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ORIGINAL RESEARCH

Diurnal Variation in Peripheral (Hair) vs Central (Saliva) HPA Axis Cortisol Concentrations

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Abstract: Cortisol concentrations in hair and saliva collected from male and female adults over a 15-hour period were compared for differences in overall level and cyclic pattern. Typical diurnal fluctuations were noted for both salivary and hair cortisol, with some individual differences that are congruent with the previous literature. Issues of the link between central and peripheral HPA axes are raised for discussion and further investigation, and hypothetical explanations for the diurnal variability shown in these two sets of cortisol secretion patterns are discussed from an evolutionary advantage perspective.

Keywords: cortisol, hair, diurnal, peripheral HPA axis

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Introduction

In its role as the principal glucocorticoid in humans, cortisol performs vital anti-inflammatory and immunosuppressive functions,[1–3] as well as a host of other effects that assist metabolism and homeostasis.[4] Although a background concentration is present at all times,[4] cortisol is elevated during times of physical or mental stress.[5,6] In addition, cortisol may exhibit a diurnal fluctuation in many persons,[7] typically with a peak concentration early in the morning (e.g. about 8.00 am) and a minimal concentration later in the afternoon or early evening.[8] However, this diurnal fluctuation is not present in everyone, with 17% of some samples showing a lack of variation over the day-night period,[8] about 30% showing variation in their diurnal cycle from day to day,[8] and up to 50% showing depression of that cycle due to a range of influences.[9] The precise points of maximum and minimum cortisol concentrations have also been shown to vary somewhat across individuals[8] (although the former is usually early in the morning and the latter later in the afternoon), thus rendering the presence of a "typical" diurnal fluctuation difficult to define. When it does occur, this diurnal fluctuation has long been considered as "adaptive"[10] in the same way that other circadian-driven physiological variations influence survival behavior by maximizing the use of physiological resources,[11] perhaps developed via evolutionary selection processes.[12]

Produced from a cascade in the hypothalamic-pituitary-adrenal (HPA) axis that commences with secretion of corticotropin releasing hormone (CRH) in the hypothalamus and then adrenocorticotropic hormone (ACTH) in the pituitary, cortisol is synthesised from cholesterol in the adrenal cortex and enters the bloodstream about eight minutes after CRH is secreted by the hypothalamus.[4] As well as being measured in blood, cortisol can also be assayed from saliva[13] and urine.[4] Cortisol produced by the adrenal glands influences the organism's preparedness for activity by gluconeogenesis, mobilization of fats and proteins from the liver and other cells, and reduction of glucose consumption by cells (leading to elevated blood glucose and stimulation of insulin).[4] Cortisol also assists survival by stabilizing lysosomal membranes, decreasing capillary permeability, depressing the immune system and reducing fever, plus making a multiple assault on inflammation via at least 17 different pathways.[14] It is therefore of particular interest when considering the latter set of capabilities to note that cortisol is also secreted in melanocytes[15,16] and hair follicles[17–24] and may perform similar immunological functions at a localized level[15] as it does when secreted from the adrenal glands to the general bloodstream.

As well its end product, the entire CRH-ACTH-cortisol sequence is replicated in melanocytes[15] and hair follicles,[25] pointing to the existence of a "peripheral" HPA axis in skin and hair[15,16,26,27] that may be related to, or independent from, the "central" HPA axis. The concentration of cortisol in hair has also been shown to vary in response to physical and psychological stressors in the same fashion as cortisol from the "central" HPA axis, although not in ways that would reflect a single connected HPA axis.[22–24] However, because peripherally-secreted cortisol has not yet been shown to enter the general bloodstream and contribute to those effects which central cortisol has upon metabolism and preparation of the organism for activity, any benefit it may convey upon the organism would most likely be in terms of its local anti-inflammatory properties, thus further demarking the peripheral HPA in terms of its effects upon behavior. The presence of a link between the central and peripheral HPA axes has been discussed by several sources,[15,26] with one recent comment[15] that "one of the major, as-yet unmet challenges in cutaneous stress research …(is)… the study of the cross-talk between peripheral and systemic (cortisol) responses" (p. 1697). That is, if there is a lack of consistency in cortisol concentrations from the peripheral and the central HPA axes, it could be concluded that they were independent sources of cortisol as suggested by Ito and colleagues,[25] a finding which would confirm hair cortisol as playing a different role to that exhibited by central cortisol in terms of behaviour and survival.

Some previous data support this hypothesis of the independence of the peripheral HPA axis by showing that cortisol responsivity to a local pain stressor applied to one hand was immediate and transient in the same arm hair, but restricted to the local area (i.e. not in the hair from the opposite leg).[24] Central cortisol reactions were also found in saliva, but about 20 minutes afterward, as expected.[6,13] That is, had central cortisol responses to the pain stressor been responsible for the cortisol responses found in hair, then the latter

should have occurred at least eight minutes after the onset of the pain stressor, rather than immediately (as was noted) or about 20 minutes later (as found in saliva). These findings suggest that the peripheral HPA axis may be uncoupled from the central HPA axis, and even be uncoupled from other HPA sites on the skin. However, those data were collected during administration of a pain stressor (ice water immersion for 1 min) which subjects described as very painful. While there may be evidence of a "regional uncoupling" of hair cortisol under stressor conditions, it remains to be seen if the same discrete responses are present under non-stressful conditions.

One way to assess the degree of linkage between central and peripheral HPA axes while subjects engage in normal daily activities is to collect hair and saliva during a day and determine (a) the existence of the diurnal fluctuation in hair cortisol and (b) whether such a fluctuation coincides with the cycle in the central HPA axis. In order to ensure a more fine-grained data-collection procedure than possible from a single observation (as reported in a previous study of "24-hour urine"),[20] and thus determine if peripheral cortisol concentrations were correlated with central cortisol diurnal fluctuations,[4] both hair and saliva should be collected at regular intervals during a typical daily period. Therefore, as a pilot investigation of the presence and nature of diurnal variation in cortisol within scalp hair, the present study measured cortisol from scalp hair samples collected simultaneously with saliva at set three-hour intervals during a 15-hour (waking) period to determine the relationship between central and peripheral HPA axes' cortisol production while the subjects were not under pain stress.

While previous studies of central cortisol have shown frequent cycles of cortisol elevation and decrease,[7] reflecting the value of very frequent collections of serum (every 20 min), that procedure was not considered appropriate here because of (a) the exploratory nature of this study and (b) the extreme unlikelihood of collecting saliva and hair samples from sleeping subjects without arousing them and thus confounding the findings due to fatigue and/or stress occasioned by frequent waking. It is worth commenting that very early explorations of central cortisol[10] also collected data at similar frequencies as that employed in this study, and this methodology may suffice at this stage of investigation of this phenomenon. Further, while it is the common procedure in much research, the use of large group research designs and group-based statistical analyses may be unjustified under two circumstances.[28] First, when exploratory studies are being conducted, ethical constraints may preclude applying the experimental procedure to the large groups of subjects that are required for adequate statistical power. Second, as mentioned above, the presence of diurnal variation in "central" HPA axis cortisol concentrations is not consistent across subjects, with about 17% of people not showing the early morning elevation and late afternoon depression in cortisol measured from blood, urine or saliva, and a further 50% being likely to experience fluctuations to that typical diurnal variation because of a range of factors.[9] Therefore, as an initial exploration of the presence of a diurnal variation in peripheral (hair) cortisol that might be parallel to that from central (saliva) cortisol, an $n = 1$ experimental design protocol was adopted.[28] Under such a design, group mean data (and relevant statistical procedures) are considered to be less important than individual subject responses as a means of forming an initial understanding of the phenomenon under scrutiny so that further (later) group studies may be conducted.

Methods

Participants

Two healthy young females aged 18 and 20 years, plus one healthy male of 21 years and the first author (to provide a comparison with an older person, age = 61 yr) volunteered to collect saliva and hair samples (of about 50 strands) at the hours of 6.00 am, 9.00 am 12.00 pm, 4.00 pm, 6.00 pm and 9.00 pm.

Sample collection and assay

Saliva was collected via subjects (Ss) using a separate and labeled (subject, time) Salivette (Sarstedt) for each time period and stored at –20 C until assayed. Hair was collected by Ss cutting with scissors from as close to the posterior vertex as possible and then placed in a labeled (S, time) paper envelope. After being emptied from the envelopes and placed in separate glass vials (20 ml), hair was weighed and then chopped with scissors (washed with methanol between chopping samples) before being extracted with 3 ml of methanol for 24 hours. The methanol was then decanted into

polypropylene tubes (3.5 mL) and evaporated under vacuum. Gel buffer (100 µL) (phosphate buffered saline, pH 7.5 containing 0.1% gelatin) was added and the solution allowed to stand at room temperature for 60 minutes prior to assay. Cortisol concentrations in both saliva and hair were determined by radioimmunoassay as previously described.[29]

Procedure

Ss were instructed in the sample-collection and storage procedures in a group. Samples were to be collected by the Ss themselves at the hours indicated above. All samples were returned to the second author within a few days and then frozen until assay. Previous examination of hair samples has shown that they may be stored at room temperature for at least a year with no effects upon cortisol concentration.[20] Similarly, saliva may be stored at room temperature for at least two weeks with no effects on stability.[30] All procedures were approved by the University of New England Human Experimentation Ethics Committee.

Results

In order to compare the cortisol concentrations from the six sample periods across each of the four Ss, each S's first sample (i.e. taken at 6.00 am) was set as a standard of 100%, and the remaining concentrations were compared to that value for each S. As an overview, Figure 1 shows the mean hair and saliva percent cortisol concentrations for the combined four Ss across all sample times, and suggests that the previously-reported diurnal fluctuation in central cortisol concentrations (i.e. saliva) were replicated in these *group mean* data, with a peak in the morning (9.00 am: 154.9%), then a drop to 90.7% at 12.00 pm and remaining at about that level for the rest of the sample period (to 9.00 pm). Cortisol concentrations in scalp hair also showed the same morning peak (9.00 am: 207.5% of 6.00 am) and a drop to 130.5% at 12.00 pm, a plateau until a further drop at 6.00 pm (88.1%) and then a rise at 9.00 pm (143.7%).

However, Figure 1 presents only mean cortisol percentage data and, as mentioned in the introduction to this paper, there are marked variations in the presence, consistency and peak/low concentration times across persons. The presence of such variability suggested that the principal examination of the data obtained from this exploratory study should be performed on an individual subject basis (i.e. from an $n = 1$ methodology perspective). Therefore, each S's hair and saliva cortisol concentrations were plotted separately and are presented in Figure 2.

It is apparent from Figure 2 that the kind of individual differences that might be expected from the wider literature[6,8,9] were also present across the four Ss in the pattern of diurnal fluctuations shown for saliva and hair cortisol. S1 showed the typical

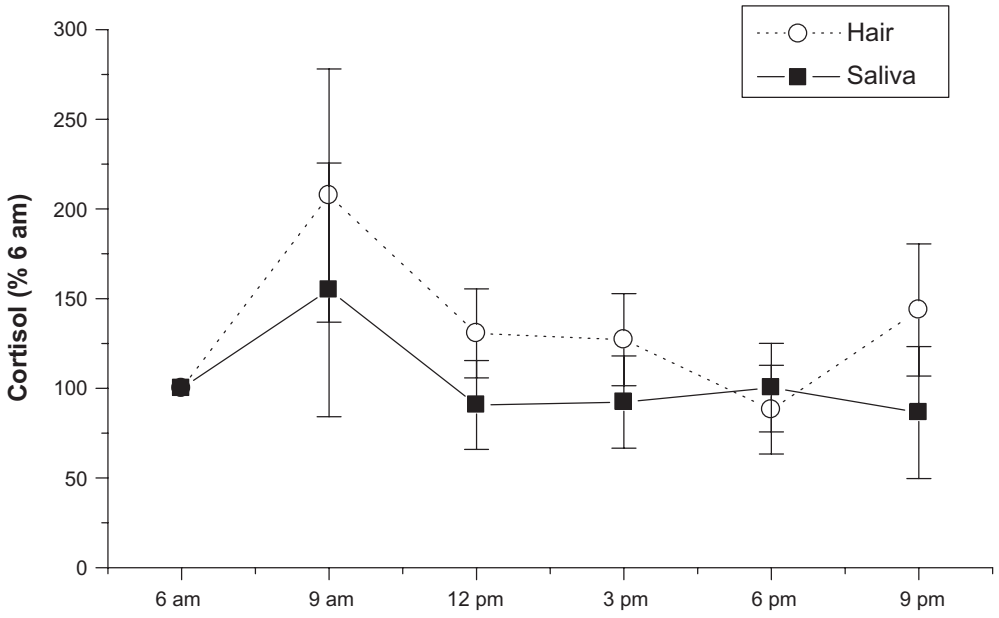

Figure 1. Mean and SE hair and cortisol concentrations for all Ss over time as% of initial (6.00 am) values.

peak cortisol concentrations for both saliva (316.7%) and hair (293.4%) at 9.00am, followed by a drop to 12.00 pm (saliva = 146.5%, hair = 169.6%) and then a gradual rise for saliva at 9.00 pm (193.6%). By contrast, hair cortisol dropped again from the 12.00 pm values to 136.2% at 6.00 pm and then rose at 9.00 pm (209.3%).

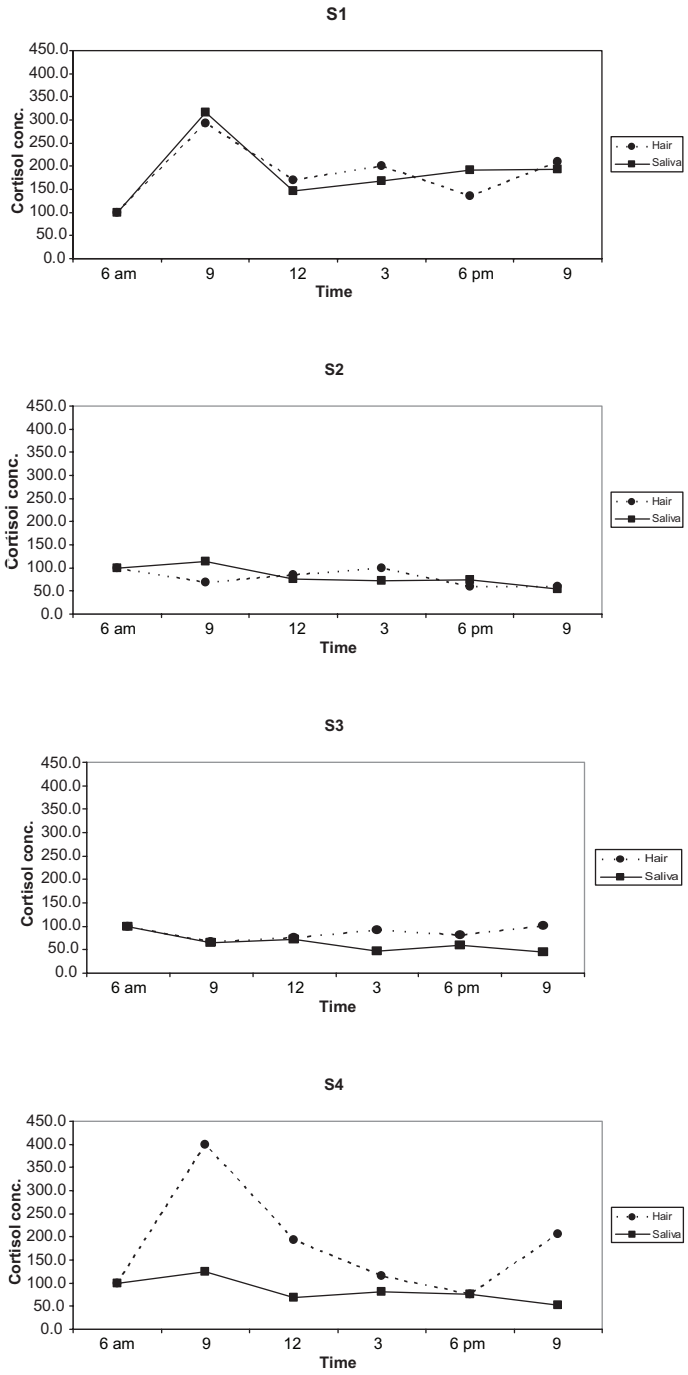

Figure 2. Individual Ss' hair and saliva cortisol concentrations over time as a percent of initial (6.00 am) values.

S2 showed the expected pattern for saliva, with a minor peak at 9.00 am = 113.5%, followed by a gradual decrease to a nadir at 9.00 pm = 54.4%) but not for hair, with a pattern for the latter that was almost the inverse of the former. The peak hair cortisol concentration for this S was 100% at 6.00 am and did not rise above this during the rest of the day, although the drop to 69.1% at 9.00 am and subsequent rise to 99.7% at 3.00 pm was suggestive of a bimodal pattern. Overall, this S's cortisol concentrations did not show the same degree of variability across the 15 hours of the study as S1.

S3 showed a peak saliva cortisol concentration at 6.00am, and generally declined gradually after that to the minimum value at 9.00 pm (45.6%). Hair cortisol concentration variations were also fairly flat for this S, with two very minor peaks, one at 6.00 am (100%) and another at 9.00 pm (100.8%), following a nadir at 9.00 am (67.6%).

S4 (the first author) showed a peak in salivary cortisol at 9.00 am (124.1%) followed by a fairly flat set of cortisol concentration values to a minimum value of 52.5% at 9.00 pm. Hair cortisol values were quite different for this S, with a dramatic increase from 100% at 6.00 am to a peak of 399.9% at 9.00 am, then a gradual decrease to 75.9% at 6.00 pm, followed by a rise to 143.0% at 9.00 pm.

Discussion

The group mean data on salivary cortisol approximated the expected single daily peak during the early morning for three of the four Ss, followed by either gradual decreases in concentrations for the rest of the day (in three Ss) or a drop to 12.00 pm and a slight decrease to the end of the day (in one S). However, group mean hair cortisol concentrations for all four Ss showed a bimodal pattern, with two peaks, one at either 6.00 am or 9.00 am and another at 9.00 pm (in three Ss) or 3.00 pm (in one S). Although the mean pattern of salivary cortisol fluctuation found here was consistent with that reported in the literature,[4,7,10] with a peak during the morning followed by a drop three hours later and then a fairly flat plateau during the rest of the day, mean hair cortisol data showed evidence of a bimodal peak in concentrations, the first at 9.00 am and the second at 9.00 pm. The central cortisol fluctuations in saliva did not show the expected steep decline during the afternoon that has been reported in

some other studies,[4,7,10] perhaps suggesting that this group of Ss experienced some exogenous stressful stimuli during the afternoon. Hair cortisol data did show that steep decline during the afternoon, with the lowest concentration being clearly at 6.00 pm.

However, while group mean data are valuable in describing general trends within large samples, they do not amplify the range of responses present within a sample and which have been previously reported in the literature.[8,9] Figure 2 shows those inter-subject differences clearly, and supports the previous literature that found variability between persons in the diurnal fluctuation pattern that was mentioned in the Introduction to this paper. Up to 17% of some samples have shown no detectable diurnal cycle,[9] 30% have inconsistent diurnal variations from day to day[8] and up to 50% have shown major variations to the standard fluctuation pattern.[8] Factors that have been shown to influence this departure from the typical diurnal cycle pattern include depression,[31] relationship functioning,[32] prenatal anxiety,[33] smoking,[34] financial strain,[35] stress and fear,[36] relative dominance-submissive position with a social group,[37] over-the-counter medications,[38] and even time of awakening.[39] Some of these factors may have been present in this sample.

Those previous reports referred to central HPA axis-produced cortisol. The current data are the first to report on the diurnal fluctuation in cortisol produced by the peripheral HPA axis in hair follicles, and the presence of inter-subject differences in this source of cortisol adds to an understanding of the peripheral secretion of this important hormone. That is, just as central cortisol diurnal fluctuation patterns vary (and are even absent in about 17% of people) across individuals, so do peripheral cortisol concentrations show some differences between persons also. The presence of inter-subject variability in both central and peripheral cortisol argues for the presence of exogenous factors in the determination of cortisol concentrations produced from the adrenal glands and also from hair follicles. While some of these that influence central cortisol secretion have been suggested above, many do not easily apply to the production of peripheral cortisol. That is, the central HPA axis is integrally involved with overall physiological regulation of many functions, and (for example) taking medications, time of awakening and psychological factors such as financial factors and depression may have direct impact upon the hypothalamus, pituitary and adrenal glands because these are key homeostatic agents for the body. However, a similar functional response in hair follicles to depression or financial stress is not so easily linked with overall functioning, and may be related to other, more localized, functions.

For example, the diurnal fluctuation in central cortisol is a function of the influence of daylight upon specialized light sensitive retinal ganglia that express the photopigment melanopsin and project directly to the suprachiasmatic nucleus in the hypothalamus, creating a "circadian clock" which generates higher cortisol levels during the early part of the morning and lower concentrations during the later afternoon and evening.[40] Described by Yates and Urquhart[10] as "adaptive", the assumed selective benefit of increased cortisol secretion in the early hours of daylight may be in terms of the effects of (central) cortisol release upon the organism's ability to move from the relative inactivity of sleep to daily activity. That is, many aspects of the body's functioning are in train to the circadian rhythm or "clock",[11,12] and cortisol has been described in this fashion.[40] As well as preparing the organism for increases in general activity levels following sleep, some of the particular physical demands faced by ancestors of *H. sapiens*' may have included foraging and general food-gathering, plus hunting. That is, under this hypothesis, the gluconeogenesis, protein mobilization, fat mobilization and associated activation effects of central cortisol secretion may be congruent with increased physical work and demands undertaken by hungry primates during the early morning.

By contrast, in terms of peripheral secretion of cortisol in hair, it is relevant to note that the central circadian clock mentioned above that controls the diurnal cycle of cortisol secretion from the central HPA is not the only such clock. In fact, it is replicated throughout the body by expression of clock genes,[41] even in non-neural tissue.[42] These peripheral clocks may function to modulate physiological dynamics in the particular tissue where they occur by secretion of selected hormones such as cortisol.[40] In this way, the difference of the hair cortisol diurnal fluctuation pattern from that shown by salivary cortisol may reflect the latter being controlled by a "local" circadian clock in ways that have particular survival value for the organism. Following the evolutionary-benefit hypothesis suggested for central cortisol diurnal cycling (and all

circadian rhythms), the survival benefit of peripheral cortisol at mid-morning (9.00 am) and mid-evening (9.00 pm) may be unrelated to those centrally-activated physiological responses (such as gluconeogenesis and mobilization of fat and protein) which prepare the organism for major physical activity and demand early, if only because the cortisol secreted in hair follicles has not yet been shown to reach the general bloodstream.[25] Instead, hair cortisol appears to be restricted to the particular site of its release, and does not generalize across body sites.[43] Therefore, any survival benefit from the release of hair cortisol is most probably restricted to those anti-inflammatory effects previously described.[14] These might be of particular value in the skin if wound punctures were experienced, especially if they were from animals' claws during hunting which might be expected to have significant pathological consequences.[44] Alternatively, the peak hair cortisol at mid-evening may coincide with the need for anti-inflammatory effects to skin during the maximum period of UV radiation received during the day and consequent skin damage and inflammation.[45,46] Thus, the comparative differences in the diurnal variation patterns shown by central vs. peripheral cortisol might be linked to the effects of cortisol secretion in each of these venues at particular times, plus the reproductive advantages which its release then and there might confer upon species that showed such responsiveness.

However, this study is exploratory and therefore this hypothetical explanation for the observed differences between the diurnal cycle patterns of central and peripheral cortisol remains untested at this stage. Further, because all the hair collected in this study was from the posterior vertex of the skull, comparisons with the diurnal fluctuation pattern shown by hair from other body sites was not possible. Recent data have shown that hair from different body sites (arms, legs) does show significantly different concentrations of cortisol, although those data were collected at a single point in time (early afternoon),[42] and replication of the present study of diurnal fluctuations in cortisol concentrations in hair from different body sites would help clarify these findings.

As acknowledged in the Introduction, this study was exploratory and used a very small sample, applying the relevant $n = 1$ methodology. On the basis of these data, further research with a larger sample is suggested to enable the application of group-based statistical procedures that might produce more informative data regarding the overall group mean trend for diurnal fluctuation in hair cortisol concentrations. However, such a study would have to accommodate the findings from the previous literature regarding quite large between-subject variations in the presence, consistency and timing of cortisol diurnal fluctuations. Therefore, as well as increasing subject numbers, replications of the current study should also conduct analyses of individual subject variability as has been done previously with such revealing outcomes.[8] Finally, although several factors which impinge upon cortisol and diurnal fluctuations were mentioned above (e.g. mood, medication, financial and social status, fear and stress, etc.), few data have been reported as yet regarding the influence that any of those factors might have upon hair cortisol production *per se* and none upon their effects on diurnal fluctuation of peripheral cortisol in particular, and this remains a fruitful avenue for future research.

Disclosures

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors report no conflicts of interest.

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