariates, hair glucocorticoids increased with age, male sex, black or brown hair color, and frequency of sweating on the scalp, and decreased with higher hair washing frequency ($P<0.05$). HairE was decreased in participants who used systemic corticosteroids (5.4 vs. 8.5 pg/mg hair, $P=0.041$), and in participants who only used local agents such as inhaled, topical and nasal corticosteroids (6.8 vs. 8.5 pg/mg, $P=0.005$). Recent life events were positively associated with HairF after adjustment for age and sex ($P=0.026$), but this association lost significance after adjustment for hair related characteristics ($P>0.05$).

Conclusions: HairE can be a useful marker to detect mild adrenal suppression due to corticosteroid use in the general population, even when only inhaled, nasal or topical corticosteroids are used, which suggests that these commonly used agents induce systemic effects.

INTRODUCTION

The human response to stress is marked by activation of the hypothalamus-pituitary-adrenal axis, which ultimately results in release of the glucocorticoid hormone cortisol from the adrenals. Cortisol binds to the glucocorticoid and mineralocorticoid receptors, and influences behavior, metabolism and immunity [1]. In addition to endogenous cortisol, medications containing corticosteroids are commonly prescribed for a wide range of diseases and health conditions, and contribute to glucocorticoid exposure in the general population [2]. Cortisol levels are usually measured in blood, saliva and urine [3], but are highly variable due to the diurnal rhythm and the influence of acute stress [4]. This high variability limits the use of cortisol measurements in body fluids as a marker for glucocorticoid exposure over longer period of time.

Over the past decade, measurements of cortisol and cortisone in scalp hair have emerged as a method to estimate long-term glucocorticoid exposure. Because scalp hair grows at a relatively stable rate of approximately 1 cm per month and steroid hormones are retained in it, hair can be used to obtain information about the exposure of glucocorticoid hormones over months of time in a single measurement [5, 6]. Increased hair cortisol (HairF) and/or cortisone (HairE) have been linked to obesity, cardiovascular disease, metabolic syndrome, and psychopathology [6]. Furthermore, we have found HairF helpful in the diagnosis of Cushing's syndrome, especially cyclic Cushing's syndrome [7], since hair analysis can be used to create retrospective timelines of cortisol exposure.

To interpret the results of hair glucocorticoid measurements in a clinical or research context, a thorough understanding of the factors that influence HairE and HairF is crucial. Hair color, use of hair products, the frequency of hair washing and hair treatments such as coloring and permanent waving have been reported to affect hair glucocorticoids in humans [6, 8, 9], and the contribution of sweating on the scalp has been debated in published literature [10, 11]. In selected populations, HairF has been shown to be increased in relation with major stressful life events [12-15], but it is unclear whether these findings can be extrapolated to the general population. One potential influencing factor that has received little attention to date, is the use of prescription corticosteroids [6]. Evidence suggests that even locally administered corticosteroids such as inhaled corticosteroids may cause a suppression of the HPA axis [16], and we hypothesize that this is reflected in decreased long-term glucocorticoids. The long-term nature of hair measurements make this matrix promising to investigate subtle influence of local corticosteroids on activity of the HPA axis.

In the present study, we aimed to study the factors that influence hair glucocorticoids in a population-based Dutch cohort, with a specific focus on the use of corticosteroids and recent major life events, as well as create population-based reference ranges for hair cortisone and cortisol.

METHODS

Participants and procedures

All study participants came from Lifelines, a multi-disciplinary prospective population-based cohort study examining in a unique three-generation design the health and health-related behaviors of 167,729 persons living in the north of the Netherlands. It employs a broad range of investigative procedures in assessing the biomedical, socio-demographic, behavioral, physical and psychological factors which contribute to the health and disease of the general population, with a special focus on multi-morbidity and complex genetics [17]. Lifelines is a facility that is open for all researchers. Information on the application and data access procedure is summarized on www.lifelines.nl. Detailed information on all collected variables can be found in the online Lifelines Data Catalogue (https://catalogue.lifelines.nl/).

For the present study, 295 adult participants of Lifelines (median age 42 [range 18 – 85], 220 females [75%]) were included in November and December of 2013. All participants came for a study site visit, which included measurements of anthropometry and vital parameters, and a fasting venipuncture. During the site visit, a scalp hair sample of approximately 100-150 hair was cut from the posterior vertex of the scalp, as close to the scalp as possible. Hair samples were taped to a piece of paper, and stored in envelopes in the dark at room temperature until further processing.

This study was approved by the Medical Ethics Review Committee of the University Medical Center Groningen. All participants provided written informed consent, and all study procedures were carried out in accordance with the declaration of Helsinki.

Hair processing and analysis

Hair sample processing and analysis was performed as described previously [18]. From each hair sample, approximately 20 mg of the proximal 3 cm was weighed and cut into 1 cm segments. Hair samples were washed in 2 mL of LC-MS grade isopropanol for 2 minutes, and left to dry. We extracted the hairs for 18 hours at 25 degrees centigrade in 1.4 mL LC-MS grade methanol and 100 uL of internal standard. Extracted samples were purified using solid phase extraction, and glucocorticoids were quantified by liquid

chromatography - tandem mass spectrometry (LC-MS/MS) using a Xevo TQ-S system (Waters, Milford, MA).

Anthropometry and blood measurements

During the site visit, weight, height, waist circumference, and blood pressure were measured. Total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, and glucose were measured using a Roche Modular system (Roche Diagnostics GmbH, Germany). HbA1c was measured using an ion exchange chromatography kit on a Tosoh G8 HPLC system (Tosoh Corporation, Tokyo, Japan).

Questionnaires

In all 295 participants, we collected a questionnaire about hair related characteristics, which included natural hair color, hair treatment in the past 3 months, hair washing frequency, frequency of sweating on the scalp (in days per months), and the use of hair products on the day of hair collection. We asked participants in a standardized manner whether they used any product containing corticosteroids in the past three months, and their route of administration (i.e., oral, intravenous, nasal, topical, inhaled, joint injections or other). Life events were evaluated using the Dutch version of the List of Threatening Experiences (LTE), which surveys the occurrence of twelve major life events in the past twelve months, such as loss of a job, or death of a first-degree relative [19].

Statistical analysis

SPSS version 21 was used for statistical analysis. Hair glucocorticoid concentrations were logarithmically transformed to achieve a normal distribution. Reference range limits were defined as the mean minus 1.96 SD (lower limit), and the mean plus 1.96 SD (upper limit) of the logarithmically transformed concentrations. Limits were then transformed back to the original unit (pg/mg hair). In order to study the influence of predictors on hair glucocorticoid levels, we used linear regression analysis. First, we performed simple linear regression between each predictor and hair glucocorticoids (model 1). Model 2 was adjusted for age and sex, while model 3 was adjusted for age, sex, corticosteroid use, and hair related variables that significantly associated with HairE or HairF in model 1 or 2. A P value below 0.05 was considered statistically significant.

RESULTS

Baseline characteristics and hair glucocorticoid concentrations

Baseline characteristics of the entire study sample are described in Table 1. The median age was 42 (range 18 - 85), and the majority of participants were female (220 out of

295, or 75%). 161 out of 277 participants reported at least one life event in the past year (58.1%, 18 missing). HairE was successfully determined in 289 hair samples (98%), and HairF in 266 (90%). Reference range values for the total group are described in Table 2.

Table 1. baseline characteristics

		Females	Males	P value
		n=220	n=75	
Age (years)		41 (34-48)	44 (37-50)	0.114
Hair characteristics				
Natural hair color	Blond	130 (46.8%)	27 (36.0%)	0.263
	Black	1 (0.5%)	11 (14.7%)	
	Brown	90 (40.9%)	25 (33.3%)	
	Red	2 (0.9%)	2 (2.7%)	
	Grey	24 (10.9%)	10 (13.3%)	
Hair washing frequency (≥3 times per week)		165 (75.0%)	61 (81.3%)	0.263
Frequency of sweating on the scalp (days per month)		0 (0 - 1)	0 (0 - 4)	**0.005**
Hair treatment in the last 3 months		108 (49.1%)	2 (2.7%)	**<0.001**
Use of hair products		179 (81.4%)	49 (65.3%)	**0.004**
Cardiometabolic characteristics				
BMI (kg/m2)		25.6 (22.9-28.9)	25.8 (23.8-28.6)	0.660
Overweight (BMI ≥25)		97 (44%)	32 (43%)	0.830
Of which obese (BMI ≥30)		49 (22%)	8 (11%)	**0.028**
Waist circumference (cm)		86 (77-94)	94 (86-100)	**<0.001**
Total cholesterol (mmol/L)		4.8 (4.2 - 5.5)	5.1 (4.6 - 5.8)	**0.021**
HDL cholesterol (mmol/L)		1.6 (1.2-1.8)	1.3 (1.2-1.5)	**<0.001**
LDL cholesterol (mmol/L)		3.0 (2.5-3.7)	3.6 (3.1-4.3)	**<0.001**
Triglycerides (mmol/L)		0.85 (0.67-1.13)	1.21 (0.89-1.75)	**<0.001**
Fasting glucose (mmol/L)		4.8 (4.6-5.1)	5.2 (4.8-5.5)	**<0.001**
HbA1c (mmol/mol)		36 (34 - 38)	37 (34 - 39)	0.358
Systolic blood pressure (mmHg)		117 (109-129)	127 (119-136)	**<0.001**
Diastolic blood pressure (mmHg)		70 (66-77)	72 (68-79)	**0.037**
Clinical characteristics				
Corticosteroid use in the past 3 months		29 (13.2%)	9 (12.0%)	0.792
Life events in the past 12 months (LTE)		1 (0 - 2)	1 (0 - 1)	0.158

Values are depicted as median (interquartile range) or n (percentage). Differences between females and males were tested using Mann Whitney U and Chi square tests.
Abbreviations: BMI, body mass index; HbA1c, glycosylated hemoglobin; HDL, high density lipoprotein; LDL, low density lipoprotein; LTE, List of Threatening Experiences.

Table 2. reference range values for hair cortisone and cortisol

	HairE (pg/mg)	HairF (pg/mg)
	n=289	n=266
Lower limit (95% CI)	3.29 (3.12 - 3.48)	0.68 (0.62 - 0.74)
Geometric mean (95% CI)	8.21 (7.78 - 8.67)	2.67 (2.45 - 2.90)
Upper limit (95% CI)	20.48 (19.41 - 21.61)	10.49 (9.64 - 11.41)

Abbreviations: CI, confidence interval; HairE, hair cortisone; HairF, hair cortisol

Associations with hair cortisone levels (HairE)

The associations between HairE and HairF levels and demographic parameters, hair related characteristics and clinical characteristics are described in Table 3. HairE increased with age (standardized regression coefficient 0.214, P<0.001; Table 1), and was higher in men (coefficient 0.172, P=0.003). The influence of age and sex remained significant after adjustment for each other, hair related characteristics, and corticosteroid use (coefficient 0.187, P=0.001; and coefficient 0.130, P=0.024, respectively).

In both simple analyses and after adjustment for age, sex, hair related variables, and corticosteroid use, HairE was higher in black (adjusted standardized regression coefficient 0.229, P=0.009) and brown hair (coefficient 0.268, P>0.001) when compared to blond hair, and was lower in participants who washed their hair at least 3 times per week (coefficient -0.175, P=0.002). The self-reported frequency of sweating on the scalp was associated with increased HairE in model 3 (coefficient 0.139, P=0.011). Hair treatment in the past 3 months or use of hair products were not associated with HairE (P>0.05).

Fasting glucose was positively associated with HairE in simple analysis (coefficient 0.190, P=0.002) and after adjustment for age, sex, hair related variables, and corticosteroid use (coefficient 0.118, P=0.041). Although HairE was positively associated with LDL cholesterol, triglycerides and HbA1c in simple analyses (P<0.05), these differences lost statistical significance after adjustment (P>0.05 in models 2 and 3). BMI, waist circumference, total cholesterol, HDL cholesterol, blood pressure and life events were not associated with HairE (P>0.05 in all models). Participants who used corticosteroids at the time of hair collection had lower HairE (coefficient -0.132, P=0.025), an association that became stronger after adjustment for age, sex, hair related variables and corticosteroid use (coefficient -0.176, P=0.001).

Associations with hair cortisol levels (HairF)

HairF increased with age (standardized regression coefficient 0.163, P=0.008; Table 1), and was higher in men (coefficient 0.153, P=0.013). After adjustment for each other, hair related characteristics and corticosteroid use, the association between HairF and

Table 3. influence of demographic, hair related and clinical characteristics on hair glucocorticoids

			Model 1		Model 2		Model 3	
			Coefficient	P value	Coefficient	P value	Coefficient	P value
Demographic parameters								
Age		HairE	0.214	**<0.001**	0.203	**<0.001**	0.187	**0.001**
		HairF	0.163	**0.008**	0.150	**0.014**	0.130	**0.036**
Sex (male vs female)		HairE	0.172	**0.003**	0.157	**0.006**	0.130	**0.024**
		HairF	0.153	**0.013**	0.139	**0.023**	0.112	0.078
Hair related characteristics								
Natural hair color	Blond	HairE	Reference		Reference		Reference	
		HairF	Reference		Reference		Reference	
	Black	HairE	0.248	**0.003**	0.168	0.060	0.229	**0.009**
		HairF	0.192	**0.030**	0.124	0.201	0.166	0.083
	Brown	HairE	0.252	**<0.001**	0.264	**<0.001**	0.268	**<0.001**
		HairF	0.142	**0.035**	0.139	**0.039**	0.150	**0.023**
	Red	HairE	0.088	0.315	0.102	0.232	0.060	0.464
		HairF	-0.004	0.963	0.006	0.950	-0.023	0.801
	Grey	HairE	0.132	0.096	-0.065	0.502	-0.043	0.642
		HairF	0.159	0.057	-0.013	0.899	-0.006	0.948
Hair washing frequency		HairE	-0.178	**0.002**	-0.147	**0.013**	-0.175	**0.002**
		HairF	-0.134	**0.029**	-0.118	0.060	-0.135	**0.029**
Frequency of sweating on the scalp		HairE	0.114	0.052	0.104	0.069	0.139	**0.011**
		HairF	0.178	**0.004**	0.160	**0.008**	0.178	**0.003**
Hair treatment in the past 3 months		HairE	0.011	0.856	0.086	0.169	0.062	0.297
		HairF	0.006	0.918	0.073	0.269	0.050	0.445
Use of hair products		HairE	-0.108	0.068	-0.071	0.220	-0.077	0.156
		HairF	-0.117	0.056	-0.088	0.152	-0.094	0.117
Clinical characteristics								
BMI (kg/m^2)		HairE	0.010	0.860	0.004	0.947	0.006	0.914
		HairF	0.078	0.206	0.065	0.290	0.029	0.637
Waist circumference (cm)		HairE	0.065	0.269	0.000	0.999	0.009	0.880
		HairF	0.130	**0.034**	0.072	0.253	0.047	0.461
Total cholesterol (mmol/L)		HairE	0.117	0.052	0.011	0.866	-0.003	0.965
		HairF	0.095	0.129	0.008	0.904	0.002	0.973
HDL cholesterol (mmol/L)		HairE	-0.045	0.456	-0.074	0.468	-0.075	0.229
		HairF	-0.016	0.800	0.003	0.959	0.010	0.881
LDL cholesterol (mmol/L)		HairE	0.130	**0.031**	0.031	0.629	0.021	0.731
		HairF	0.089	0.158	0.002	0.979	-0.006	0.930
Triglycerides (mmol/L)		HairE	0.133	**0.027**	0.075	0.223	0.090	0.129
		HairF	0.118	0.059	0.064	0.336	0.062	0.344
Fasting glucose (mmol/L)		HairE	0.190	**0.002**	0.126	**0.039**	0.118	**0.041**

Table 3. influence of demographic, hair related and clinical characteristics on hair glucocorticoids (continued)

		Model 1		Model 2		Model 3	
		Coefficient	P value	Coefficient	P value	Coefficient	P value
	HairF	0.150	**0.018**	0.097	0.134	0.086	0.176
HbA1c (mmol/mol)	HairE	0.173	**0.006**	0.086	0.200	0.083	0.192
	HairF	0.095	0.146	0.023	0.745	0.010	0.881
Systolic blood pressure (mmHg)	HairE	0.098	0.095	0.002	0.968	-0.008	0.890
	HairF	0.132	**0.031**	0.058	0.372	0.045	0.494
Diastolic blood pressure (mmHg)	HairE	0.094	0.109	0.032	0.587	0.023	0.677
	HairF	0.063	0.306	0.004	0.953	-0.021	0.742
Corticosteroid use in the past 3 months	HairE	-0.132	**0.025**	-0.132	**0.020**	-0.176	**0.001**
	HairF	-0.023	0.705	-0.021	0.729	-0.057	0.339
Life events in the last year (LTE)	HairE	0.026	0.670	0.044	0.465	-0.033	0.580
	HairF	0.131	**0.039**	0.139	**0.026**	0.068	0.306

Coefficients are standardized linear regression coefficients. Model 1 is a simple linear regression model. Model 2 is adjusted for age and sex. Model 3 is adjusted for age, sex, black hair, brown hair, hair washings, sweating on the scalp and corticosteroid use.
Abbreviations: BMI, body mass index; HairE, hair cortisone; HairF, hair cortisol; HbA1c, glycosylated hemoglobin; HDL, high density lipoprotein; LDL, low density lipoprotein; LTE, List of Threatening Experiences.

age remained significant (coefficient 0.130, P=0.036), but the association with sex was reduced to a trend (coefficient 0.112, P=0.078).

In both simple analyses and after adjustment for age, sex, hair related variables, and corticosteroid use, HairF was higher with brown hair (adjusted coefficient 0.150, P=0.023) and the frequency of sweating on the scalp (coefficient 0.178, P=0.003), and was lower in participants who washed their hair at least 3 times per week (coefficient -0.135, P=0.029). Black hair was associated with higher HairF in simple analysis (coefficient 0.192, P=0.030), but this association lost statistical significance after adjustment (coefficient 0.166, P=0.083). Hair treatment in the past 3 months or use of hair products were not associated with HairF (P>0.05).

HairF was positively associated with waist circumference (coefficient 0.130, P=0.034), fasting glucose (coefficient 0.150, P=0.018) and systolic blood pressure (coefficient 0.132, P=0.031), but all of these associations lost statistical significance after adjustment for age, sex, hair-related variables, and corticosteroid use (P>0.05). BMI, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, HbA1c, diastolic blood pressure and corticosteroid use were not associated with HairF (P>0.05).

The number of recent life events (LTE) was associated with HairF in both simple analyses (coefficient 0.131, P=0.039) and after adjustment for age and sex (coefficient 0.139, P=0.026). However, this association lost significance in the final model 3. Exploratory analyses showed that the association between HairF and life events only lost statistical significance when sweating on the scalp was added to the model (data not shown). We therefore performed a mediation analysis, which provided evidence for effect modification by sweating on the scalp (Figure S1, supplementary appendix).

Use of corticosteroids and HairE

Because corticosteroid use significantly decreased HairE (adjusted coefficient -0.176, P=0.001), we investigated whether this decrease was specific for use of systemic corticosteroids (i.e., oral or intravenous), or could also be detected when they were administered locally (i.e., inhaled, nasal, topical, joint injections or other local corticosteroids). Out of 295 participants, 38 (12.9%) reported corticosteroid in the past three months, of which four (1.7%) used systemic corticosteroids, and 34 (11.1%) only used locally administered corticosteroids: eight used only inhaled, nine used nasal, thirteen used topical corticosteroids, one received a joint injection in the past three months, and three participants used a combination of local corticosteroids. HairE could be quantified in all corticosteroid users, except for one participant who used nasal steroids. Compared to none-users (adjusted geometric mean 8.5 pg/mg, 95%CI 8.0 - 8.9), both users of systemic corticosteroids (5.4 pg/mg, 95%CI 3.5 - 8.3, P=0.041), and users of only local corticosteroids (6.8 pg/mg, 95%CI 5.9 - 7.9, P=0.005) had decreased HairE (Figure 1). When stratified by route of administration, users of nasal, inhaled or topical corticosteroids had lower HairE on average, but these differences were not statistically significant compared to none-users (P>0.05, Figure 1).

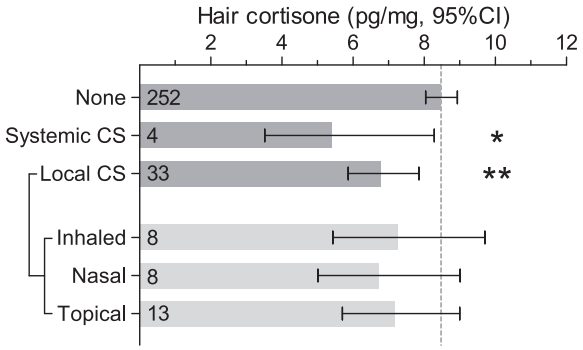

Figure 1. Hair cortisone levels and corticosteroid use
Numbers in bars represent the number of subjects. Abbreviations: CI, confidence interval; CS, corticosteroids. *P<0.05, **P<0.01, compared to no use of corticosteroids.

DISCUSSION

We present a study in which the glucocorticoids cortisone and cortisol were analyzed in scalp hair of 295 participants from a population-based cohort in the north of the Netherlands. Long-term glucocorticoid levels were significantly affected by age, sex, hair-related factors, corticosteroid use, and major stressful life events.

For the first time, we report that users of corticosteroids had lower HairE, but not HairF. Not only did individuals who used systemic corticosteroids had lower HairE, but also individuals who used only local corticosteroids (e.g. inhaled, topical, nasal). This is an important finding, because it suggests that a subtle suppression of the HPA axis due to these commonly used agents occurs in the general population. In a recent meta-analysis, it was reported that all forms of administration of corticosteroids may result in adrenal insufficiency, even at low doses [16]. Together with the results in our study, this indicates that despite being locally administered, local corticosteroids enter the bloodstream in sufficient quantities to cause systemic effects. This suggests that long-term use of these medications may put patients at risk of the sequelae of increased systemic corticosteroid exposure. Previous studies have investigated the association between hair cortisol and corticosteroid use. One small study found that hair cortisol levels were decreased in asthmatic children using inhaled corticosteroids, compared to children without asthma [20]. In contrast, Wells *et al.* reported increased hair cortisol in association with corticosteroid use in a pooled adult sample, of which the route of administration was not further specified [21]. However, it should be noted that the latter study employed an immunoassay, which may be affected by cross-reactivity. Therefore, it is possible that the increased hair cortisol levels in this study represent false elevations due to detection of administered corticosteroids, rather than increases in endogenous cortisol production.

It remains unclear why in the present study, corticosteroid use was associated with lower cortisone, but not with lower cortisol. One possible explanation for this discrepancy could be that the conversion of cortisol into cortisone is rate-limited by the capacity of 11-beta hydroxysteroid dehydrogenase type 2 [22]. Therefore, it is conceivable that acutely peaking levels of cortisol mainly increase HairF in favor of HairE. In contrast, HairE may be predominantly affected by total long-term glucocorticoid output, and therefore more reflective of baseline cortisol production. A potential limitation in our study is that we used self-reported corticosteroid use, and we did not have information on corticosteroid use from medical records. In our study, we specifically asked participants about the different administration forms of corticosteroids, and we provided examples of the most commonly prescribed agents in the questionnaire. However, we still may have underestimated corticosteroid use. On the other hand, self-reported use may form a better

reflection of the real-life exposure to corticosteroid medications, since compliance with prescriptions for these medications is known to be low. For instance, adults with asthma who have been prescribed inhaled corticosteroids have been reported to not use them on a daily basis in more than half of cases [23].

In line with previous findings, we showed that hair glucocorticoid levels increase with age, and are higher in men than in women. Furthermore, we confirmed previous findings that hair glucocorticoid levels are higher with dark hair color, and decreased by hair washing [8, 9]. These observations indicate that hair glucocorticoids measured using LC-MS/MS may be influenced by structural differences between different hair colors, and hair damage caused by frequent washing. Of the cardiometabolic parameters, only fasting glucose levels were independently associated with HairE, but not HairF. None of the other cardiometabolic parameters, including BMI, waist circumference, lipid levels and HbA1c, was independently associated with HairE of HairF. This is surprising, because multiple studies to date have found that hair glucocorticoids are positively associated with BMI or waist circumference, and are higher in obese individuals [9, 24-26]. Furthermore, hair glucocorticoids have been shown to be increased with presence of metabolic syndrome and its individual components, and diabetes mellitus [8, 9, 27]. Like with any epidemiological study, care must be taken not to extrapolate our findings to all populations. Although the Lifelines is a population-based cohort study, it is performed in a region of the Netherlands with a largely Caucasian population [17]. Our findings may therefore not be valid in other ethnic groups, since hair glucocorticoids have been reported to be influenced by ethnicity [28, 29].

For the first time, we report an association between recent major life events and HairF in a population-based cohort. Other studies have reported life events to be associated with HairF in specific populations, including elementary school girls, college students, bipolar disorder patients, and in users of crack cocaine [12-15]. In our study, the association between life events and HairF remained significant after adjustment for age and sex, but disappeared when hair-related variables were added as covariates. An exploratory stepwise analysis showed that the association between HairF and life events only disappeared when sweating on the scalp was added, and that a significant association exists between sweating on the scalp and HairF (supplementary appendix, Figure S1). This indicates that there may be effect modification by sweating on the scalp.

To the best of our knowledge, this is the first published epidemiological study which reports an association between the self-reported frequency of sweating on the scalp, and hair glucocorticoid concentrations. Higher frequency of sweating was independently associated with increased HairE and HairF. Two previous studies have been published,

which studied the acute influence of sweating on HairF in an experimental design. Russell *et al.* reported in 17 subjects that cortisol could be detected in sweat after exercise, and immersion of hair in a hydrocortisone solution mimicking sweat for 60 minutes increased HairF as measured using ELISA [10]. A more recent study by Grass *et al*. found contrasting results. Sweating was induced using a treadmill challenge in 42 subjects, and a sauna bathing challenge in 52 subjects, both of which did not affect HairF measured using LC-MS/MS [11]. It is conceivable that one acute episode of sweating does not significantly impact hair glucocorticoid content, but that repeated exposure of the hair to sweat may result in incremental increases. However, our results must be interpreted with caution, because we did not measure the actual sweating on the scalp, but estimated sweating by questionnaire ("*Do you regularly sweat excessively on your head? A) Yes, on average … days per month; B) No (or rarely)*"). The answer to this question may not only be influenced by sweating itself, but also by an individual's perception of bodily sensations.

In conclusion, we found that in a population based cohort, the use of local corticosteroids such as inhaled, nasal and topical corticosteroids, is associated with a decreased long-term cortisone as measured in scalp hair. This indicates that the use of local corticosteroids results in a subtle suppression of the HPA axis in response, and may have systemic effects next to their desired localized therapeutic benefits. Furthermore, we found that major stressful life events may increase long-term cortisol levels in the general population.

REFERENCES

1. Quax, R.A., et al., *Glucocorticoid sensitivity in health and disease.* Nat Rev Endocrinol, 2013. **9**(11): p. 670-86.
2. van Staa, T.P., et al., *Use of oral corticosteroids in the United Kingdom.* Qjm, 2000. **93**(2): p. 105-11.
3. Nieman, L.K., et al., *The diagnosis of Cushing's syndrome: an endocrine society clinical practice guideline.* The Journal of Clinical Endocrinology & Metabolism, 2008. **93**(5): p. 1526-1540.
4. Tsigos, C. and G.P. Chrousos, *Hypothalamic–pituitary–adrenal axis, neuroendocrine factors and stress.* Journal of psychosomatic research, 2002. **53**(4): p. 865-871.
5. Gao, W., et al., *LC-MS based analysis of endogenous steroid hormones in human hair.* J Steroid Biochem Mol Biol, 2015.
6. Wester, V.L. and E.F. van Rossum, *Clinical applications of cortisol measurements in hair.* Eur J Endocrinol, 2015. **173**(4): p. M1-10.
7. Manenschijn, L., et al., *A novel tool in the diagnosis and follow-up of (cyclic) Cushing's syndrome: measurement of long-term cortisol in scalp hair.* The Journal of Clinical Endocrinology & Metabolism, 2012. **97**(10): p. E1836-E1843.
8. Stalder, T., et al., *Cortisol in hair and the metabolic syndrome.* J Clin Endocrinol Metab, 2013. **98**(6): p. 2573-80.
9. Staufenbiel, S.M., et al., *Determinants of hair cortisol and hair cortisone concentrations in adults.* Psychoneuroendocrinology, 2015. **60**: p. 182-94.
10. Russell, E., et al., *The detection of cortisol in human sweat: implications for measurement of cortisol in hair.* Therapeutic drug monitoring, 2014. **36**(1): p. 30-34.
11. Grass, J., et al., *Sweat-inducing physiological challenges do not result in acute changes in hair cortisol concentrations.* Psychoneuroendocrinology, 2015. **53**: p. 108-116.
12. Vanaelst, B., et al., *Cortisone in hair of elementary school girls and its relationship with childhood stress.* European journal of pediatrics, 2013. **172**(6): p. 843-846.
13. Karlén, J., et al., *Cortisol in hair measured in young adults-a biomarker of major life stressors?* BMC clinical pathology, 2011. **11**(1): p. 12.
14. Staufenbiel, S.M., et al., *Recent negative life events increase hair cortisol concentrations in patients with bipolar disorder.* Stress, 2014. **17**(6): p. 451-459.
15. Grassi-Oliveira, R., et al., *Hair cortisol and stressful life events retrospective assessment in crack cocaine users.* The American journal of drug and alcohol abuse, 2012. **38**(6): p. 535-538.
16. Broersen, L.H., et al., *Adrenal Insufficiency in Corticosteroids Use: Systematic Review and Meta-Analysis.* J Clin Endocrinol Metab, 2015. **100**(6): p. 2171-80.
17. Scholtens, S., et al., *Cohort Profile: LifeLines, a three-generation cohort study and biobank.* Int J Epidemiol, 2015. **44**(4): p. 1172-80.
18. Noppe, G., et al., *LC-MS/MS-based method for long-term steroid profiling in human scalp hair.* Clinical endocrinology, 2015. **83**(2): p. 162-166.
19. Rosmalen, J.G., E.H. Bos, and P. de Jonge, *Validation of the Long-term Difficulties Inventory (LDI) and the List of Threatening Experiences (LTE) as measures of stress in epidemiological population-based cohort studies.* Psychol Med, 2012. **42**(12): p. 2599-608.
20. Kamps, A.W., et al., *Children with asthma have significantly lower long-term cortisol levels in their scalp hair than healthy children.* Acta Paediatr, 2014. **103**(9): p. 957-61.
21. Wells, S., et al., *Associations of hair cortisol concentration with self-reported measures of stress and mental health-related factors in a pooled database of diverse community samples.* Stress, 2014. **17**(4): p. 334-342.

22. Ferrari, P., *The role of 11beta-hydroxysteroid dehydrogenase type 2 in human hypertension.* Biochim Biophys Acta, 2010. **1802**(12): p. 1178-87.
23. Chambers, C., et al., *Health beliefs and compliance with inhaled corticosteroids by asthmatic patients in primary care practices.* Respiratory medicine, 1999. **93**(2): p. 88-94.
24. Younge, J.O., et al., *Cortisol levels in scalp hair of patients with structural heart disease.* Int J Cardiol, 2015. **184**: p. 71-8.
25. Stalder, T., et al., *Cortisol in hair, body mass index and stress-related measures.* Biol Psychol, 2012. **90**(3): p. 218-23.
26. Wester, V.L., et al., *Long-term cortisol levels measured in scalp hair of obese patients.* Obesity (Silver Spring), 2014. **22**(9): p. 1956-8.
27. Feller, S., et al., *Predictors of hair cortisol concentrations in older adults.* Psychoneuroendocrinology, 2014. **39**: p. 132-40.
28. Abell, J.G., et al., *Assessing cortisol from hair samples in a large observational cohort: The Whitehall II study.* Psychoneuroendocrinology, 2016. **73**: p. 148-156.
29. Rippe, R.C., et al., *Splitting hair for cortisol? Associations of socio-economic status, ethnicity, hair color, gender and other child characteristics with hair cortisol and cortisone.* Psychoneuroendocrinology, 2016. **66**: p. 56-64.

SUPPLEMENTARY DATA

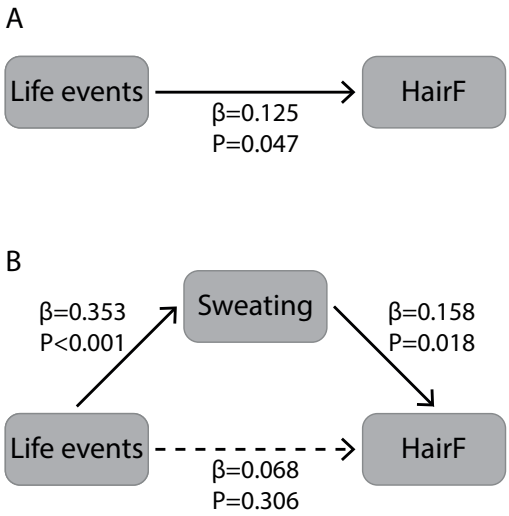

Figure S1. (A) the association between life events and hair cortisol, and (B) a mediation analysis between life events, hair cortisol and frequency of sweating on the scalp
Life events were evaluated using the List of Threatening Experiences (LTE). Analyses were adjusted for age, sex, black hair, brown hair, hair washings, and corticosteroid use
Abbreviations: β, standardized linear regression coefficients; HairF, hair cortisol.