

# Physiological Research Pre-Press Article

Hair cortisol concentration differs across site and person: Localisation and consistency of responses to a brief pain stressor.

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Running title: Hair cortisol responses

### Summary

Although *in vitro* studies have shown that cortisol concentrations in human and animal hair respond to environmental stressors, few data have been reported regarding the *in vivo* variability of hair cortisol to brief pain stressors. As an extension of a previous study, hair was collected and assayed for cortisol concentrations from each of three sites (elbow, mid-forearm, wrist) before and after participants immersed their hand in ice water for 1 min. Results showed that the “localization” boundary of hair cortisol responses previously reported was able to be reduced to only 250 mm between sites. Further, all participants showed considerable variability in hair cortisol across the three sites at each collection period, although consistency across participants in overall responsiveness of hair cortisol to the pain stressor was observed.

Key words: cortisol, hair, stress, peripheral HPA axis

## Introduction

Cortisol is the principal glucocorticoid in humans and has vital anti-inflammatory and immunosuppressive effects on all tissues including the skin (Klaiman & Almog, 2003; Weissman & Thomas, 1963). As part of the hypothalamic-pituitary-adrenal axis (HPA), cortisol is produced by the adrenal cortex following ACTH stimulation from the pituitary and is regulated by the hypothalamic peptide CRH which arises in the hypothalamus (Aron, Findling & Tyrrell, 2007). Although a basal concentration of cortisol is required at all times (Guyton & Hall, 2006), various physical or mental stressors can elevate central cortisol concentration (Dickerson & Kemenyi, 2004; Sharpley & Mclean, 1992).

Cortisol has traditionally been assayed from serum, urine and saliva in various studies as a measure of physiological response to stress (Umeda et al, 1981) but has recently been shown to be synthesised in skin (Arck et al, 2006) and hair (Davenport et al, 2006; Klein et al, 2004; Koren et al, 2002; Raul et al, 2004; Sauve et al, 2007; Van Uum et al, 2008; Yamada et al, 2007; Yang et al, 1998; Wheeler et al, 1998). The production of cortisol in the hair follicle, as evidenced by its presence along the hair shaft, is particularly interesting because it has been assumed that the hair shaft above skin level is dead (Harkey, 1993). However, some studies have challenged this view by showing that sex steroid levels within the whole hair shaft vary in parallel with serum steroid concentrations during the menstrual cycle in women (Yang et al, 1998). These concentrations are not affected by washing of the hair, which has been shown to remove less than 7% of the total hormone extracted (Cirimele et al, 2000; Raul et al, 2004).

These findings have established the existence of a “peripheral” HPA axis in skin and hair (e.g., Arck et al, 2006; Paus et al, 2006; Slominski et al, 2005). That is, as well as being *targets* for HPA axis activity, skin and hair are also *producers* of cortisol via their own neuroendocrine systems which include CRH, POMC, ACTH (Aron, et al, 2007) and cortisol itself, all of which have been shown to be secreted by human hair follicles (Ito, et al, 2005; Kalra, et al, 2005; Klein, et al, 2004). However, much of the data supporting this model were collected from *in vitro* studies which used expurgated human hair follicles in which the secretion of cortisol in response to ACTH stimulation was delayed by at least 48 hours (Ito et al, 2005). In addition, where hair cortisol concentrations have been shown to be linked to stress, data were gathered from comparison groups (e.g., depressed vs non-depressed pregnant women: Kalra, et al, 2005) or from a single collection (babies vs adults: Klein, et al, 2004) over relatively static periods of time. Although valuable in clarifying the presence of hair cortisol concentration differences between stressed and non-stressed individuals, those data do not indicate how quickly hair cortisol concentrations change in response to stressors, nor whether the cortisol response is general or localized to the site of the stressor (e.g., in the case of pain).

Therefore, in a previous paper, we described the timing and the localization of hair cortisol responses to a brief pain stressor by comparison of data collected from hair on the site of pain (a participant’s hand which was immersed in ice water) and their opposite lower leg (no immersion or pain) (Sharpley, Kauter & McFarlane, 2009). Hair cortisol concentrations increased dramatically by over 320 % and almost immediately (within one minute) of participants immersing their hands in ice water, much faster than the

commonly-reported 18 to 20 min lag between onset of stress and salivary cortisol concentration increases (Sharpley & McLean, 1992). These hair cortisol responses were brief, lasting for less than five minutes after the pain stressor was removed. In addition, while the immersed hand showed increases in hair cortisol concentrations, the opposite (non-immersed) lower leg did not, suggesting that the hair cortisol response was localized. Finally, by reference to salivary cortisol data collected during that experiment, it was clear that the “central” and “peripheral” cortisol responses were independent of each other.

However, the distance between an individual’s forearm and lower opposite leg is considerable and, while showing that there is localization of hair cortisol response to pain, differences in hair cortisol responses between these two sites do not indicate the degree of localization of those responses. Therefore, in the current study, we gathered hair cortisol data from three sites along the forearms of five men who immersed only the hand of their selected forearm into ice water. These sites are described below, but were within 250mm of each other, thus allowing for a more precise test of the degree of localization in hair cortisol responses to a pain stressor.

## Methods

### Participants

Data were collected from five healthy males, none of whom was taking medication. Ages were 22, 35, 52, 54 and 61. All were volunteers for “a study about how you react to ice water”.

### Stressor task

Mild pain was evoked by use of the Cold Pressor Test (CPT). In this procedure, participants immerse their hand in ice water at 1<sup>0</sup> to 4<sup>0</sup> C for 1.0 min. The CPT has been shown to induce sympathetic responses, including cortisol (Sharpley, Kauter & McFarlane, in press) but does not cause any injury.

### Sample collection and Assay

Hair was collected by shaving samples of about 6 cm<sup>2</sup> from three sites on the nondominant forearm of each participant. The first site (wrist) was at the head of the ulna, extending towards the elbow for 45 mm; the third site (elbow) was at the head of the radius, also extending for 45 mm, but towards the wrist; the second site (mid-arm) was centered on the midpoint of the distance between the head of the ulna and the head of the radius, and extended for 22.5 mm either side of this midpoint. Each of these three sites was bounded by two parallel lines drawn across the forearm and 45 mm apart and each site was further divided into three equally-sized sub-areas so that each of the three sub-areas was parallel to the arm itself and named *a, d, g* (wrist), *b, e, h* (mid-arm) and *c, f, i* (elbow). These sub-areas were sampled sequentially for each of the three sites as described below.

After shaving, each razor and the hair collected was placed in a labeled 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 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 buffer [0.05 M], saline [0.15 M], pH 7.5 containing 0.1 %

gelatin) were added and allowed to stand at room temperature for 60 minutes prior to assay. Cortisol concentrations were determined by radioimmunoassay as previously described (Yuen et al, 2004).

### Procedure

Each participant was treated separately. After greeting and signing of appropriate consent forms, each participant sat quietly and read a recent issue of *New Scientist* for 10 min. After this period, the first hair samples were taken from sites *a*, *b*, and *c* by the first two authors acting in concert and the razors and hair were stored in their envelopes. These samples were labeled “Rest”. The participant was then asked to insert his hand in ice water ( $1^{\circ}$  to  $4^{\circ}$  C) for 1.0 min so that the entire hand was immersed to the head of the ulna but short of the first hair sample area. Immediately after withdrawing his hand and having it quickly dried, the second set of samples (sub-areas *d*, *e*, *f*) was collected (“CPT”) and stored and the participant was asked to resume reading. After nine min of reading, a further sample was collected from each site (*g*, *h*, *i*) (“Post-CPT-Rest”) and stored. Participants were then thanked and given AUD\$10.00 for taking part in the experiment. All procedures were approved by the University of New England Human Experimentation Ethics Committee.

### Results

Figure 1 shows each of the five participants’ cortisol data from the three sites at each of the three sampling periods. Several findings emerge from these graphical presentations of the hair cortisol data. First, each participant showed variability in hair cortisol concentrations across the three sites after 10 min of silent reading (Rest), again after CPT and also after Post-CPT-Rest. Second, variability in terms of hair cortisol

concentrations was present between participants. Third, four (P: 1, 2, 3, 5) of the five participants showed immediate increases in hair cortisol from Rest to CPT on at least one of the three sites sampled (wrist, mid-arm, elbow), with two (P: 3, 5) showing increases at two sites. Further, at the Post-CPT-Rest sampling, all five participants showed increases in hair cortisol on at least one site, two sites (P: 1, 3) or all three sites (P: 2, 4).

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Insert Figure 1 here

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### Discussion

The variability in hair cortisol collected from the same sites between participants **may be expected**, since cortisol concentrations (like other glucocorticoids) reflect individual responsiveness to external stimuli. **Variability in hair cortisol concentrations within participants** and between sites was found for all participants on all three experimental data collection periods (after rest, immediately following CPT and after nine further minutes of rest), suggesting **that the synthesis of cortisol in specific hair sites may be relatively independent from that in other sites.**

While previous data showed that the peripheral cortisol production (in hair) was independent of the central HPA cortisol production (in saliva) and also that the stressed forearm showed different hair cortisol responses to hair cortisol from the opposite and unstressed lower leg, the present data suggest that even quite close hair cortisol production sites (i.e., within 250 mm of each other) **may** act independently of each other in the absence and presence of stressors. This “localization” effect may be more finely



apparent than shown here, and further data need to be collected to test the limits of this phenomenon within closer distances than those measured in this study.

Following from the variability shown across sites and between participants, the pattern of responses to CPT was consistent with that which might be expected from an ideographically-based glucocorticoid system. That is, while four of the five participants showed immediate increases in cortisol concentrations in hair from at least one of the nearby sites (i.e., wrist, mid-arm, elbow) and all five participants showed elevations in hair cortisol by nine minutes after the end of the CPT immersion, there was **an indication of some** variability across participants' hair cortisol concentrations from the three sampling sites during each of the three data collection periods, as well as in the number of sites which showed cortisol elevations. These data **may provide** support the hypothesis of individual differences in hair cortisol responses suggested from the data discussed above, plus the finding that CPT produces uniform (across participants) hair cortisol elevations within a reasonable time (i.e., 10 min) after the stressor onset.

In our previous paper, we collected data at more frequent intervals (every 5 min for 30 min) in order to compare forearm with opposite leg hair cortisol responses and salivary cortisol, but the sites of hair collection were much larger than those used herein because eight samples needed to be collected rather than three, necessitating a larger area to be shaved. In fact, in order to collect sufficient hair for eight assays of hair cortisol and compare them with data from the opposite leg, the arm sampling area in that study spread over the whole of the three sampling areas used in this study, and therefore the variability noted between the wrist, mid-arm and elbow sites in this study may have been overlooked in previous research.

These data **provide some support for previous data on** the responsivity of hair cortisol concentrations to relatively brief pain stressors and **suggest that this** glucocorticoid activity **may be** relatively locally-oriented. While further investigations may enable the localization boundaries to be reduced, these data suggest that areas of 250 mm **may** represent separate response sites which may have related activity to a pain stressor. CPT (and other pain) may have different effects upon different sections of the forearm across individuals so that the variability shown here may reflect an overall consistent, but locally variable, response to a site-specific stressor, and this is currently being investigated.

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Figure captions

Figure 1: Hair cortisol concentrations across three sites prior to and following CPT of Participants' hands.

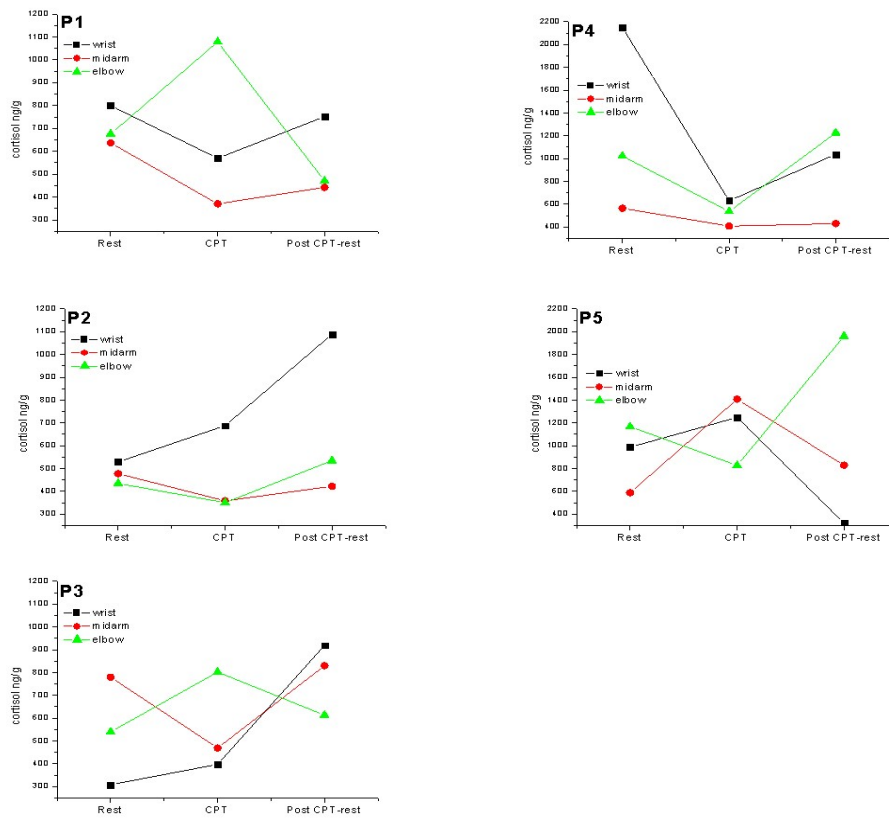


Figure 1: Hair cortisol concentrations across three sites prior to and following CPT of Participants' hands.