

Concerns regarding hair cortisol as a biomarker of chronic stress in exercise and sport science

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Abstract

Hair cortisol has the potential to fill the methodological void of long-term cortisol assessment while becoming a widely accepted measure in biopsychology. This review critically examines the applicability and relevance of hair cortisol measurement specifically within the field of exercise and sport science. Current measures of the HPA axis only cover a brief time period, whereas hair cortisol is a unique, non-invasive means to capture long-term cortisol secretion. Studies have shown that individuals who have elevated cortisol secretion (e.g. due to diseases associated with a disturbed activation of the HPA axis or exposure to stressful life events) reveal increased hair cortisol. By contrast, only weak correlations exist between hair cortisol and perceived stress, and the direction of the relationship between hair cortisol levels and mental disorders is unclear. Acute exercise, however, results in increased levels of cortisol that eventually is reflected in higher levels of cortisol in hair samples and studies have shown that exercise intensity is related to hair cortisol level. Thus, elevated hair cortisol levels found among regular exercisers are not necessarily pathological. Thus, one should practice caution when associating athletes' elevated hair cortisol with poor mental health or disease. Hair cortisol analysis can contribute to a more complete understanding of how long-term cortisol elevation mediates stress-related effects on the health and performance of recreational exercisers and elite athletes. Nevertheless, it is crucial for exercise and sport scientists to consider whether their research questions can be adequately addressed, given that regular intense exercise results in substantially augmented hair cortisol levels.

Key words: Exercise, hair cortisol, physical activity, review, stress.

Introduction

During the last ten years, hair cortisol measurement has become an increasingly accepted approach in endocrinology and biopsychology. Scholars have argued that hair cortisol measurement has the potential to fill the methodological void regarding the assessment of long-term cortisol levels (Gow et al., 2010; Stalder and Kirschbaum, 2012). Hair cortisol has been described as an indicator of chronic stress exposure, and studying chronic stress has a long-standing tradition in exercise and sport science. As a biomarker of chronic stress, hair cortisol could provide supplementary and objective insight beyond self-reported accounts of stress. In exercise and sport science, hair

cortisol could be used by researchers interested in stress fractures (e.g. Fredericson et al., 2005), injury (e.g. Albinson and Petrie, 2003), sources of organizational stress (e.g. Fletcher and Hanton, 2003), competitive stress (e.g. Mellalieu et al., 2009), or the interplay between stress, recovery, overtraining and burnout among elite athletes (e.g. Kellmann, 2010). Hair cortisol might also be useful for discerning the relationships between stress and health-related behaviors (e.g. Lutz et al., 2010), exercise-based stress-buffer effects (e.g. Gerber et al., 2010), and the effects of exercise on patients suffering from stress-related disorders (e.g. de Assis et al., 2008).

Moreover, using a biopsychological perspective to examine various phenomena in athletic populations and recreational exercisers has become increasingly popular during recent years (Acevedo and Ekkekakis, 2006; Ehrlenspiel and Strahler, 2012). It is well known, however, that intense exercise results in substantial increases of salivary and plasma cortisol concentrations (Luger et al., 1988; Borer, 2003), and a recent study by Skoluda and colleagues (2012) is the first to reveal increased hair cortisol levels in endurance athletes compared to controls. More importantly, they showed a dose response relationship between training volume and hair cortisol levels. This pioneer study on hair cortisol in athletes raises some important issues regarding the complexity of interpreting cortisol data in both elite athletes and recreational exercisers. A large part of the retrospective cortisol level measured in hair is likely due to repeated cortisol elevations associated with vigorous exercise participation and, to a lesser extent, cognitive, emotional or psychosocial stress exposure.

Hair cortisol can be used as a retrospective calendar of cortisol levels (Detterborn et al. 2010; Russell et al., 2012). However, even though hair cortisol measurements offer great possibilities in stress research, using this method in exercise and sport science, and particularly with elite athletes, introduces a new challenge regarding the interpretation of cortisol levels. Another issue to consider is that increments of cortisol due to metabolic demands associated with exercise-based stress are different with regard to physiological and metabolic load and presumably also differ regarding the adverse consequences for health when compared to what has been described for cognitive, emotional or psychosocial stress or for different diseases resulting in increased levels of cortisol (McE-

wen, 1998; Chrousos, 2009).

This review raises several questions regarding the applicability and utility of hair cortisol measurement as a biological marker of chronic stress in the field of exercise and sport science. It also highlights several issues pertaining to athletes and exercisers with repeated exposure to high cortisol secretion rates during their everyday intense physical training. This review is warranted because the interest in biopsychological aspects of exercise and psychosocial stress in athletes is increasing (Ehrlenspiel and Strahler, 2012) and assessments utilizing biomarkers is now more accessible to researchers with a limited psychoneuroendocrinological background. Many published reviews describe the role of cortisol in health and disease and the use of different cortisol measures in stress research (Chrousos, 2009; Gow et al., 2010; McEwen, 1998; Russell, 2012; Stalder and Kirschbaum, 2012). We therefore, briefly mention the methodological considerations of using hair cortisol for assessing long-term cortisol secretion (including sampling and analysis) and discuss how strongly hair cortisol is associated with salivary, plasma, and urinary cortisol. We also examine the validity of hair cortisol as a marker of chronic stress. The main focus of the present review is to discuss the role of hair cortisol in exercise and sport science research and the limitations associated with hair cortisol analysis among regular exercisers and athletes. The relationship between hair cortisol and stress is complex, particularly when discussed from a health perspective. Researchers in exercise and sport science face new problems that were not pertinent in cortisol assessment via saliva, plasma or urine.

Cortisol: A Stress Hormone

Cortisol is an end product of the hypothalamic-pituitary-adrenal (HPA) axis, one of the main endocrine stress axes (Dettenborn et al., 2012b). Cortisol has a wide influence on the regulation of other bodily functions including gluconeogenesis, lipolysis, insulin resistance, blood pressure regulation, immunity, wound healing, neural survival, neurogenesis, sleep regulation, and cognitive performance (Gow et al., 2010; McEwen, 1998). Cortisol is also necessary for optimal exercise performance, which is particularly evident in sufferers of chronic cortisol deficiency (Borer, 2003). During acute psychological or physical challenges, the metabolic, behavioral, cognitive and immunological effects of cortisol have adaptive functions, allowing the human organism to maintain a state of homeostasis (Chrousos, 2009).

Exercise itself can be regarded as a physical stressor, and there is a plethora of studies that examined the influence of acute bouts of exercise on physiological stress reactions in children and adults, as well as in healthy and clinical populations (cf. Delcorral et al., 1994; Negro et al., 2000; Shephard and Sidney, 1975). Numerous hormones and regulating systems are involved in the neuroendocrinology of acute stress responses and similar to psychosocial stress, the main systems for acute exercise responses are the sympathetic-adrenomedullary system with the release of catecholamines (epinephrine, norepi-

nephrine) and the HPA axis with the corticotropin-releasing hormone (CRH) as the principal hypothalamic regulator of the adrenocorticotrophic hormone (ACTH) which stimulates the release of cortisol from the adrenal cortex.

The role played by cortisol during intense exercise is vital because of its catabolic, proteolytic and anti-inflammatory characteristics, and it also functions to maintain blood pressure and plasma volume. Generally, the rise of cortisol secretion follows the ACTH release with a lag of 15 to 30 minutes (Borer, 2003 for review). Prior studies showed that acute bouts of high intensity exercise (above ~70% VO₂max) result in a substantial increase in salivary and plasma cortisol from before to after exercise (e.g. Hill et al., 2008). Studies have explored various factors moderating the exercise-cortisol response relationship such as gender (e.g. Wall and Rudisill, 2007), intake of specific substances (e.g. Beaven et al., 2008), hydration state (e.g. Maresh et al., 2006), and the use of oral contraceptives (e.g. Kirschbaum et al., 1996). Recently, depressed participants have shown decreased cortisol reactions after a standardized ergometer test when compared to controls (Krogh et al., 2010). A substantial body of research also exists regarding the influence of single bouts of exercise prior to experimentally induced stress on cortisol reactivity (Hamer et al., 2006 for review). Additional research has examined the influence of physical fitness on cortisol reactivity during laboratory stress (cf. Jackson and Dishman, 2006 for review).

Hair Cortisol: A Novel Marker of Long-Term Cortisol Levels

In prior research, scientists have frequently used salivary, plasma and urinary samples to measure cortisol levels. Particularly, the use of salivary cortisol measurements has increased, as this is a convenient non-invasive and relatively cost effective method, and samples can be collected by non-medical personnel or even participants themselves (Karlen et al., 2011). Saliva or plasma samples are snapshots of HPA axis activity and reveal acutely circulating cortisol levels, whereas urine samples measure mean cortisol secreted over a relatively short period of time (usually 24h) (D'Anna-Hernandez et al., 2011; Dettenborn et al., 2012b).

Saliva, plasma and urinary cortisol have also been used to obtain valid information on patterns of long-term cortisol secretion (Stalder and Kirschbaum, 2012). For instance, salivary cortisol was used to investigate the association between cortisol levels and regular physical activity (e.g. Hansen et al., 2010) and structured exercise (e.g. Karacabey, 2009). Researchers have however, argued that these measures are not ideal indicators of long-term cortisol secretion under naturalistic circumstances (cf. Dettenborn et al., 2010; Steudte et al., 2011a). First, the HPA axis is highly reactive, and the mere fact that a participant faces a stressor before the assessment can impact adrenocortical activity (Dowlati et al., 2010). Second, circadian rhythms can result in considerable individual variability of baseline cortisol levels between

and within subjects (Hellhammer et al., 2007). Third, it is methodologically difficult to assess cortisol via blood and salivary samples during the second half of sleep; a key period for cortisol secretion (Clow et al., 2004). Fourth, acute assessments of cortisol (saliva and plasma) are influenced by food intake, smoking and physical activity done immediately before sampling (Hamer et al., 2006; Dettenborn et al., 2010). Fifth, participants might not adhere to timely saliva collection protocols (Kudielka et al., 2003).

Hair cortisol is a novel marker of long-term cortisol elevation free from many of the methodological difficulties associated with plasma, salivary and urinary cortisol (Dowlati et al., 2010; Steudte et al., 2011a). Segmental hair analysis has been successfully used in forensic, toxicologic and doping research (e.g. Villain et al., 2004). However, researchers have only recently discovered that corticosteroids in human hair can be substantiated by means of high-performance liquid chromatography-ion spray mass spectrometry (HPLC/MS) (Cirimele et al., 2000) and that endogenously produced cortisol can be analyzed in human hair (Raul et al., 2004).

Hair cortisol sampling is characterized by non-invasive collection, easy storage (room temperature) and dispatch (postal mail), and the samples do not decompose like bodily fluids or tissues. Moreover, sampling is simple and can be executed by a non-professional at any time of the day (D'Anna-Hernandez et al., 2011; Dettenborn et al., 2012b; Gow et al., 2010). Researchers have also shown that hair cortisol concentration does not depend on natural hair color, use of oral contraceptives and smoking status (Dettenborn et al., 2012b). However, increased hair cortisol levels were found in men, young children and older adults when compared to women, adolescents and younger adults, respectively (Dettenborn et al., 2012b). Given this knowledge, it is recommended that analyses be adjusted for social and demographic factors such as age and gender. It is, however, clear that many methodological advantages are associated with hair sampling compared to e.g. blood or saliva, the most important ones being frequency of sampling, invasiveness of the procedure and less considerations during sampling, i.e. sleep, acute exercise and food intake.

A particular strength of hair cortisol measurements is that alterations of the HPA axis, based on diurnal variations or the measurement process, do not taint the findings. In contrast, hair cortisol provides information regarding the total amount of cortisol rather than its function or dynamics. For instance, Halford et al. (2012) have suggested that the steepness of diurnal slope in cortisol concentration might be related to mental well-being. This also precludes the use of hair cortisol as an indicator of acute stress reactivity and recovery. Since hair cortisol is a marker of long-term cortisol elevation, moderately high test-retest reliabilities are expected. In support of this, Stalder et al. (2012) demonstrated a high intra-individual stability of hair cortisol concentrations with strong correlations of repeated assessments from two months to one year, $r = 0.68 - 0.79$.

Methodological Aspects of Segmental Hair Corti-

sol Analysis

After having highlighted possible benefits of hair cortisol analysis, the following sections briefly discuss how researchers can collect hair samples and how cortisol concentration is established in subsequent analyses.

Hair sampling

Hair strands (approximately 3 mm diameter) are usually taken scalp-near from a posterior vertex position. It is important not to include the hair follicle (Gow et al., 2010; Ito et al., 2005) and to take hair samples from the posterior vertex position of the scalp as vertex cortisol levels have a lower variability compared to other areas (Sauvé et al., 2007; Stalder and Kirschbaum, 2012). Thus, training of research personnel is important as hair cortisol concentrations vary depending on where on the head the sample is taken. Hair samples are cut using fine-tipped surgical scissors (not pulled out). Afterwards, hair samples are placed on aluminum foil, and the researcher marks the root end of the hair sample. Samples are stored at room temperature and mailed to the laboratory in a sealed envelope.

It is still debated how far back in time one can measure hair cortisol (Gow et al., 2010). Some studies found a linear decline from scalp-near to distal hair segments, indicating that cortisol gradually dissipates with the increased exposure of several cortisol content-altering factors such as chemicals present in shampoos, dyes, and perms, permanent waving or straightening, use of hair products (spray, mousse, gel and wax), or sunlight and heat (see Kirschbaum et al., 2009; Dettenborn et al., 2010). Nevertheless, a decline did not occur across all investigations (Thomson et al., 2010; Manenschijn et al., 2011a); possibly due to different sample preparation techniques (Dettenborn et al., 2012b). Given this background, Kirschbaum et al. (2009) recommended only using the first two 3-cm segments towards the scalp. This might eliminate the possibility of bias from the leaching effects of hair samples that are older than six months (average hair growth is around 1cm per month) (Wenning, 2000).

Methods used to analyze cortisol in hair

Methods to quantify hair cortisol include enzyme linked immunosorbent assays (ELISA) and high performance-liquid chromatography-mass spectrometry (HPLC/MS) (Gow et al., 2010). Gow et al. (2010) have described mass spectrometry as the 'golden standard' of hair analysis, but these methods are relatively expensive. This may explain why researchers seldom use them to quantify hair cortisol (e.g. Cirimele et al., 2000; Raul et al., 2004).

Most scientists have used ELISA methods to establish hair cortisol levels in humans (e.g. Dettenborn et al., 2012b; Kalra et al., 2007; Kirschbaum et al., 2009; Thomson et al., 2010; Yamada et al., 2007; van Uum et al., 2008). Gow et al. (2010) concluded that the ELISA methods are promising, given their sensitivity, cost-effectiveness and speedy procedure. Coefficients of variation for ELISA methods are generally below 10% (Kramer et al., 2009; Sauvé et al., 2007; van Uum et al., 2008), which is similar to the coefficients found with

HPLC/MS (Raul et al., 2004). Hair cortisol concentration is generally reported in pg/mg. The hair cortisol concentration in a sample of 99 young healthy Swedish adults in the first 3-cm segment was $M = 19.93 \text{ pg}\cdot\text{mg}^{-1}$ ($SD = 33.35$) ranging from 1.45 to 212.03 $\text{pg}\cdot\text{mg}^{-1}$ (Karlen et al., 2011).

Associations between Hair Salivary, Plasma, and Urinary Cortisol

In most early hair cortisol studies, it was important to provide evidence of construct validity. According to Stalder and Kirschbaum (2012), the most direct validation of hair cortisol is to correlate data accumulated from repeated assessments of cortisol in hair, saliva, plasma, and urine. Among healthy Chinese graduate students, a significant correlation of $r = 0.38$ was found between hair cortisol analyzed in the most proximal centimeter (representing the most recent month) and average salivary morning cortisol derived from three weekly measurement days over four weeks (Xie et al., 2011). Hair cortisol and diurnal salivary cortisol (three samples per day over three days) were also correlated in pregnant women, $r = 0.24$ – 0.57 (D'Anna-Hernandez et al., 2011). Steudte et al. (2011a) revealed a non-significant correlation, $r = 0.27$, between hair cortisol in the first hair segment and diurnal salivary cortisol in participants with generalized anxiety disorder (six samples per day over two days). Lastly, Sauvé et al. (2007) found significant correlations between hair cortisol and 24h-urinary cortisol, $r = 0.33$, but not with plasma, $r = 0.06$, or morning salivary cortisol obtained at a single time point, $r = 0.31$.

In conclusion, results from the aforementioned studies regarding validation of cortisol levels in hair through comparison with other cortisol measures are not compelling, since the data are quite variable with low to moderate correlations. Nevertheless, as salivary, urinary and hair cortisol assessments reflect different time frames (current state, one day, 3–6 months respectively), strong associations are not expected.

Is Chronic Stress Reflected in Hair Cortisol Levels?

Besides direct validation, researchers have used several different strategies to indirectly validate hair cortisol as a biomarker of chronic stress, which are detailed below.

Hair cortisol levels in individuals with diseases associated with disturbed HPA activity or altered cortisol levels due to biological changes

Thomson et al. (2010) revealed that patients with Cushing's syndrome, characterized by an excessively high cortisol production, had elevated hair cortisol levels compared to controls. Hair cortisol concentration varied in accordance with the clinical course and thus declined after surgical intervention. Comparable findings were reported by Manenschijn et al. (2011a) in a study with 195 healthy individuals, nine hypercortisolemic participants (one person with Cushing's syndrome) and one hypocortisolemic patient. Specifically, the hair cortisol level of the

patient with Cushing's disease decreased after ketoconazole treatment and surgery. Additionally, Kirschbaum et al. (2009) and D'Anna-Hernandez et al. (2011) both confirmed that hair cortisol levels increase during the third trimester of pregnancy.

Hair cortisol in individuals who were exposed to major life events or severe stressful circumstances

Yamada et al. (2007) reported that term and preterm infants hospitalized at a neonatal intensive care unit had significantly elevated hair cortisol concentrations compared to healthy term counterparts. Higher hair cortisol concentrations were also found in long-term unemployed versus employed individuals, with hair cortisol explaining considerable variance (7.1–8.5%) (Dettenborn et al., 2010). Manenschijn et al. (2011b) further reported that shift workers had higher hair cortisol than day workers, and Stalder et al. (2010) found that alcoholics in acute withdrawal had substantially elevated hair cortisol levels compared to abstinent alcoholics or controls. Steudte et al. (2011b) demonstrated that traumatized individuals from the Ugandan civil war (diagnosed with post-traumatic stress disorder, or PTSD) had higher hair cortisol levels than traumatized controls without PTSD. Moreover, the number of lifetime traumatic events was positively correlated with hair cortisol levels in this study.

Associations between hair cortisol levels and self-perceived stress

Kalra et al. (2007) found that the Perceived Stress Scale (PSS) (Cohen et al., 1983) scores were significantly correlated with hair cortisol in pregnant women near the end of the first or the beginning of the second trimester. Insignificant correlations with the PSS were found in patients with coronary artery disease (Dowlati et al., 2010), pregnant women (Kramer et al., 2009), endurance athletes (Skoluda et al., 2012), and alcoholics (Stalder et al., 2010). In a regression analysis, Karlen et al. (2011) showed that only serious life events (not the PSS scores) predicted hair cortisol in young healthy adults.

The stronger association between hair cortisol and life events supports previous research showing that serious stressful events are associated with increased hair cortisol levels (Dettenborn et al., 2010; Yamada et al., 2007). Several explanations are possible why hair cortisol concentrations are more closely associated with life events than perceived stress. One basic explanation is that cortisol may poorly reflect perceived stress. Alternatively, different timeframes assessed via hair cortisol and stress questionnaires may account for the non-significant relationships in the above studies. Some further explanations should be considered: Firstly, life events are mostly studied from a between-group perspective, in which individuals who were exposed to a stressor (e.g., long-term unemployment) are compared with healthy controls. In contrast, the relationship between hair cortisol and perceived stress is mostly studied from a within-group perspective, in which hair cortisol and perceived stress are correlated in relatively homogeneous populations. Within this approach, however, low correlation coefficients can be due to low variations in terms of stress and hair cortisol. Sec-

only, life event inventories may have a higher discriminative power per se, since fewer individuals face major life events, whereas most individuals encounter minor daily stressors (Twisk et al., 1999).

Associations between hair cortisol and stress-related mental disorders

As the most indirect approach towards validation, researchers have studied whether hair cortisol is associated with stress-related mental health problems. Significant relationships were found between hair cortisol and psychological disorder. For example, a comparison of 23 depressed individuals and 64 controls revealed that depressed individuals had higher cortisol levels in near-scalp and adjacent hair segments (Dettenborn et al., 2012b). Similarly, Karlen et al. (2011) reported in a study with young adults that two participants with very high hair cortisol levels had serious psychological problems (including depressive symptoms). In contrast, a study with 42 exercise and health science students showed that students with elevated hair cortisol levels reported lower depressive symptoms (Gerber et al., unpublished observation). Moreover, Dowlati et al. (2010) did not find differences in hair cortisol between patients with coronary heart disease independent of whether major depressive disorder (MDD) was present or not. Furthermore, Stalder and colleagues (2010) were unable to demonstrate a significant relationship between hair cortisol and the Beck Depression Inventory in a combined sample of abstinent and non-abstinent alcoholics and healthy controls. On the other hand, Steudte and colleagues (2011a; 2011b) showed that patients suffering from generalised anxiety disorder had significantly lower (50-60%) hair cortisol concentrations compared to age- and gender-matched healthy controls. Hence, this study corroborates that decreased levels of cortisol could be related to pathological situations.

In summary, evidence has accumulated over the last decade to confirm that hair cortisol levels are increased among individuals who have been exposed to stressful life events or who are expected to have high cortisol levels due to disturbed cortisol secretion. On the other hand, most studies showed, at best, a weak association between hair cortisol and perceived stress, which is in line with research on salivary cortisol (Kristenson et al., 2012). Finally, the direction of association between hair cortisol and mental health is not clear, and findings point towards hyper- and hypocortisolism.

The Challenge of Measuring and Interpreting Hair Cortisol in Physically Active Individuals

Hair cortisol levels of physically active individuals compared to controls

Preliminary evidence exists that levels of physical exercise are associated with cortisol levels measured in hair. Skoluda et al. (2012) compared hair cortisol concentrations in the first three 3-cm hair segments (reflecting the last three months) of 304 amateur endurance athletes including long-distance runners (10-km runners, half-marathon runners, and marathoners), triathletes, cyclists,

and 70 controls. Independent of gender, endurance athletes had 46% higher cortisol levels in the first 3-cm hair segment. Elevated cortisol levels were also found in the second and third segments. Among athletes, positive significant correlations with hair cortisol were found for training distance, $r = 0.32$, training hours, $r = 0.22$, and number of competitions per year, $r = 0.29$. Using accelerometer data, our research group confirmed that differences in hair cortisol levels depended upon participation in vigorous physical activity (Gerber et al., unpublished observation). Specifically, participants with high vigorous physical activity had elevated hair cortisol concentrations and decreased stress perceptions compared to peers with low levels. In contrast, no differences were found for moderate physical activity. In conclusion, these findings are in line with studies showing that acute bouts of vigorous exercise are associated with a considerable increase in salivary and plasma cortisol concentrations (e.g. Hill et al., 2008; Wall and Rudisill, 2007). Hence, repeated exercise-induced HPA activation seems to be reflected in overall heightened hair cortisol levels. While the findings of these studies can be interpreted as proof-of-concept that increased (physical) stress is associated with augmented hair cortisol levels, this fact raises a major question regarding the utility to use hair cortisol as a biopsychological measure in athlete populations. The behavioral pattern for this population performing several bouts of intense exercise each week makes it difficult to evaluate the different source of cortisol. Studies on elite athletes with equal training loads and different perceived stress levels could help to elucidate whether it is possible to distinguish physical stress from emotional, cognitive or psychosocial stress.

Are high cortisol levels a health risk for athletes?

The interpretation of data will ultimately lead to discussion regarding the association of hair cortisol with health and disease. Caution is needed as high levels of cortisol measured in athlete populations and exercisers should be considered differently in respect to both metabolic consequences during exercise as well as the lasting adverse health effects that are associated with chronically high levels of cortisol (Chrousos, 2009). Activating the neuroendocrinological stress response during exercise is considered beneficial in the long run. Based on Selye's (1950) cross-stressor adaptation hypothesis, researchers have argued that adaptation of the stress systems due to regular exercise may not only influence the physiological stress reactivity to physical stress, but also have a positive impact on non-exercise stressors, such as psychosocial stress (Rimmele, 2012). Thus, measuring a high level of cortisol does not necessarily equate to poor health for athletes and exercisers.

To illustrate the complexity of the so-called 'pathogenic elements' of stress responses, high hair cortisol levels constitute a risk factor for myocardial infarctions (Gow et al., 2009; Pereg et al., 2011). However, previous investigations have shown that regular exercise and high cardiorespiratory fitness are associated with a reduced risk for cardiovascular diseases (e.g. Carnethon et al., 2003). Similarly, van Uum et al. (2008) studied pa-

tients with severe chronic pain who had significantly higher hair cortisol when compared to controls. Again, this contradicts the notion that regular exercise is typically associated with decreased pain perception (e.g. Nilsen et al., 2011). High hair cortisol has also been associated with psychological disorders (Dettenborn, et al., 2012b; Karlen et al., 2011), whereas regular exercise is related to better mental health (e.g. Jonsdottir et al., 2010). Finally, high cortisol levels are related to obesity (Manenschijn et al., 2011b), whereas exercise training is considered to be one of the primary preventive factors (Holmes et al., 2010).

Consequently, information regarding exercise volume, including intensity, duration and frequency is crucial for the interpretation of hair cortisol level in athletes and regular exercisers. The aspect of training history and current level of fitness is of particular interest as equal training volumes could mean different things for different athletes depending on their fitness levels. Fitness status is related to stress reactivity during exercise; thus aerobically fit persons show less cardiovascular and neuroendocrinological activation in a response to exercise (e.g. Wittert et al., 1996). By contrast, it remains unclear if physically active persons have attenuated HPA-axis activation in response to psychological stress, hence, more randomized controlled trials are needed (Jackson and Dishman, 2006).

Even with detailed information regarding training history and fitness, it will be a challenge to estimate the impact of psychosocial stress on the hair cortisol level of athletes and exercisers. This is due to possible ceiling effects associated with exercise-based long term-cortisol elevation. Therefore, multiple assessment tools are needed, including medical history, questionnaires reflecting perceived stress levels, exposure to critical life events, mental health and quality of life.

In summary, the fact that athletes have higher hair cortisol concentration than controls indicates that a person's hair cortisol concentration is related to his/her exercise involvement, and this higher level may not necessarily contain pathological aspects. In view of the fact that hair cortisol condenses information from various life domains including physical, cognitive, emotional as well as psychosocial stressors that all can activate the HPA axis, it is crucial to scrutinize hair cortisol data, training history, and other life stressors in order to apply findings. Hence, researchers should use caution when associating athletes' elevated hair cortisol with health or disease, particularly among athletes who often exercise above 70% of VO_2max .

Basic research considerations

The fact that the relationships between hair cortisol, stress, vigorous exercise and health are more complex than expected underlines that researchers should (i) have a priori defined hypotheses before data collection, (ii) have a clear idea how to use hair cortisol in their study (e.g. as independent, dependent, moderating or mediating variable), and (iii) be aware that both hyper- and hypocortisolism are possible patterns when studying associations with stress and mental disorders. For instance, hypocortisolism is common in several somatic and psychiatric

disorders including fibromyalgia, PTSD, chronic fatigue, and chronic pain and it is likely that lower hair cortisol concentrations can be seen in these populations (Fries et al., 2005).

Researchers should also be aware that the relationship between hair cortisol and other variables such as exercise, stress and health might not be linear. Therefore, choosing appropriate methods of data analysis is important. Finally, as vigorous exercise appears to have a substantial influence on hair cortisol, stress researchers should place more emphasis on this factor as a source of potential confound to gain deeper insights regarding the relationships between hair cortisol, non-physical stress and mental health. Strategies to control for exercise levels include focusing on untrained individuals, on athletes with similar training workloads or including exercise as a covariate or moderating variable.

A considerable amount of pilot work is needed before the usefulness of hair cortisol as a biological marker of chronic stress can be judged accurately in this specific field of research. From a general point of view, it would be interesting to know whether a high-intensity exercise program with healthy untrained individuals leads to increased hair cortisol concentrations as compared to a program with low or moderate intensity. Similarly, researchers could explore whether hair cortisol concentrations change among athletes who stop training due to an injury or whether an athlete's hair cortisol concentration changes in relation to his/her reported training intensity, number and importance of competitions and perceived stress throughout the season. From an elite athlete perspective, researchers could further ask whether symptoms of burnout or overtraining are associated with altered hair cortisol levels among athletes with similar training workloads, whereas from a health perspective, exercise scientists could examine whether levels of exercise or physical fitness moderate the relationship between hair cortisol, mental disorders and physical diseases. Appendix provides a checklist with recommendations for exercise and sport scientists interested in including hair cortisol in their own research.

Further Considerations Associated with Hair Cortisol Analysis in Exercise and Sport Science

Although hair cortisol offers a novel approach for exercise and sport scientists to examine long-term cortisol secretion, as mentioned in the previous sections, this research is in its infancy. Additionally, hair cortisol is only weakly associated with other cortisol measures, only low correlations exist between hair cortisol and perceived stress, and many other issues are unexplored.

Most importantly, we wanted to raise several other issues related to hair sampling, cortisol analysis and their interpretation within athlete populations. There may be some problems with collecting hair samples from athletes, including the idea that; (i) elite athletes are reluctant to provide hair samples as they might fear misuse of the data (e.g. doping control), (ii) short hair length/baldness might be common in some sports (e.g. swimmers) or specific populations (e.g. elderly men), (iii) hair length may

change across time, which introduces a source of possible dropout, (iv) at least one face-to-face contact is necessary with the participants, (v) athletes might frequently use substances related to body hygiene, (vi) pool chemicals may affect results for swimmers and divers, (vii) some athletes might be exposed more frequently to water which increases the risk for a wash-out effect (e.g. surfers), and (viii) some athletes might be exposed to more sunlight than others which may influence their hair cortisol concentrations.

Although Stalder and Kirschbaum (2012) argued that hair cortisol concentrations are mainly attributable to systemic cortisol secretion, sweat may constitute a possible confounding factor in elite athletes and contribute to their elevated hair cortisol levels. Nevertheless, knowledge is still limited about how cortisol is incorporated into the hair shaft. Stalder and Kirschbaum (2012) have recently suggested five ways how cortisol can be incorporated in human hair (cp. Gow et al., 2010; van Uum et al., 2008) including: (a) passive diffusion from blood capillaries into the growing hair cells (through adrenal cortex secretion), (b) sweat, (c) sebum secretion from the sebaceous gland into the completed hair shaft, (d) incorporation of substances from external sources (contamination through cortisol-containing creams or medical ointments residing on the hands), and (e) local synthesis through intrafollicular HPA axis secretion. With regard to local synthesis, researchers argue that hair follicles are independent peripheral neuroendocrine organs capable of synthesizing relatively small amounts of cortisol in the pilosebaceous unit located in the hair follicles in response to ACTH (D'Anna-Hernandez et al., 2011; Ito et al., 2005; Skoluda et al., 2012). Additionally, Slominski et al. (2005) have shown in vitro that normal human epidural melanocytes from moderately pigmented skin can stimulate cortisol production, and that stress leads to a similar activation sequence in the single cell as in the whole body.

Conclusion

The intention of this review was to show the potential relevance of hair cortisol as a marker of long-term cortisol secretion for exercise and sport scientists, but also to reveal possible limitations and areas of concern. The present review points out that hair cortisol research is in an early stage, that many pieces in the puzzle are available, but that a clear picture of how to interpret and use the findings is only just emerging. Hence, we are aware that this review may raise just as many questions as it provides answers. Nevertheless, the investigation of chronic stress is a fundamental topic in the area of exercise and sport science. The need for practical non-invasive measures of cumulative stress is critical in athletes involved in sports with long seasons, short rest periods between games, constant travel (especially across seasons) and high physical and metabolic demand. Potential use of hair cortisol measurements in sport sciences includes prevention of stress fractures among athletes, improvement of the early detection of athlete overtraining, or testing the impact of physical activity interventions

on the well-being of healthy or stress impaired participants. Pilot studies are needed to examine whether hair cortisol analysis can contribute to a more complete understanding of how long-term cortisol secretion mediates stress-related effects on health and performance of recreational exercisers and elite athletes. Most importantly, it will be crucial for exercise and sport scientists to consider whether their specific research questions can be addressed in a meaningful way, given that regular intense exercise results in substantially augmented hair cortisol levels.

References

- Acevedo, E.O. and Ekkekakis, P. (2006) *Psychobiology of physical activity*. Champaign: Human Kinetics.
- Albinson, C.B. and Petrie, T.A. (2003) Cognitive appraisals, stress, and coping: Preinjury and postinjury factors influencing psychological adjustment to sport injury. *Journal of Sport Rehabilitation* **12**, 306-322.
- Beaven, C.M., Gill, N.D. and Cook, C.J. (2008) Salivary testosterone and cortisol responses in professional rugby players after four resistance exercise protocols. *Journal of Strength and Conditioning Research* **22**, 426-432.
- Borer, K.T. (2003) *Exercise endocrinology*. Champaign: Human Kinetics.
- Carnethon, M.R., Gidding, S.S., Nehgme, R., Sidney, S., Jacobs, D.R., Jr. and Liu, K. (2003) Cardiorespiratory fitness in young adulthood and the development of cardiovascular disease risk factors. *JAMA* **290**, 3092-3100.
- Chrousos, G.P. (2009) Stress and disorders of the stress system. *Nature Reviews Endocrinology* **5**, 374-381.
- Cirimelle, V., Kintz, P., Dumestre, V., Goulle, J.P. and Ludes, B. (2000) Identification of ten corticosteroids in human hair by liquid chromatography-ion spray mass spectrometry. *Forensic Science International* **107**, 381-388.
- Clow, A., Thorn, L., Evans, P. and Hucklebridge, F. (2004) The awakening cortisol response: Methodological issues and significance. *Stress* **7**, 29-37.
- Cohen, S., Kamarck, T. and Mermelstein, R. (1983) A global measure of perceived stress. *Journal of Health and Social Behavior* **24**, 385-396.
- D'Anna-Hernandez, K.L., Ross, R.G., Natvig, C.L. and Laudenslager, M.L. (2011) Hair cortisol levels as a retrospective marker of hypothalamic-pituitary axis activity throughout pregnancy: Comparison to salivary cortisol. *Physiology & Behavior* **104**, 348-353.
- de Assis, M.A., de Mello, M.F., Scorza, F.A., Cadrobbi, M.P., Schoedel, A.F., da Silva, S.G., de Albuquerque, M., da Silva, A.C. and Arida, R.M. (2008) Evaluation of physical activity habits in patients with posttraumatic stress disorder. *Clinics* **63**, 473-478.
- Delcorral, P., Mahon, A.D., Duncan, G.E., Howe, C.A. and Craig, B.W. (1994) The effect of exercise on serum and salivary cortisol in male-children. *Medicine and Science in Sports and Exercise* **26**, 1297-1301.
- Dettenborn, L., Tietze, A., Bruckner, F. and Kirschbaum, C. (2010) Higher cortisol content in hair among long-term unemployed individuals compared to controls. *Psychoneuroendocrinology* **35**, 1404-1409.
- Dettenborn, L., Tietze, A., Kirschbaum, C. and Stalder, T. (2012a) The assessment of cortisol in human hair: Associations with socio-demographic variables and potential confounders. *Stress* **15**(6), 578-588.
- Dettenborn, L., Muhtz, C., Skoluda, N., Stalder, T., Steudte, S., Hinkelmann, K., Kirschbaum, C. and Otte, C. (2012b) Introducing a novel method to assess cumulative steroid concentrations: Increased hair cortisol concentrations over 6 months in medicated patients with depression. *Stress* **15**, 348-353.
- Dienstbier, R.A. (1989) Arousal and physiological toughness: Implications for mental and physical health. *Psychological Review* **96**, 84-100.
- Dowlati, Y., Herrmann, N., Swardfager, W., Thomson, S., Oh, P.I., van Uum, S., Koren, G. and Lancôt, K.L. (2010) Relationship between hair cortisol concentrations and depressive symptoms in

- patients with coronary artery disease. *Neuropsychiatric Disease and Treatment* **6**, 393-400.
- Ehrlenspiel, F. and Strahler, K. (2012). *Psychoneuroendocrinology of sport and exercise: Foundations, markers, trends*. Oxford: Routledge.
- Fletcher, D. and Hanton, S. (2003) Sources of organizational stress in elite sports performers. *Sport Psychologist* **17**, 175-195.
- Forcier, K., Stroud, L.R., Papandonatos, G.D., Hitsman, B., Reiches, M., Krishnamoorthy, J. and Niaura, R. (2006) Links between physical fitness and cardiovascular reactivity and recovery to psychological stressors: A meta-analysis. *Health Psychology*, **25**, 723-739.
- Fredericson, M., Ngo, J. and Cobb, K. (2005) Effects of ball sports on future risk of stress fracture in runners. *Clinical Journal of Sport Medicine* **15**, 136-141.
- Fries, E., Hesse, J., Hellhammer, J. and Hellhammer, D.H. (2005) A new view on hypocortisolism. *Psychoneuroendocrinology* **30**, 1010-1016.
- Gerber, M., Kellmann, M., Hartmann, T. and Pühse, U. (2010) Do exercise and fitness buffer against stress among Swiss police and emergency response service officers? *Psychology in Sport and Exercise* **11**, 286-294.
- Gow, R., Pereg, D., Koren, G., Rieder, M. J. and van Uum, S. (2009) Elevated hair cortisol levels in patients with acute myocardial infarction. *Canadian Journal of Clinical Pharmacology* **16**, E301.
- Gow, R., Thomson, S., Rieder, M., van Uum, S. and Koren, G. (2010) An assessment of cortisol analysis in hair and its clinical applications. *Forensic Science International* **196**, 32-37.
- Halford, C., Jonsdottir, I.H. and Eek, F. (2012) Perceived stress, psychological resources and salivary cortisol. In: *The role of saliva cortisol measurement in health and disease*. Eds: Kristenson, M., Garvin, P. and Lundberg, U. Oak Park: Bentham Science Publisher. 67-86.
- Hamer, M., Taylor, A. and Steptoe, A. (2006) The effect of acute exercise on stress related blood pressure responses: A systematic review and meta-analysis. *Biological Psychology* **71**, 183-190.
- Hansen, A.M., Blangsted, A.K., Hansen, E.A., Sogaard, K. and Sjogaard, G. (2010) Physical activity, job demand-control, perceived stress-energy, and salivary cortisol in white-collar workers. *International Archives of Occupational and Environmental Health* **83**, 143-153.
- Hellhammer, J., Fries, E., Schweisthal, O.W., Schlotz, W., Stone, A.A. and Hagemann, D. (2007) Several daily measurements are necessary to reliably assess the cortisol rise after awakening: State and trait components. *Psychoneuroendocrinology* **34**, 32-37.
- Hill, E., Zack, E., Battaglini, C., Viru, M., Viru, A. and Hackney, A.C. (2008) Exercise and circulating cortisol levels: The intensity threshold effect. *Journal of Endocrinological Investigation* **31**, 587-591.
- Holmes, M.E., Ekkekakis, P. and Eisenmann, J.C. (2010) The physical activity, stress and metabolic syndrome triangle: a guide to unfamiliar territory for the obesity researcher. *Obesity Reviews*, **11**, 492-507.
- Ito, N., Ito, T., Kromminga, A., Bettermann, A., Takigawa, M., Kees, F., Straub, R.H. and Paus, R. (2005) Human hair follicles display a functional equivalent of the hypothalamic-pituitary-adrenal (HPA) axis and synthesize cortisol. *The FASEB Journal* **19**, 1332-1334.
- Jackson, E. M. and Dishman, R. K. (2006) Cardiorespiratory fitness and laboratory stress: A meta-regression analysis. *Psychophysiology* **43**, 57-72.
- Jonsdottir, I.H., Rödger, L., Hadzibajramovic, E., Börjeson, M. and Ahlberg, G. (2010) A prospective study of leisure-time physical activity and mental health in Swedish health care workers and social insurance officers. *Preventive Medicine* **51**, 373-377.
- Kalra, S., Einarson, A., Karaskov, T. van Uum, S. and Koren, G. (2007) The relationship between stress and hair cortisol in healthy pregnant women. *Clinical & Investigative Medicine* **30**, E103-E107.
- Karacabay, K. (2009) The effect of exercise on leptin, insulin, cortisol and lipid profiles in obese children. *Journal of International Medical Research* **37**, 1472-1478.
- Karlen, J., Ludvidsson, J., Frostell, A., Theodorsson, E. and Faresjo, T. (2011) Cortisol in hair measured in young adults – A biomarker of major life stressors? *BMC Public Health* **11**(1), 12.
- Kellmann, M. (2010) Preventing overtraining in athletes in high-intensity sports and stress/recovery monitoring. *Scandinavian Journal of Medicine & Science in Sports* **20**, 95-102.
- Kirschbaum, C., Platte, P., Pirke, K.M. and Hellhammer, D. (1996) Adrenocortical activation following stressful exercise: Further evidence for attenuated free cortisol responses in women using oral contraceptives. *Stress Medicine* **12**, 137-143.
- Kirschbaum, C., Tietze, A., Skoluda, N. and Dettenborn, L. (2009) Hair as a retrospective calendar of cortisol production – Increased cortisol incorporation into hair in the third trimester of pregnancy. *Psychoneuroendocrinology* **34**, 32-37.
- Kramer, M.S., Lydon, J., Séguin, L., Goulet, L., Kahn, S.R., McNamara, H., Genest, J., Dassa, C., Chen, M.F., Sharma, S., Meaney, M. J., Thomson, S., van Uum, S., Koren, G., Dahhou, M., Lamoureux, J. and Platt, R.W. (2009) Stress pathways to spontaneous preterm birth: The role of stressors, psychological distress, and stress hormones. *American Journal of Epidemiology* **169**, 1319-1326.
- Kristenson, M., Garvin, P. and Lundberg, U. (2012) *The role of saliva cortisol measurement in health and disease*. Bentham Science Publishers: Oak Park.
- Krogh, J., Nordentoft, M., Mohammad-Nezhad, M. and Westrin, A. (2010) Growth hormone, prolactin and cortisol response to exercise in patients with depression. *Journal of Affective Disorders* **125**, 189-197.
- Kudielka, B.M., Broderick, J.E. and Kirschbaum, C. (2003) Compliance with saliva sampling protocols: Electronic monitoring reveals invalid cortisol daytime profiles in noncompliant subjects. *Psychosomatic Medicine* **65**, 313-319.
- Luger, A., Deuster, P. A., Kyle, S. B., Gallucci, W.T., Montgomery, L. C., Gold, P.W., Loriaux, D.L. and Chrousos, G.P. (1988) Acute hypothalamic-pituitary-adrenal responses to the stress of treadmill exercise. Physiologic adaptation to physical training. *New England Journal of Medicine* **316**, 1309-1315.
- Lutz, R., Stults-Kolehmainen, M. and Bartholomew, J. (2010) Exercise caution when stressed: Stages of change and the stress-exercise participation relationship. *Psychology of Sport and Exercise* **11**, 560-567.
- Manenschijn, L., Koper, J.W., Lamberts, S.W.J. and van Rossum, E.F.C. (2011a) Evaluation of a method to measure long term cortisol levels. *Steroids* **76**, 1032-1036.
- Manenschijn, L., van Kruysbergen, R. G. P. M., de Jong, F. H., Koper, J. W. and van Rossum, E. F. C. (2011b) Shift work at young age is associated with elevated long-term cortisol levels and Body Mass Index. *Journal of Clinical Endocrinology and Metabolism* **96**, E1862-E1865.
- Maresh, C.M., Whittlesey, M.J., Armstrong, L.E., Yamamoto, L.M., Judelson, D.A., Fish, K.E., Casa, D.J., Kavouras, S.A. and Castrocane, V.D. (2006) Effect of hydration state on testosterone and cortisol responses to training-intensity exercise in collegiate runners. *International Journal of Sports Medicine* **27**, 765-770.
- McEwen, B.S. (1998) Protective and damaging effects of stress mediators. *New England Journal of Medicine* **338**, 171-179.
- Mellalieu, S.D., Neil, R., Hanton, S. and Fletcher, D. (2009) Competition stress in sport performers: stressors experienced in the competition environment. *Journal of Sports Sciences* **27**, 729-744.
- Negrao, A.B., Deuster, P.A., Gold, P.W., Singh, A. and Chrousos, G.P. (2000) Individual reactivity and physiology of the stress response. *Biomedicine & Pharmacotherapy* **54**, 122-128.
- Nilsen, T.I., Holtermann, A. and Mork, P.J. (2011) Physical exercise, body mass index, and risk of chronic pain in the low back and neck/shoulders: longitudinal data from the Nord-Trøndelag Health Study. *American Journal of Epidemiology* **174**, 267-273.
- Pereg, D., Gow, R., Mosseri, M., Lishner, M., Rieder, M., van Uum, S. and Koren, G. (2011) Hair cortisol and the risk for acute myocardial infarction in adult men. *Stress* **14**, 73-81.
- Raul, J.S., Cirimele, V., Ludes, B. and Kintz, P. (2004) Detection of physiological concentrations of cortisol and cortisone in human hair. *Clinical Biochemistry* **37**, 1105-1111.
- Rimmele, U., Davachi, L. and Phelps, E.A. (2012) Memory for time and place contributes to enhanced confidence in memories for emotional events. *Emotion* **12**(4), 834-846.
- Russell, E.W. (2012) Methodological challenges and clinical applications of hair cortisol analysis. Electronic Thesis and Dissertation Repository. The University of Western Ontario. Paper 630. Available from URL: <http://ir.lib.uwo.ca/etd/630>.

- Sauvé, B., Koren, G., Walsh, G., Tokmakejian S. and Van Uum S. H. (2007) Measurement of cortisol in human hair as a biomarker of systemic exposure. *Clinical and Investigative Medicine* **30**, E183-E191.
- Shephard, R.J. and Sidney, K.H. (1975) Effects of physical exercise on plasma growth hormone and cortisol levels in human subjects. *Exercise and Sport Sciences Reviews* **3**, 1-30.
- Skoluda, N., Dettenborn, L., Stalder, T. and Kirschbaum, C. (2012) Elevated hair cortisol concentrations in endurance athletes. *Psychoneuroendocrinology* **37**, 611-617.
- Slominski, A., Zbytek, B., Szczesniowski, A., Semak, I., Kaminiski, J., Sweatman, T. and Wortsman, J. (2005) CRH stimulation of corticosteroids production in melanocytes is mediated by ACTH. *American Journal of Physiology - Endocrinology and Metabolism* **288**, E4701-E4706.
- Stalder, T. and Kirschbaum, C. (2012) Analysis of cortisol in hair – State of the art and future directions. *Brain, Behavior, and Immunity* **26**(7), 1019-1029.
- Stalder, T., Kirschbaum, C., Heinze, K., Steudte, S., Foley, P., Tietze, A. and Dettenborn, L. (2010) Use of hair cortisol analysis to detect hypercortisolism during active drinking phases in alcohol-dependent individuals. *Biological Psychology* **85**, 357-360.
- Stalder, T., Steudte, S., Miller, R., Skoluda, N., Dettenborn, L. and Kirschbaum, C. (2012) Intraindividual stability of hair cortisol concentrations. *Psychoneuroendocrinology* **37**, 602-610.
- Steudte, S., Stalder, T., Dettenborn, L., Klumbies, E., Foley, P., Beesdo-Baum, K. and Kirschbaum, C. (2011a) Decreased hair cortisol concentrations in generalised anxiety disorder. *Psychiatry Research* **186**, 310-314.
- Steudte, S., Kolass, I.-T., Stalder, T., Pfeiffer, A., Kirschbaum, C. and Elbert, T. (2011b) Increased cortisol concentrations in hair of severely traumatized Ugandan individuals with PTSD. *Psychoneuroendocrinology* **36**, 1193-1200.
- Thomson, S., Koren, G., Fraser, L.A., Rieder, M., Friedman, T.C. and Van Uum, S. H. (2010) Hair analysis provides a historical record of cortisol levels in Cushing's syndrome. *Experimental and Clinical Endocrinology & Diabetes* **118**, 133-138.
- Twisk, J.W., Snel, J., Kemper, H.C. and van Mechelen, W. (1999) Changes in daily hassles and life events and the relationship with coronary heart disease risk factors: A 2-year longitudinal study in 27-29-year-old males and females. *Journal of Psychosomatic Research* **46**, 229-240.
- van Uum, S., Sauvé, B., Fraser, L.A., Morley-Forster, P., Paul, T.L. and Koren, G. (2008) Elevated content of cortisol in hair of patients with severe chronic pain: A novel biomarker for stress. *Stress* **11**, 483-488.
- Villain, M., Cirimele, V. and Kintz, P. (2004) Hair analysis in toxicology. *Clinical Chemistry and Laboratory Medicine* **42**, 1265-1272.
- Wall, S.J. and Rudisill, M.E. (2007) Gender differences in cortisol response to physical activity: Comparing young children with adults. *Research Quarterly for Exercise and Sport* **78**, A22-A23.
- Wenning, R. (2000) Potential problems with the interpretation of hair analysis results. *Forensic Science International* **107**, 5-12.
- Wittert, G.A., Livesey, J.H., Espiner, E.A. and Donald, R.A. (1996) Adaptation of the hypothalamopituitary adrenal axis to chronic exercise stress in humans. *Medicine and Science in Sport and Exercise* **28**, 1015-1019.
- Yamada, J., Stevens, B., de Silva, N., Gibbins, S., Beyene, J., Taddio, A., Newman, C. and Koren, G. (2007) Hair cortisol as a potential biologic marker of chronic stress in hospitalized neonates. *Neonatology* **92**, 42-49.
- Xie, Q., Gao, W., Li, J., Qiao, T., Jin, J., Deng, H. and Lu, Z. (2011) Correlation of cortisol in 1-cm hair segment with salivary cortisol in human: Hair cortisol as an endogenous biomarker. *Clinical Chemistry and Laboratory Medicine* **49**, 1-6.

Key points

- Hair cortisol is a unique, non-invasive and painless means to capture long-term cortisol secretion.
- Individuals expected to have elevated cortisol secretion (e.g. due to trauma) have increased hair cortisol.
- Preliminary evidence shows that exercisers have higher hair cortisol levels as well.
- Hair cortisol analysis can contribute to a more complete understanding of how long-term cortisol secretion mediates stress-related effects on health and performance.
- There is a great dearth of knowledge about the relationship between sport, exercise and hair cortisol.

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Appendix

Recommendations for exercise and sport scientists interested in using hair cortisol measurements

Elaboration of the study question

- Decide whether you are interested in acute cortisol responses or long-term cortisol elevations. Only use hair cortisol if you are interested in long-term cortisol elevations.
- Decide which function hair cortisol has in your study: independent, dependent, moderating, mediating or confounding variable.
- Reflect whether you expect positive or negative relationships; consider that both hyper- and hypocortisolism are conceivable.
- Decide whether your study question can be answered with cross-sectional data or whether longitudinal or experimental designs are necessary. Almost all prior studies were based on cross-sectional data.
- If you plan a longitudinal study, consider that you may not take hair from exactly the same vertex position for several months as the hair needs to grow back.
- Reflect whether and how the inclusion of hair cortisol can further your field of research. If you are interested in validity issues, describe why your study is important: Make sure that you provide information about the practical relevance and possible implications of your expected findings.
- If relating hair cortisol to stress, do not only assess levels of general perceived stress, but also gather information about objective or self-reported life events.

Role of vigorous exercise

- Consider how and whether differing levels of vigorous exercise influence the study findings.
- Decide how and whether you will control for vigorous exercise (e.g. inclusion as a covariate, focus on inactive participants or athletes with equal training loads).
- Clearly define exercise intensity, i.e. light, moderate and vigorous exercise. Vigorous exercise is assumed to have a stronger influence on hair cortisol than light and moderate exercise.
- Decide whether to assess vigorous exercise via self-report or objective assessments.
- Consider not only the role of vigorous exercise, but also the one of physical fitness. The relative exercise intensity depends on the fitness level of a participant.

Instruction of participants

- Elite athletes may expect data misuse: Provide adequate information about the purpose and the importance of your study and confirm in writing that the hair cortisol samples are only examined for scientific purposes.
- Inform participants that they should not cut their hair shorter than 3-6cm before the assessment (depending on the length of the retrospective period being studied).
- Ask participants not to colour their hair between baseline and post measurement in longitudinal or experimental studies.

Controlling sources of potential confound

- Use multiple assessment tools that include medical history, questionnaires on perceived stress, life events, mental health or quality of life (to get information about different sources of stress).
- Assess information about participants' social and demographic background (e.g. gender, age, race).
- Assess information about frequency of showering, contact with (chemically treated) water, and use of hair cortisol-altering products (e.g. shampoo, other hair products).
- Assess information about sauna use (heat) and sunlight exposure.
- Assess information about diseases related to altered cortisol secretion (i.e. Cushing's syndrome), pregnancy and use of cortisol-containing medication.
- Do not use more than two 3cm hair segments (representing the last 6 months).
- Ensure that the periods measured via hair cortisol correspond with those assessed via self-report measures.

Sampling

- Ensure that your variables vary sufficiently: envisage using stratified sampling methods.
- Use power analysis to calculate the minimal/optimal sample size.

Data collection and hair cortisol analysis

- Make sure that the study personnel is well-trained and with sufficient equipment. They must work quickly and be precise (e.g. all samples must be taken at the same vertex position, as near to the scalp as possible).
- Be aware of cultural issues. For instance, the religion of some female participants may not allow uncovering their head in front of a man.
- Make sure that enough hair is taken to analyze hair cortisol concentrations.
- Get in contact with the laboratory before you start data collection; inform the laboratory about the purposes of your study and make sure that the analysis procedures remain unchanged in longitudinal data assessments; discuss the total costs of the requested analyses.

Data analysis

- Consider that relationships between hair cortisol and other variables may not be linear; consider using data analysis methods that allow examining dose-effect relationships.
- Check your data for univariate outliers; if you find outliers do not simply exclude them, but examine carefully why they have markedly increased hair cortisol concentrations.
- Explore whether your data is normally distributed. Otherwise, perform log-transformations before conducting statistical tests.

Data interpretation

- Seek collaboration with and ask for advice by experts who have founded endocrinologic knowledge.
 - Be careful when relating hair cortisol levels to health and disease; ensure that you do not over-interpret your findings.
 - Clearly define your point of reference; whether you find hypocortisolism or hypercortisolism may depend on the reference group/norm you apply; compare the mean and range of your study with other investigations.
-