Hair cortisol and cortisone are decreased by natural sunlight

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ABSTRACT

Background and aims: Hair glucocorticoids (cortisol and cortisone) are increasingly used as measures of long-term integrated exposure to glucocorticoid hormones. Glucocorticoids gradually disappear from the hair shaft, which may result from exposure to ultraviolet (UV) radiation in natural sunlight. We aimed to study the influence of sun exposure on hair glucocorticoids.

Material and methods: Scalp hair samples were obtained from nine volunteers (median age 33 [range 21 - 81], 7 females), and part of each hair sample was exposed to three experimental conditions: repeated exposure to natural sunlight for 40 hours (natural UV), exposure to a high amount of artificial UV radiation, and storage in the dark (control). Hair cortisol (HairF) and cortisone (HairE) were quantified by liquid chromatography - tandem mass spectrometry.

Results: When compared to control, HairF was decreased in 9 out of 9 hair samples after natural sunlight exposure (median decrease -3.1 pg/mg or -54%, $P<0.001$) and artificial UV radiation (-4.7 pg/mg or -75%, $P=0.003$). HairE decreased in 8 out of 9 samples, both after natural sunlight (-7.6 pg/mg or -32%, $P=0.012$) and artificial UV (-10.7 pg/mg or -52%, $P=0.026$).

Conclusions: Exposure to natural sunlight decreases the glucocorticoid content of scalp hair, apparently through UV radiation, and is therefore an important confounder that should be considered in studies involving the measurement of hair glucocorticoids.

INTRODUCTION

Over the past decade, the measurement of cortisol and cortisone in scalp hair has emerged as a method to estimate the long-term exposure to glucocorticoid hormones in humans [1, 2]. Increased hair cortisol (HairF) and/or cortisone (HairE) have been linked to psychopathology, physical and psychological stressors, and the cardiovascular risk profile [2]. Furthermore, they are promising markers in the evaluation of endocrine diseases of the HPA axis (e.g. Cushing's syndrome) [3], and may in future prove useful in evaluating changes in long-term glucocorticoid exposure in response to (behavioral) interventions [2].

Various situational factors have been found to influence HairE and HairF, many of which are hair-related, such as hair treatment, hair color and the use of hair products [2, 4-6]. Furthermore, it has been shown repeatedly that hair glucocorticoids show a gradual decrease from proximal to distal hair [7-9]. Possible mechanisms of this 'wash-out' effect include wear and tear of the hair shaft and repeated washing of the hair. The latter explanation is further substantiated by the observation that a higher frequency of hair washing decreases cortisol content [6]. However, steroid hormones are known to be degraded under the influence of ultraviolet (UV) radiation and sunlight [10, 11]. Furthermore, in an experimental design hair cortisol was decreased after artificial UV radiation [12]. It is currently unclear how this photodegradation translates to the physiological situation in which hair is exposed to natural sunlight, and how this influences hair glucocorticoids measured in clinical research.

We hypothesized that exposure to UV radiation present in natural sunlight decreases hair glucocorticoids, and may therefore be a causative factor in the 'wash-out' effect observed. Therefore, we devised an experimental study to evaluate the influence of natural sunlight on hair glucocorticoids, and compared this with exposure to a supraphysiological amount of UV radiation.

METHODS

Participants and procedures

Scalp hair samples were collected from nine healthy volunteers, as close to the scalp as possible, at the posterior vertex. The entire length of each hair sample was divided in three equal parts, in such a way that the entire length of the hair sample was exposed to each experimental condition: the first part was stored in the dark (control condition), the second and third part were taped to a different piece of cardboard to be subjected to

natural sunlight (experiment 1) or extreme amounts of artificial UV radiation (experiment 2). All participants provided written informed consent, and this study was approved by the Institutional Review Board of the Erasmus MC.

Experiment 1: natural sunlight exposure

During 28 days between June 26 and July 28, 2015, hair samples were exposed to natural sunlight, for 60 minutes per day, around solar noon. Hair samples were exposed to a further six hours of sun light on two days, between 11 and 17 pm, on August 29 and 30, 2015. In total, hair samples were subjected to 40 hours of sunlight. On the days of sunlight exposure, the median peak UV index at a nearby location (Bilthoven, The Netherlands) was 6.4, with a range of 4.7 to 7.1 (UV station data based on satellite data (SCIAMACHY, GOME-2). http://www.temis.nl/uvradiation/archives/overpass/uv_Bilthoven_Netherlands_ENS_M8.dat).

Experiment 2: artificial UV light exposure

Hair samples were exposed to UV radiation, using a new 26W 10.0 UVB spiral lamp (Reptile Technologies, Gorinchem, the Netherlands), for a total duration of 137.55 hours, spread over 16 days. The lamp was placed at 3.5 cm of distance from the hair samples. We evaluated the UV radiation of our lamp using a calibrated USB2000+ spectrometer (Ocean Optics, Inc., Dunedin, FL). At 3.5 cm distance, we measured a total irradiance of

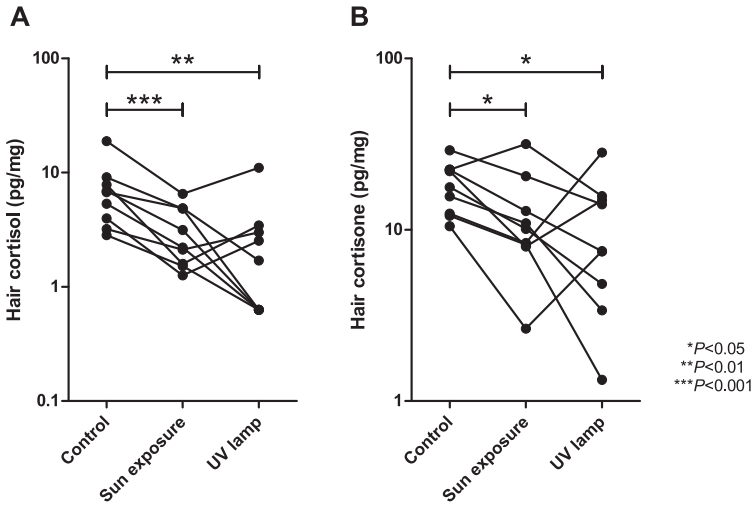

Figure 1. Hair cortisol (A) and cortisone (B) levels in control conditions, after exposure to sunlight, and artificial UV light

Every line represents cortisol or cortisone levels in a hair sample from one individual, plotted on a logarithmic scale. Part of each hair sample was exposed to control condition (storage in the dark), 40 hours of natural sunlight, or 137.55 hours of high-dose artificial UV. Differences between experimental conditions were tested using paired student's t tests.

10817 µW/cm2, most of which was UVA (320 - 400 nm; 7202 µW/cm2) and UVB (280-320 nm; 1195 µW/cm2). These values correspond to a UV index of 56, which exceeds the highest UV index ever measured on earth surface [13].

Hair processing and steroid hormone analysis

After the experiments described above, at least 5 mg of the most proximal 3 cm of hair was weighed for each hair sample. Samples were then processed and analyzed as described previously [9]. HairF and HairE were quantified by liquid chromatography - tandem mass spectrometry using a Xevo TQ-S system (Waters, Milford MA).

Statistical analysis

SPSS statistics version 21 and GraphPad Prism version 5 were used for statistical analysis. Hair glucocorticoid values were log transformed to achieve normality. Differences in hair glucocorticoid levels were tested using paired student's t tests. A P value lower than 0.05 was accepted to indicate statistical significance.

RESULTS

Out of nine subjects, seven were female (78%). The median age of subjects was 33 (range 21 - 81). Three participants had blond hair, three had black hair, and three had grey hair. In the hair samples not exposed to UV light (controls), the geometric mean for HairF was 6.1 pg/mg hair (95% CI [confidence interval] 3.9 - 9.6), and HairE was 17.3 (95% CI 13.3 - 22.6).

Compared to control conditions, exposure to natural UV light decreased HairF in all samples (median decrease -3.1 pg/mg, range -12.4 – -1.1; $t(df=8)=6.28$, $P<0.001$; median percentage decrease -54%; Figure 1A), as did exposure to artificial UV light (-4.7 pg/mg, range -8.5 – -0.2; $t(df=8)=4.30$, $P=0.003$; median percentage decrease -75%; Figure 1A). HairE decreased in all but one hair sample after natural sunlight exposure (median decrease -7.6 pg/mg, range -14.0 – 9.2; $t(df=8)=3.25$, $P=0.012$; median percentage decrease -32%; Figure 1B), and in all but one hair sample after artificial UV light (-10.7 pg/mg, range -15.0 – 15.8; $t(df=8)=2.73$, $P=0.026$; median percentage decrease -52%; Figure 1B). No difference was found between natural and artificial UV exposure for HairF or HairE ($P>0.05$).

DISCUSSION

To the best of our knowledge, this is the first study to investigate the influence of natural sunlight on hair glucocorticoid levels. Although we only studied nine hair samples, our

experiment is well controlled, and showed a consistent decrease in hair cortisol after both natural sunlight and artificial UV exposure in all samples. For hair cortisone, a decrease was shown after both sunlight and artificial UV in 8 out of 9 samples.

Remarkably, the physiologic UV exposure consisted of a relatively low amount of sun exposure of 40 hours, indicating that differences in sun exposure that may occur in the general population, impact an individual's hair glucocorticoid contents. Furthermore, a much higher amount of artificial UV light was not associated with a further decrease in hair glucocorticoids, which appears to indicate that most of the effect was already achieved after the physiological exposure. Our study highlights that sunlight might be an important factor to consider in studies examining hair cortisol and cortisone, and may confound results in such studies. This is especially important when a variable of interest associates with sunlight exposure.

In this light, it is important to consider that season has been associated with hair cortisol levels. We recently reported in a cohort of 760 adults that hair cortisol levels were lower in winter compared to other seasons [6]. This result appears contradictory to the present study, since a decreased UV light exposure in winter would be expected to be associated with higher hair glucocorticoid levels. However, it should be noted that winter season is associated with changes in mood and diurnal rhythm, which may result in an altered HPA axis activity [14].

In our present study, we did not investigate the mechanism by which cortisol and cortisone were decreased. It has been reported that hydrocortisone 21-acetate, an acetate of cortisol, in a methanol solution undergoes degradation under the influence of UVB light [10]. Besides photodegradation, UV induced crosslinking between glucocorticoids and the hair matrix could also contribute to the decrease in the extractable and measurable concentrations of cortisol and cortisone. UV radiation has been shown to induce crosslinking between the corticosteroid flumethasone and the cytoskeletal protein spectrin [15]. A similar mechanism could induce crosslinking between endogenous glucocorticoids and hair matrix components.

In conclusion, we showed that not only an extreme dose of UV light, but also a repeated sunlight exposure decreases the cortisol and cortisone content of scalp hair. Researchers should therefore be aware of exposure to natural UV light as a potential confounder in studies investigating hair glucocorticoids.

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