Published in final edited form as:
Ann Epidemiol. 2013 December ; 23(12): 797–811.e2. doi:10.1016/j.annepidem.2013.09.006.

Correlates of Cortisol in Human Hair: Implications for Epidemiologic Studies on Health Effects of Chronic Stress

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Abstract

Assessment of cortisol concentrations in hair is one of the latest innovations for measuring long-term cortisol exposure. We performed a systematic review of correlates of cortisol in human hair to inform the design, analysis and interpretation of future epidemiologic studies. Relevant publications were identified through electronic searches on PubMed, WorldCat, and Web of Science using keywords, “cortisol” “hair” “confounders” “chronic” “stress” and “correlates.” Thirty-nine studies were included in this review. Notwithstanding scarce data and some inconsistencies, investigators have found hair cortisol concentrations to be associated with stress-related psychiatric symptoms and disorders (e.g., PTSD), medical conditions indicating chronic activation of the hypothalamic-pituitary-adrenal axis (e.g., Cushing´s syndrome) and other life situations associated with elevated risk of chronic stress (e.g., shiftwork). Results from some studies suggest that physical activity, adiposity, and substance abuse may be correlates of hair cortisol concentrations. In contrast to measures of short-term cortisol release (saliva, blood, and urine), cigarette smoking and use of oral contraceptives appear to not be associated with hair cortisol concentrations. Studies of pregnant women indicate increased hair cortisol concentrations across successive trimesters. The study of hair cortisol presents a unique opportunity to assess chronic alterations in cortisol concentrations in epidemiologic studies.

Keywords

hair; cortisol; chronic stress; correlates; assessment; analysis; determinants; psychiatric disorders

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Conflicts of Interest: None Declared

INTRODUCTION

> "It is not stress that kills us; it is our reaction to it."
>
> *-Hans Selye (stress research pioneer)*

Overview of the Stress Response

Despite its extensive study, psychosocial stress (hereafter called stress) remains challenging to describe. Investigators acknowledge stress as a process whereby a stimulus imposes demands or overwhelms an organism's ability to cope, causing physiological and psychological responses[1–3]. The magnitude, duration and quality of these responses are contingent upon the individual´s interpretation of the stressor and how well the individual adapts or copes with the stressor[3].

Two neuroendocrine pathways are activated in stress response: one, the sympathetic-adrenal-medullary axis is activated rapidly–its activation has been described elsewhere[1]. The other, the hypothalamic-pituitary-adrenal (HPA) axis, is activated less swiftly: first, the hypothalamus discharges corticotrophin-releasing hormone, stimulating the dispensation of adrenocorticotropin-releasing hormone by the pituitary into systemic circulation, which then triggers the release of cortisol by the adrenal cortex[1]. Cortisol has emerged as an important objective biological measure of stress.

Cortisol and Health

Cortisol helps maintain homeostasis in the body by aiding metabolism and immune response[4–6]. The human body secretes approximately 10mg of cortisol daily; however, excess or insufficient amounts of cortisol are synthesized and released into systemic circulation under stressful conditions[1, 7–9]. Although acute alterations in cortisol concentrations may not immediately cause disease, long-term alterations of the HPA axis are associated with adverse health outcomes. For example, high concentrations of cortisol have been associated with Cushing's disease[10], diabetes complications[11], adverse perinatal outcomes[12], and myocardial infarction[13]. Low cortisol concentrations have been associated with Addison's disease[14], endometriosis[15], chronic pelvic pain[15, 16], and psychiatric disorders including post-traumatic stress disorder (PTSD)[17].

Measuring Chronic Cortisol Concentrations: Traditional Matrices of Cortisol

Saliva, blood, and urine, the predominant matrices from which cortisol is obtained, have been useful in establishing the diurnal profile of circulating cortisol and in elucidating the relationship between aberrations in diurnal cortisol patterns and various disorders. But these matrices have several limitations. First, they only inform about short-term cortisol concentrations, i.e. cortisol released within a few hours or one day[18]. Second, cortisol concentrations obtained using these specimens are easily influenced by a host of factors including study procedures[19], time of the day[20] and food consumption[21]. Third, the process of specimen collection can be invasive, particularly for blood, which in itself may induce stress and corresponding cortisol levels. Fourth, to deal with variability and the need to obtain approximate measurements of chronic cortisol concentrations, researchers need to take repeated measurements during 24 hours for several days[22]. This need for repeat sampling is expensive, burdensome for study participants and likely to increase incomplete sample collection and loss to follow-up. Given these limitations, investigators have searched for matrices that can ensure better, less invasive measurements of long-term cortisol concentrations. The most recent innovation is the isolation of cortisol from hair.

Why Hair? Why Now?

To the best of our knowledge, Raul *et al* were the first to examine cortisol concentrations in human hair[23]. Previously, three investigative teams[24–26] provided evidence of hair as a matrix for various glucocorticoids—for example, hair was used to detect exposure to corticosteroids, amphetamines and anabolic steroids in athletes[26]. Hair allows for retrospective assessment of long-term cortisol concentrations because it grows over weeks, months and years (e.g., 18 month hair cortisol concentrations were assessed in [27, 28]). Collecting hair is less invasive than obtaining blood and hair can be stored easily (Table 1). The proposed mechanisms by which cortisol is incorporated into hair is beyond the scope of this review. However, we refer interested readers to a highly relevant review on this topic[29].

Correlations of Hair Cortisol Concentrations with Other Measures of Chronic Stress

In efforts to assess the validity of hair cortisol as a biological marker of chronic stress, investigators have examined the correlations of hair cortisol concentrations (HCC) with cortisol concentrations from repeated samplings of other matrices. Van Holland *et al* found that HCC was moderately correlated with mean salivary cortisol concentrations taken on three days ($r=0.41$, $p=0.03$, samples were taken at six time points on each day)[30]. Similarly, Vanaelst *et al* found that HCC was significantly correlated with area under the curve for salivary cortisol collected over two consecutive days (samples were taken at four time points on each day)[31]. Statistically significant correlation between 24-hour urinary cortisol concentrations and HCC has been reported[32], though no statistically significant correlations were found between HCC and cortisol in one-time samples of morning blood serum[32] and blood serum collected after an overnight fast[31]. Additionally, investigators have examined correlations of HCC with scores on the perceived stress scale (PSS), a widely used self-reported measure of chronic stress over a 4-week period. Overall, results have been mixed with some investigators reporting correlation coefficients of <0.10 in studies of young adults[33] and in a racially diverse adult sample[34]; and correlations of 0.2 in adrenal insufficiency patients[35], 0.24 in chronic pain patients and controls[36] and 0.47 in pregnant women[37]. On balance, available evidence suggests positive associations of HCC with PSS scores and repeated measures of cortisol from other matrices.

Correlates of Hair Cortisol

A previous review from 2012[29] found limited data on relevant correlates of HCC. Yet, as this is a rapidly developing field, we performed a systematic review of all relevant literature to shed light on correlates of HCC, broadly encompassing factors that may be important determinants, confounders, effect modifiers, interactions and mediators that could influence the relationship between HCC and covariates of interest in epidemiologic studies.

Firstly, it is inevitably of principal interest to understand how hair-related factors (e.g., natural hair color, frequency of hair wash) might affect cortisol concentrations in hair. Second, it is important to examine whether hair cortisol concentrations reflect an individual ´s subjective experience of chronic psychological stress and related morbidities or disorders. Lastly, the relationships between cortisol concentrations in hair and important socio-demographic and lifestyle factors should be assessed. Our systematic review summarizes evidence from existing literature on the correlates and determinants of HCC. The findings will be used to inform the design, analysis and interpretation of clinical and population-based studies that may use HCC as measures of chronic hypo or hyper-activation of the HPA axis.

METHODS

Literature Search and Paper Identification

Pertinent publications on hair cortisol, published before September 2013, were identified from PubMed, WorldCat, and Web of Science using keywords, “cortisol” “hair” “confounders” “chronic” “stress” “correlates” and “determinants.” We also perused references of identified publications to find other relevant papers. We included papers published in English and in peer-reviewed journals.

Paper Selection, Data Extraction and Data Synthesis

Human studies were the primary consideration in this systematic review. For studies to be included, the investigators had to have specified the length and weight of hair analyzed, the cortisol extraction methods, participant recruitment procedures, and comprehensible quantitative figures on the primary relationships of interest. Lastly, hair must have been taken from the posterior vertex region. Hair from the posterior vertex region is the standard for hair cortisol analysis[38]. It has been shown to have higher cortisol content and lower inter-individual variability compared to hair sampled from other regions[39]. We grouped the findings according to the following themes: hair-specific characteristics, stress-related correlates, lifestyle and behavioral factors.

RESULTS

Characteristics of Studies Included

After elimination of duplicates, abstracts and papers that did not meet the inclusion criteria, 39 studies were chosen for inclusion in this review (Figure 1). Studies were conducted in multiple countries: Brazil, Canada, China, France, Germany, Israel, The Netherlands, Switzerland, Uganda, and the United States (Supplement Table). Approximately 33% of the studies were from Germany, published by pioneers who integrated hair cortisol measurement in their clinical and population-based studies[40–52]. In the sections that follow, we provide summary results of studies that assessed HCC in relation to hair-specific characteristics, stress-related correlates, lifestyle and behavioral factors.

Hair-Related Characteristics

Natural Hair Color, Frequency of Hair Washing, Hair Dyeing and Treatment— Natural hair color appears not to be associated with HCC in humans. In their early study, Raul *et al* reported no association between natural hair color and HCC[23]. Other investigators have confirmed these findings[27, 32, 41, 42, 53]. On balance, it appears reasonable to conclude that natural hair color is not a correlate or determinant of HCC.

Investigators have speculated that the frequency and temperature at which hair is washed may be inversely associated with HCC. However, most[27, 43, 46, 51, 53], though not all[39] studies on this topic indicated no association between HCC and hair washing. Li *et al* found that in a subsample of three participants, mean HCC decreased from 30.5±9.6 pg/mg to 6.5±5.9 pg/mg in hair immersed in shampoo solution for four hours[39]. Furthermore, in a subsample of eight participants, cortisol concentrations progressively declined after immersion of hair in hot water at 40°C, 65°C, and 80°C (Table 2)[39].

As with frequency of hair washing, findings on the relationship between hair treatment and HCC have been inconsistent. Some investigators[27, 32, 51, 54] but not all[33, 46, 55] have documented substantial influences of hair dye and other hair treatments on HCC. Manenschijn *et al* found that treated hair had lower HCC compared with untreated hair while use of hair product (e.g., spray, mousse, gel and wax) on the day of hair sample

collection was not significantly associated with mean HCC[27]. In aggregate, available data suggest modest influence of hair dyes and other treatments on HCC. Hence, investigators may consider collecting information on these behaviors in future large-scale epidemiologic studies that rely on HCC as a biomarker of chronic stress.

Hair Segment—HCC has been shown to decrease as one moves distally from the scalp[42, 44, 49, 56–58]. This attenuation in cortisol concentration has been attributed to exposure to water, sunlight and other elements. Of note, Steudte *et al* reported that HCC decreased 17.4% from the proximal 3-cm segment to the second 3-cm segment, then decreased 18.3% from the second 3-cm segment to the third[49]. Manenschijn *et al*, however, found no difference in HCC among six consecutive 3-cm segments of hair from healthy women (Table 2)[27].

Other Factors—Other hair-related factors that have been studied include hair texture and ultraviolet (UV) irradiation. While HCC appears to not be influenced by curls, hair curvature, or waves[42, 43], investigators have reported that 9-hour UV irradiation, compared with no exposure, is associated with statistically significant reductions in HCC (Table 2)[39]. More studies are needed to further examine the effect of hair texture and UV irradiation on HCC.

Taken together, while the available data suggest that natural hair color and texture are not associated with HCC, data are still scarce or inconclusive on the relationship between the various hair treatments and HCC. Thus, investigators may consider collecting information on these behaviors along with precisely defining hair segment for collection and analysis in future large-scale epidemiologic studies that rely on HCC as a biomarker of chronic stress.

Stress-Related Correlates

Demographic, Social and Occupational Stressors—Socio-economic factors such as income, educational level, occupation, and neighborhood characteristics are important determinants of health and are closely related with psychosocial stress[59]. Results of studies exploring the association between these factors and cortisol concentrations in saliva, urine and blood serum have been mixed[60]. To date, only a few studies have examined the relationship between HCC and socioeconomic factors. In a sample of 333 children from 23 neighborhoods in Vancouver, Canada, Vaghri *et al* observed that maternal and paternal education were both inversely correlated with HCC ($r=-0.18$, $p=0.001$). However, in a subsample of 275 preschoolers, annual family income was not significantly correlated with HCC ($r=-0.07$, $p=0.235$) (Table 2)[61]. Chen *et al* observed no relationship between HCC and educational level in a study of adults in Nanjing, China[62].

With regard to employment-related factors, shiftwork is of increasing importance in health studies, particularly because chronic shiftwork alters the circadian rhythm of cortisol production leading to HPA dysfunction[63]. In a small but important early study, investigators observed elevated mean HCC in participants who had a fast-forward rotating shift schedule compared with those who only worked during the day (47.32 vs. 29.72 pg/mg, $p<0.001$)[64]. However, there appeared to be an effect of age on the relationship between HCC and shiftwork (Table 2)[64]. Unemployment has also been examined in relation to HCC, however with mixed results. Dettenborn *et al* found higher HCC in unemployed individuals compared to those employed[41].

Age—Results of studies on the relationship between age and cortisol in other matrices have been quite mixed with investigators reporting both positive[66, 67] and negative associations[68]. With regard to the hair cortisol literature, some[23, 27, 34, 43, 46, 53, 54,

61, 62, 65, 69, 70], but not all[42, 51] investigators have reported no relationship between age and HCC. However, inferences from most studies documenting no associations are hindered by limited variability in the age of study participants. A study of 360 individuals aged 1 to 91 years suggests a complex non-linear relationship between age and HCC[42]. Across the age spectrum, the authors found that HCC were elevated in children <10 years old and in adults aged 50–91 years[42]. Furthermore, HCC was inversely related with age (in months) of children ≤5 years ($r=-0.428$, $p=0.023$).

In a smaller study with a comparable age range (2–90 years old), Raul *et al* found no association of age with HCC[23]. Available data suggest a complex relationship of HCC with age. Studies of individuals across broader age spectrums are needed to more thoroughly explore the relationship between age and HCC.

Sex—Findings on the relationship between sex and cortisol concentrations in other matrices have been inconsistent with some investigators finding higher concentrations in men[71] and others finding higher cortisol concentrations in women[66]. HCC studies on this topic have also been mixed with some investigators finding associations between HCC and sex[34, 42, 44, 46, 69, 72] and others finding none[23, 27, 33, 51–54, 61, 62, 65, 70, 73]. Dettenborn *et al* found higher HCC among males than among females in two age groups: adults aged 18–49 years and children <10 years of age. However, no differences were found in adults >49 years nor in individuals aged 10–17 years (Table 2)[42].

Race and Ethnicity—Race and ethnicity are important indicators of exposure to social stressors[74]. Equally vital, they are determinants of hair texture and hair growth rate[75]. Yet race and ethnicity have not been adequately studied in HCC studies. The average rate of hair growth used in most hair cortisol studies and other hair analysis is 1-cm/month, however, hair growth is known to vary between 0.7cm and 3.6cm per month[76]. Loussouarn *et al* observed lower hair density and hair growth rate in Africans compared with Caucasians[75]. These findings are consistent with racial differences in hair growth characteristics. In a study by O'Brien *et al*, participants were classified into two groups: minorities (African-Cuban, Afro-Cuban, Asian, Brazilian, Indian, Latino-Hispanic and Pacific Islander) and non-minorities (European or white American), and investigators observed no relationship between racial category and HCC[34]. However, the authors found a significant interaction of race and socio-economic status (SES) on HCC with minorities having elevated HCC at low and high SES[34]. Additional studies are needed to ascertain variations in hair growth rate among diverse populations. Considering inconsistencies in findings concerning HCC in relation to gender and given the importance of race and ethnicity as important indicators of social stressors, hair texture, and hair growth rate, investigators should account for sex, race and ethnicity in the design, analysis, and interpretation of hair cortisol studies.

Psychiatric Symptoms and Disorders

Despite some inconsistencies, findings from studies examining cortisol concentrations in saliva, blood, and urine generally point to a relationship between HPA dysregulation, psychiatric disorders, and aberrations in cortisol concentrations or diurnal cycles[17, 56, 77, 78]. A meta-analysis of 47 studies that examined salivary, blood plasma/serum, and urinary cortisol showed that compared with no-trauma controls, PTSD patients had suppressed morning cortisol concentrations and afternoon/evening cortisol concentrations[17]. Studying the association of HCC with stressful events and psychological symptoms or disorders is particularly important for establishing the validity of hair cortisol as a biomarker of chronic stress.

Using hair samples that corresponded to 1 to 2 months after the 2008 Wenchuan earthquake, Luo *et al* determined that HCC were elevated in PTSD and traumatized controls (TC) compared with non-traumatized controls (NTC) (Table 2). Furthermore, for an additional 2 to 4 months after the earthquake, TC had the most elevated average HCC compared with the other two groups (Table 2)[56]. Steudte *et al* also observed higher HCC in individuals with PTSD compared to TC (60% of PTSD patients and 22.2% of TC experienced traumatic events in the past year)[48]. However, in a separate study, Steudte *et al* observed lower HCC in PTSD patients and TC compared to NTC (75% of participants had experienced their most traumatic event >5 years ago) and no difference in HCC of PTSD patients and TC[50]. Furthermore, they found no associations between salivary cortisol levels and traumatization or PTSD[50]. On balance, available data indicate that trauma exposure and PTSD are associated with aberrant HCC, and that while cortisol levels may rise shortly after trauma, they may decrease over time. Like trauma, serious adverse life events, frequently precursors to impaired mental health, have also been associated with HCC[33, 79].

Depression has also been studied in relation to HCC, however the results have been mixed. One study found that depressed patients had elevated HCC compared with healthy controls[40]. In another study of cardiac rehabilitation patients, investigators observed no significant difference in HCC between depressed and nondepressed subjects[55]. The authors suggested that the absence of an association may be due to already elevated stress and cortisol levels in the cardiac rehabilitation population[55]. Investigations of the relationship between HCC and scores on the Beck Depression Index (BDI) have found both negative associations[80] and no associations[50, 80]. Saleem et al found no difference in prevalence of depression between normal HCC and high HCC subjects [65].

Other psychiatric conditions that have been studied in hair cortisol research include bipolar disorder and generalized anxiety disorder (GAD). Manenschijn *et al* found no overall difference between mean HCC of bipolar disorder patients, most of whom were receiving pharmacological treatment, compared with healthy controls[73]. However in a subsample, it was observed that individuals with older age of onset (≥30 years) had higher HCC compared with those with early onset (<30 years old) (Table 2). The authors suggested that stressful life events and HPA axis dysregulation may play a role in later development of bipolar disorder while early onset may be related to genetics or fluctuations in sex hormones[73]. Finally, Steudte *et al* examined cortisol concentrations for GAD patients and controls. While they found no difference in area under the diurnal curve for 24-hour salivary cortisol concentrations between the two groups, GAD patients had lower HCC compared with controls (Table 2)[49]. The investigators suggested that the experience of participating in a study might have contributed to acute fluctuations in salivary cortisol concentrations such that no differences in salivary cortisol concentrations were detectable. They also reasoned that HCC might be a more accurate measure of long-term HPA axis activity as it is not easily susceptible to acute influences[49].

Finally, in a sample of young school children, HCC significantly increased after school entry, but only in children who scored high on the fearfulness subscale of the Child Behavior Questionnaire[53]. Collectively, findings suggest that trauma exposure, psychiatric disorders, serious averse life events and symptoms of negative moods and emotions may be associated with HCC. More studies are needed to firmly establish HCC as a clear biomarker of chronic stress, and to elucidate the temporal relationship of HCC deviations with psychiatric disorders while jointly considering the mediating effects of stressors and stress response.

Other Medical Conditions

Adrenocorticoidal Conditions—Cushing's syndrome, Addison's disease and other conditions affecting the hypothalamus, pituitary or adrenal cortex are associated with aberrations in cortisol concentrations or diurnal patterns in saliva, blood serum/plasma and urine[81]. Studies examining HCC and adrenocorticoidal conditions have been consistent with these findings. In a study of adrenal insufficiency (AI) patients receiving hydrocortisone replacement therapy and household partner controls, AI patients had higher HCC than controls. HCC was also positively correlated with glucocorticoid dose[35]. The authors speculated that patients with adrenal insufficiency may be over-treated and could be at risk for adverse effects of elevated cortisol concentrations.

Investigators examining the relationship between Cushing's syndrome and HCC have found significantly higher HCC in Cushing's patients compared with healthy controls (Table 2)[27, 28, 54]. In their study of non-obese healthy controls and patients with cyclic Cushing's syndrome, Manenschijn *et al* observed 86% sensitivity and 98% specificity for Cushing's syndrome, using the upper-limit reference range for non-overweight healthy controls (75.9 pg/mg hair)[54]. These findings on Cushing's syndrome lend support to the capability of hair as a valid matrix of chronic cortisol exposure.

Chronic Pain, Cardiovascular Disease and Metabolic Syndrome—Results support an association between aberrations in cortisol concentrations from other matrices and cardiovascular disease[82–84], cardio-metabolic syndromes[85] and chronic pelvic pain[15, 16]. Positive significant associations have been observed of high HCC with severe chronic pain[36], one year verbal memory and history of coronary artery bypass graft surgery in cardiac rehabilitation patients[65], cardiovascular disease and events[13, 69].

The results on cardio-metabolic parameters have been mixed. HCC has not been associated with diastolic blood pressure (BP)[27, 34, 65], and while O'Brien *et al* found a significant positive correlation between HCC and systolic BP ($r=0.25$, $p<0.01$)[34], two other studies observed no significant association[27, 65]. Stalder *et al* observed a positive correlation in unadjusted analysis of HCC and mean arterial pressure, but after adjustment, the correlation did not exist ($r=0.047$, $p>0.05$). They also observed mixed results between HCC and cardio-metabolic parameters (Table 2)[51]. In one study, mean values of various cardio-metabolic fitness parameters did not differ between normal and high HCC groups [65]. To date, HCC has not been associated with cancer, osteoporosis and chronic nonspecific lung diseases[69].

On balance, HCC appears to be associated with chronic disorders, particularly disorders that are strongly associated with stress (e.g., chronic pain and cardiovascular disease). There is a need for prospective studies to further elucidate the relationship of HCC with incident disease and disease progression. For example, epidemiologic studies that investigate the relationship between HCC and adrenocorticoidal conditions should take into account the duration for which individuals have had the condition, the duration of treatment, and the type of treatment that individuals receive as all these factors may influence the relationship between HCC and adrenocorticoidal conditions.

Pregnancy—Evidence of increased cortisol production during pregnancy is well-established[43, 86]. Findings on the relationship between HCC and pregnancy have been consistent with a positive association. D'Anna-Hernandez *et al* studied HCC among pregnant women and found that HCC was significantly higher in the third trimester compared with the first, and then decreased during the first 2 to 3 months postpartum (Table 2)[87]. In their study of mothers of newborns 2–4 days of age, mothers of toddlers aged 3–9 months, and control women who were nulliparous and non-pregnant, Kirschbaum *et al* found that mean HCC among mothers of newborns 2–4 days of age (their hair samples

corresponded with the third trimester) was two-fold higher than that of non-pregnant nulliparous women (Table 2)[43]. Krumbholz *et al* also found significantly elevated HCC in hair segment reflecting the last month of pregnancy and first month of delivery[58]. Given well-documented evidence of a relationship between pregnancy and cortisol increase, investigators should exclude pregnant women from hair cortisol studies unless of course when pregnancy is the focus of the research.

Early Life Adversity—In their study of term infants and preterm infants who were >25 weeks gestational age at birth, Yamada *et al* found that neonatal intensive care unit (NICU) infants had significantly higher HCC compared with healthy term (≥37 weeks) infants (mean±SD: 2.06±2.05 vs. 0.11±0.42 nmol/g, p=0.004). Total number of days on the ventilator was associated with HCC and there was no significant difference in HCC for NICU term infants and NICU preterm infants[88]. In a study of depressed patients and healthy subjects, Hinkelmann *et al* observed lower mean HCC in subjects who experienced childhood maltreatment[52]. However, Grassi-Oliveira *et al* did not observe a correlation between severity of early life stress and HCC in a sample of women seeking treatment for substance abuse[79].

Although studies on early life adversity and HCC in humans have been few, compelling results have been observed in studies of non-human primates. Two studies that examined monkeys showed that those separated from their mothers in early life had aberrant HCC when placed in new social environments compared with those that were reared by their mothers[89, 90]. Additionally, Laudenslager *et al* observed that monkeys whose social groups were relocated during the perinatal period had elevated HCC compared with monkeys raised in a constant environment[91]. On balance, results from studies of humans and non-human primates suggest that early life adversity may influence cortisol concentrations, and have long-term negative effects on development. Thus, investigators should consider early life adversity in studies of HCC where necessary. There is a need for more studies to elucidate the relationship between HCC and perinatal and other early life experiences.

Adiposity—Results of studies examining relationships between adiposity and cortisol in blood, urine, and saliva have been varied. Investigators have reported no association[92], inverse[93–95] and positive associations[96, 97] of cortisol concentrations with a full range of measures meant to reflect body fatness or adiposity. In Table 2, we provide a summary of the relatively sparse literature devoted to assessing HCC in relation to different measures of adiposity. Of note, some[27, 46, 51, 64], but not all[34, 73] available studies have found positive relationships between HCC and at least one adiposity measure including body mass index (BMI), waist circumference (WC) and waist-to-hip-ratio (WHR).

Positive associations between HCC and BMI have been reported in various populations including fast-forward rotating shift workers[64], university students and adults[51]. However, other studies have found no relationship between HCC and BMI[27, 65, 69, 73] or WC[65]. Still, others have shown HCC to be positively correlated with WC[27, 51, 69] and WHR[27, 51] but not with hip circumference[27]. Despite some inconsistencies, available data suggest that HCC may be associated with adiposity in some, but not all populations studied to date. Studies with objective measures of adiposity and central obesity are needed to further clarify suggested associations.

Lifestyle and Behavioral Factors

Alcohol Use—Studies of other matrices suggest that aberrations in cortisol concentrations are associated with alcoholism[98] and alcohol relapse[99]. Two studies have found positive

associations between HCC and high alcohol intake[45, 69]. A third study reported no associated between HCC and regular alcohol consumption (≥3 days/week), but observed positive associations between HCC and serum γ-glutamyltransferase (γGT)[51]. Overall, these findings suggest a positive relationship between high alcohol intake and HCC. More studies are needed to explore the relationship between HCC and alcohol use or abuse. Investigators should ascertain alcohol consumption among study participants and examine alcohol's effect on HCC where necessary.

Cigarette Smoking—Evidence suggests that nicotine alters the HPA axis and acutely increases cortisol concentrations in saliva, urine and blood plasma[100–104]. However, the association may only be acute[101]. Thus far, HCC has not been associated with cigarette smoking[42, 44, 46, 51, 69]. More studies are needed to provide further evidence about the relationship between cigarette-smoking status and HCC, especially since smokers report higher levels of stress than non-smokers[105].

Oral Contraceptives and other Medications—The results of studies examining the effect of oral contraceptive use on cortisol concentrations in other matrices have been inconsistent. A summary of the relationship between oral contraceptive use and cortisol concentrations in blood plasma and saliva is given by Dettenborn *et al*[42]. So far, investigators have reported no significant positive or negative relationships between HCC and oral contraceptive use[42, 46, 50]. See Table 2 for report of findings on the association between HCC and other medications.

Physical Activity—Investigators have previously reported on the influence of moderate to high intensity physical activity to acutely raise concentrations of circulating cortisol in blood[106]. Positive associations have been observed for HCC and participation in endurance sports[44] and vigorous physical activity. However, no associations have been observed for moderate physical activity[70]. Further, Stalder *et al* observed no association between HCC and physical activity level as assessed by summing up 5-point frequency ratings in the areas of mild, moderate and vigorous physical activity over the preceding 6 months[51]. On balance, evidence suggests an association between HCC and vigorous physical activity.

DISCUSSION

The study of correlates of hair cortisol concentrations is still at an early stage. Yet, available data obtained through this systematic review are suggestive of the validity of this biomarker. Importantly, our findings suggest that hair cortisol may be associated with early life adversity, stress-related psychiatric conditions, and medical conditions indicating chronic activation of the HPA axis or high stress levels. Further research is needed to underpin the validity of the biomarker as well as to obtain deeper understanding of its potentially different associations to reactive psychiatric conditions versus psychiatric disorders with stronger hereditary etiology.

The literature suggests that some factors (e.g., natural hair color, cigarette smoking, oral contraceptive use, and medication use) may not have important influences on HCC. Other factors, such as sex, adiposity, hair treatments, and substance abuse, may be potential determinants of HCC and thus investigators may want to include these measures in future studies. For a number of other factors (e.g., age, physical activity and hair texture) available evidence are insufficient, particularly since most existing hair cortisol studies assess the effect of socio-demographic and hair-specific behaviors secondary to some other primary outcomes. As such, studies were not usually conducted in ways that maximized opportunities for comprehensive assessments of these secondary covariates[42].

Over the last decade, hair as a measure of cortisol has emerged as a promising biomarker of chronic stress and alterations of the HPA axis. Hair cortisol concentrations have shown favorable intra-individual variability in some studies. In one study, Stalder *et al* observed a relatively strong correlation between HCC after an average 375.3 day interval ($r=0.78$, $p<0.0001$, adjusted for perceived stress scores and number of competitions per year)[47]. Three repeated measurements at two month intervals showed correlation coefficients ranging from 0.53–0.79 ($p<0.001$)[47]. These findings support the reliable intra-individual stability of HCC.

Many gaps in understanding the fidelity and determinants of hair cortisol remain. One major limitation of current hair cortisol research is that most studies have been cross-sectional. Little is known about how HCC change across the human life-course; about the degree with which HCC changes across preclinical and clinical manifestations of disease; how HCC change with pharmacological and non-pharmacological treatments of conditions including mood and anxiety disorders; and how time since last episode and severity of illness influence HCC.

Other under-studied areas include the influences of diet, hair loss, and seasonal variations on HCC and the extent to which different methods of laboratory analysis affect HCC observed and reported in studies[75, 76, 107]. To date, there have been few studies of large representative populations and few studies have included ethnically, racially and economically diverse populations. Studies conducted in increasingly diverse populations will enrich the literature and provide investigators with much needed information that can be used to design, analyze and interpret HCC in clinical and population based studies.

Our assessment of correlates of HCC suggests that hair cortisol may indeed constitute an important biomarker of HPA-axis dysregulation and chronic psychosocial stress. Investigators who elect to integrate this emerging biological marker into their studies should be strategic about accounting for covariates that may confound or modify associations of primary interests. Results of hair cortisol studies should be interpreted with consideration of appropriate factors including study size and segments of hair studied. The assessment of cortisol in hair presents unique strengths—and challenges that if overcome may revolutionize the study of the potential health effects of chronic stress in large-scale epidemiologic studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research was supported by grants from the National Institutes of Health (NIMHD: T37-MD001449; and NCRR/NCATS: 8UL1TR000170).

LIST OF ABBREVIATIONS

AI	Adrenal Insufficiency
AMI	Acute Myocardial Infarction
BDI	Beck Depression Index
BMI	Body Mass index
BP	Blood Pressure

CS	Cushing's Syndrome
γGT	γ-Glutamyltransferase
HCC	Hair Cortisol Concentrations
HPA	Hypothalamic-Pituitary-Adrenal
NICU	Neonatal Intensive Care Unit
NTC	Non-Traumatized Controls
PTSD	Post-Traumatic Stress Disorder
SD	Standard Deviation
TC	Traumatized Controls
WC	Waist Circumference
WHR	Waist-to-Hip Ratio

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Limitations of Cortisol Concentrations Obtained from Saliva, Blood and Urine

- Only inform about short-term cortisol concentrations (24 to 36 hours)
- Easily influenced by individual and environmental characteristics
- Can be invasive, especially for blood
- Require repeated measurements during 24 hours over several days to obtain average chronic concentrations of cortisol

Gaps in Current Hair Cortisol Research

- Lack of prospective studies to elucidate the association of HCC with major disease risk as well as how HCC change over preclinical and clinical manifestations of disease, and with pharmacological and non-pharmacological treatments
- Other under-studied areas include the effects of diet, hair loss, and seasonal variations on HCC, and the extent to which the different methods of laboratory analysis affect HCC observed and reported in studies
- Little racial/ethnic variation in current studies

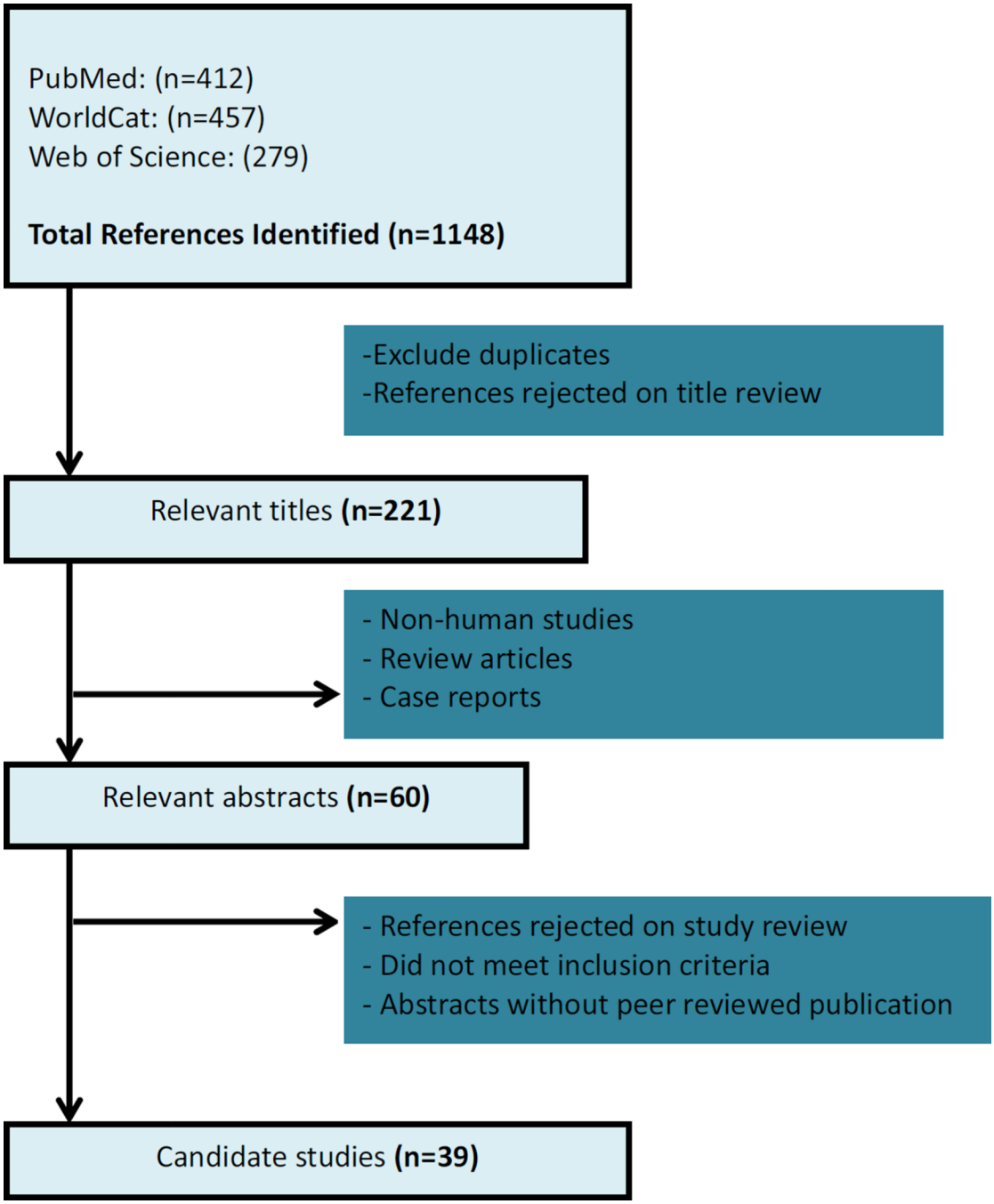

Figure 1.
Assessment of Eligibility

Table 1

Comparison of hair with other matrices of cortisol

Characteristics	Hair	Other Matrices (saliva, blood and urine)
Length of cortisol information	Chronic	Acute
Storage	Can be stored at room temperature	Require specific storage (e.g., refrigeration or freezing)
Situational and intra-individual variability	Cortisol in hair is not easily influenced by acute situational and individual factors—although evidence suggests that it might be influenced by hair hygiene and cosmetic related behavior (e.g., hair treatment and hair dyeing)	Measurements are vulnerable to factors such as time of the day, cigarette smoking, and acute stress
Repeated Measurements	Less need for repeated measurements—although some individuals might be unwilling to give hair	Need for repeated measurements—expensive, burdensome for study participants and likely to increase incomplete sample collection and loss to follow-up
Invasiveness	Small amount of hair is needed; not invasive	Although saliva and urine are less invasive, obtainment of blood can be invasive and painful for participants.

(Adapted from Stalder *et al* (2012) [9] and Russell *et al* (2012) [22]).

Table 2

Correlates of Hair Cortisol Concentrations

Study	Sample size for analysis	Summary of Findings
Hair-Related Factors		
Natural Hair Color		
Raul *et al* (2004)	8 black, 3 blond, 24 brown, 7 grey	No differences in HCC of different hair colors (p=0.9773) [AB] Mean±SD: (black: 16.63±7.99; blond: 16.97±6.84; brown: 15.52±7.85; grey: 15.86±5.21) pg/mg
Sauvé *et al* (2007)	4 blond, 4 brown, 4 black	No difference in HCC of different hair colors[CD]
Kirschbaum *et al* (2009)	142 women[E]	No difference in HCC of different hair colors (p>0.20)[CD]
Manenschijn *et al* (2011)	10 black, 75 brown, 94 blond, 6 red, 9 grey	No difference in HCC of different hair colors (p=0.413)[D]
Dettenborn *et al* (2012)	33 light blond, 36 dark brown	No difference in HCC of different hair colors (p=0.524) Mean±SD: (light blond: 20.22±11.2; dark brown: 21.88±10.4) pg/mg
Dettenborn *et al* (2012)	79 light-middle blond, 71 dark blond-light brown, 84 middle-dark brown	No significant difference in HCC of different hair colors $F_{(2,232)}$=2.538, p=0.081[D]
Groeneveld *et al* (2013)	42 children (mean age, 50.1 months, SD=0.42 months, 45% boys)[E]	HCC was not significantly related to hair color[CD]
Hair Wash Frequency and Temperature		
Kirschbaum *et al* (2009)	142 women[E]	No significant association between frequency of hair washes per week and HCC (p>0.20)[CD]
Manenschijn *et al* (2011)	50 hair samples (≤2 times per week) 143 hair samples (≥3 times per week)	No significant association between frequency of hair washes per week and HCC (p=0.673)[D]
Dettenborn *et al* (2012)	Frequency of hair washing: (0–1, 1.5–2, 2.5– 3, 3.5–4, 4.5–5, 5.5–6, 6.5–9 times per week)	No significant association between frequency of hair wash and HCC in first two segments: (first 3-cm segment: r=−0.061, p=0.335, n=249), (second 3-cm segment: r=−0.102, p=0.168, n=184). However, in third segment, HCC was inversely correlated with frequency of hair wash (r=−0.248, p=0.008, n=113)
Li *et al* (2012)	3 participants	After immersion in shampoo solution for four hours, HCC decreased from 30.5±9.6 to 6.5±5.9 pg/mg[C] Mean±SD cortisol loss ratio was 75.5±27.4%, range: 45.1–98.4%
	8 participants	After 20 hour immersion, mean HCC decreased from (at 40°C: 13.8±5.8 to 4.4±2.9 pg/mg, p<0.001), (at 65°C: 8.8±4.1 to 1.4±0.4 pg/mg, p<0.01) and (at 80°C: 8.8±4.1 to 0.6±0.2 pg/mg, p<0.01)
Stalder *et al* (2012)	155 individuals in study I (mean±SD of number of hair washes in the entire sample: 3.1±2.3)[E] 58 individuals in study II (mean±SD of number of hair washes in the entire sample: 3.7±1.6)[E]	No significant association between frequency of hair washes per week and HCC (p>0.10 in study I, p>0.30 in study II)[CD]
Groeneveld *et al* (2013)	42 children (mean age, 50.1 months, SD=0.42 months, 45% boys)[E]	HCC was not significantly related to hair washing frequency[CD]
Stalder *et al* (2013)	1258 men and women[E]	HCC was not correlated with number of hair washes per week (r = −0.046, p>0.05)[C]
Hair Dyeing and other Hair Treatments		
Sauvé *et al* (2007)	25 untreated hair, 14 dyed hair	Dyed hair had lower HCC (p=0.036)[D]

Study	Sample size for analysis	Summary of Findings
Dowlati *et al* (2010)	5 dyed hair, 78 non-dyed hair	No difference in mean HCC of dyed and non-dyed hair (4.77±0.70 vs. 4.95±0.57, p=0.49)
Karlén *et al* (2011)	95 students (40 students had colored/permed hair)	There seemed to be a difference between mean HCC of colored/ permed hair vs. non colored/permed hair (mean±SD 13.0±16.0pg/mg vs. 24.8±40.9 pg/mg, p=0.09), but it was not statistically significant
Manenschijn *et al* (2011)	152 untreated hair, 43 treated hair (women) 90 used hair products, 104 did not use hair products	Treated hair (dyed, bleached, permanent waved/straightened) had lower mean HCC than untreated hair (24.27 vs. 29.38 pg/mg, p=0.051) Use of hair product (hair spray, mousse, gel, wax) on the day of hair collection was not significantly associated with HCC: 24.83 pg/mg (product use) vs. 28.44 pg/mg (no product use), p=0.109
Manenschijn *et al* (2012)	96 healthy, non-obese controls, 14 patients with Cushing's Syndrome, 6 patients suspected of cyclic Cushing's syndrome[E]	HCC was slightly lower in healthy women with treated hair, i.e. hair dyeing or bleaching (p=0.06)[D] No difference between HCC of treated and untreated hair in Cushing's patients[CD]
Stalder *et al* (2012)	Study I: 59 treated hair, 96 untreated hair Study II: 21 treated hair, 37 untreated hair	Study I: No difference between treated semi-permanent color, coloration or permanent wave) and untreated hair (p>0.10)[CD] Study II: No difference between treated (as described above) and untreated hair (p>0.30)[CD]
Stalder *et al* (2013)	1258 men and women[E] (15.5% reported use of hair treatment)	HCC was negatively associated with hair treatment after adjustment for sex ($F_{1, 1195}$=4.22, p=0.04, ηp^2=0.0004)
Hair Segment		
Xie *et al* (2011)	8 female participants	Average linear HCC decline rate of 2.9±0.6 pg/mg (r=−0.948, p<0.05) per centimeter in the first five 1-cm hair segments
Manenschijn *et al* (2011)	28 healthy women	HCC was not significantly different in six consecutive 3-cm segments (p=0.249)[D]
Steudte *et al* (2011)	30 participants	HCC decreased 17.4% from the proximal 3-cm segment to the second 3-cm segment, then decreased 18.3% from the second 3-cm segment to the third
Dettenborn *et al* (2012)	155 individuals	HCC decreased by 16.32% from first 3-cm hair segment to the second hair segment (p<0.001). HCC also decreased 12.29% from second 3-cm hair segment to third (p<0.001)
Skoluda *et al* (2012)	273 individuals	HCC decreased from proximal to distal hair segments ($F_{(1.55, 273.05)}$=131.9, p<0.001)[D]
Luo *et al* (2012)	20 non-traumatized controls[E]	HCC linearly declined from proximal to distal in the nontraumatized control group (F=41.07, p <0.0001)
Krumbholz *et al* (2013)	1 woman (hair examined across pregnancy)	HCC decreased by >50% after 4 months of hair growth
Hair Texture and UV-Irradiation		
Kirschbaum *et al* (2012)	142 women[E]	Hair curvature was not significantly related to HCC (p>0.20)[D]
Dettenborn *et al* (2012)	360 individuals (70% of whom were aged 18–49 years old)[E]	There was no influence of curls (p=0.468)[D] or waves (p=0.633)[D] on HCC when compared with straight hair in individuals aged 18–49 years old
Li *et al* (2012)	12 hair samples exposed to 9-hour UV irradiation	HCC decreased from 26.1±8.8pg/mg to 18.9±8.0pg/mg (p=0.047) The average loss ratio in HCC was 26.5±20.9% (r=0.616, p<0.05)
Stress-Related Factors		
Demographic, Social and Occupational Factors		

Study	Sample size for analysis	Summary of Findings
Dettenborn *et al* (2010)	31 unemployed, 28 employed	Unemployed individuals had higher HCC for first 3-cm segment ($p<0.05$)[D] and second 3-cm segment ($n=52$, $p<0.05$)[D]
Manenschijn *et al* (2011)	33 shift workers, 89 day workers	Workers who had a fast-forward rotating shift schedule had higher mean HCC compared with those who only worked during the day (mean(95% CI): 47.32(38.37–58.21) vs. 29.72(26.18–33.73) pg/mg, $p<0.001$). However, in individuals <40 years of age, shift workers had higher HCC (mean(95%CI): 48.53(36.56–64.29) vs. 26.42(22.91–30.55) pg/mg, $p<0.001$) while for those >40 years, there was no effect of shiftwork. There were no individuals who were exactly 40 years old
Chen *et al* (2013)	Cohort A (13 had elementary school education, 34 had high school education or below, 6 had college education or above) Cohort B (4 had elementary school education, 27 had high school education and below, 19 had college education or above)	No significant difference in HCC among all three educational groups in cohort A ($F=0.31$, $p=0.74$) and in cohort B ($F=1.98$, $p=0.15$)
Groeneveld *et al* (2013)	42 children (mean age, 50.1, SD=0.42 months); mean parental educational was 14.8 years (SD=1.9)[E]	HCC was not significantly related to parents' educational level[CD]
Saleem *et al* (2013)	56 cardiac rehabilitation patients with CAD (26 with normal HCC (median 111 ng/g) and 30 with elevated HCC (median 263 ng/g)	Normal vs. high HCC group: total years of education (mean±SD: 16.5±3 vs 17±3.5 years, $p=0.45$), married (88.5% vs. 73.3% married, $p=0.20$)
Vaghri *et al* (2012)	333 preschoolers	Parental income was inversely correlated with HCC from 1–2cm hair segment ($r=-0.18$, $p=0.001$); in a subsample of 275 preschoolers, annual family income was not correlated with HCC ($r=-0.07$, $p=0.235$)
Age		
Raul *et al* (2004)	44 individuals	Linear regression predicting HCC from age ($\beta=0.0296$, standard error=0.0541, $p=0.139$)[AB] There was no relationship between HCC and age category (mean ±SD: 0–15 years: 15.2±7.1; 16–30 years: 14.4±8.4; 31–50 years: 16.2±7.6; 51–90 years: 17.3±6.3 pg/mg, $p=0.878$)
Manenschijn *et al* (2011)	195 individuals (18–63 years); Mean age: 36 years	HCC was not correlated with participant age ($p=0.388$)[D]
Dettenborn *et al* (2012)	360 individuals (1–91 years)	HCC was positively related with age ($R^2=0.010$, $p=0.030$) For children 0–5 years, age in months was negatively correlated with HCC ($r=-0.428$, $p=0.023$). Children (1–9 years old, n=28) had significantly higher HCC compared with adults (n=34, 18–38 years old) ($p<0.001$)[D]
Kirschbaum *et al* (2012)	103 mothers of newborns 2–4 days of age (mean±SD age: 29.7 ±5.5years), 19 mothers of toddlers 3–9 months (mean±SD age: 30.4± 2.8years) and 20 nonpregnant nulliparas (mean±SD age: 25.8±6.4years)	HCC was not significantly associated with age across the entire group ($p>0.20$) [CD]
Manenschijn *et al* (2012)	14 Cushing's syndrome patients, 6 individuals with suspected cyclic Cushing's Syndrome, 96 healthy controls[E]	HCC was not significantly related with age in either the patient or control group[CD]
O'Brien *et al* (2012)	135 adults (18–66 years old)	No correlation of age and HCC ($r=0.07$, $p>0.05$) HCC was not significantly correlated with tertiles of age (age ranges: young=18–21 years, n=44; middle=22–29 years, n=45; older=30–66 years, n=43)
Stalder *et al* (2012)	155 individuals (18–46 years) Mean±SD age: 24.1±14.2 years	HCC was not correlated with participant age ($r=-0.02$, $p>0.05$) [CD]

Study	Sample size for analysis	Summary of Findings
Stalder *et al* (2012)	58 individuals (20–70 years) Mean±SD age: 30.5±12.1 years	HCC was not correlated with participant age (r=−0.06, p>0.05) *CD*
Vaghri *et al* (2012)	339 preschoolers (mean±SD age: 4.6±0.5 years)	No statistically significant correlation between age and log-transformed, winsorized HCC variable (r=−0.09, p=0.098)
Chen *et al* (2013)	Cohort A (23 participants 21–40 years; 29 participants >40 years); Cohort B (21 participants aged 21–40 years; 23 participants >40 years)	No significant difference in HCC of those aged 21–40 years and those >40 years in cohort A (z=−0.179, p=0.86), and in cohort B (z=−0.51, p=0.61)
Gerber *et al* (2013)	42 university male and female students	Age was not significantly associated with HCC *CD*
Manenschijn *et al* (2013)	283 elderly adults (65–85 years)	HCC was not correlated with age (r=−0.02; p=0.76)
Saleem *et al* (2013)	56 cardiac rehabilitation patients with CAD (26 in normal HCC (median 111 ng/g) and 30 in the elevated HCC (median 263ng/g)	Age was not significantly associated with HCC
Stalder *et al* (2013)	1258 men and women *E* (age: 16–64 years)	HCC was positively correlated with age (r=0.11, p<0.0001) *C*
Sex		
Raul *et al* (2004)	16 males, 26 females	No differences in HCC of males and females (mean±SD: 15.6±8.8 vs. 16.1±6.3 pg/mg, p=0.859)
Karlén *et al* (2011)	95 students (24 males, 71 females)	No difference in HCC of males and females (mean±SD: 17.8±13.4 pg/mg vs 20.6±37.7 (p=0.73)
Manenschijn *et al* (2011)	90 men, 105 women	No significant effect of gender (p=0.353) *D*
Manenschijn *et al* (2012)	Bipolar patients: 34 men, 62 women Healthy controls: 90 men, 105 women	No sex differences in HCC of bipolar patients (p=0.12) *D* and healthy controls (p=0.87) *D*
Steudte *et al* (2011)	PTSD patients: 4 men, 6 women Controls: 11 men, 6 women	No significant effects of gender on HCC ($F_{(1,23)}$=1.01, p=0.33, ηp^2=0.04) *D*
Dettenborn *et al* (2012)	252 adults (18–49 years) 52 children (1–9 years) 25 adolescents (10–17 years) 31 elderly (50– 91 years)	Males had higher HCC in first 3-cm segment compared with females for adults 18–49 years ($F_{(1,251)}$=9.573, p=0.002, ηp^2=0.037). *D* Males had higher in first 3-cm segment HCC compared with females for children 1–9 years ($F_{(1,51)}$=5.304, p=0.025, ηp^2=0.078). *D* No sex differences were seen in HCC of adolescents ($F_{(1,24)}$=0.837, p=0.370) *D* or elderly ($F_{(1,30)}$=0.064, p=0.803) *D*
Manenschijn *et al* (2012)	14 Cushing's syndrome patients, 6 individuals with suspected Cushing's Syndrome, 96 healthy controls *E*	HCC was not significantly related with sex in either the patient or control group *CD*
O'Brien *et al* (2012)	135 adults (60% female)	Men had slightly higher HCC compared with women $t_{(129)}$ = 2.91, p<0.01
Skoluda *et al* (2012)	Athletes: 304 individuals (58.9% female), Controls: 70 individuals (82.9% female)	No significant differences in HCC between the sexes in athletes (p=0.06) *D* nor in controls (p=0.62) *D* Across the entire sample, males had higher HCC ($F_{(1,372)}$=8.13, p<0.01) *D*
Stalder *et al* (2012)	Study I: N=155 (73.5% females) Study II: N=58 (67.2% females)	Study I: No significant difference in HCC of males and females ($F_{(1.154)}$=0.006, p>0.05) *CD* Study II: Non significant linear trend for higher HCC in males compared with females ($F_{(1,57)}$=3.58, p=0.06, ηp^2=0.06) *D*
Vaghri *et al* (2012)	167 preschool boys, 172 preschool girls	No statistically significant correlation between age and log-transformed, winsorized HCC variable (biserial correlation=−0.01, p=0.790)

Study	Sample size for analysis	Summary of Findings
Chen *et al* (2013)	Cohort B (41 men, 9 women)	No significant gender difference in HCC (z=−1.25, p=0.22)
Gerber *et al* (2013)	42 university students (22 women, 20 men)	Sex was not significantly associated with participants' HCC[CD]
Groeneveld *et al* (2013)	42 children (mean age, 50.1 months, SD=0.42 months, 45% boys)	HCC was not significantly related to gender[CD]
Hinkelmann *et al* (2013)	43 major depressive disorder patients, 41 age- and sex-matched controls[E]	HCC was not significantly associated with sex (p>0.10)[CD]
Manenschijn *et al* (2013)	283 elderly adults (66.1% female)	HCC were significantly higher in men than in women (median, 26.3 pg/mg hair [interquartile range (IQR), 20.6 – 35.5 pg/mg hair] vs 21.0 pg/mg hair [IQR, 16.0 – 27.0 pg/mg hair]; p<0.001)
Saleem *et al* (2013)	56 cardiac rehabilitation patients with coronary artery disease (26 in normal HCC group (median 111.4 ng/g) and 30 in the elevated HCC group (median 262.8 ng/g)	Gender was not significantly different between normal HCC group (92.3% male) and high HCC group (80% male) (p=0.19)
Stalder *et al* (2013)	1258 men and women[E] (about 84.8% were men)	HCC was not significantly with sex ($F_{1, 1216}$=1.83, p>0.05)[CD]
Race and Ethnicity		
O'Brien *et al* (2012)	135 adults categorized into two groups: minorities (African-Cuban, Afro-Cuban, Asian, Brazilian, Indian, Latino-Hispanic and Pacific Islander (n=67)); and non-minorities (European or White American (n=68))	HCC was not correlated with race (r=−0.09, p>0.05). However, there was significant interaction of race and SES on HCC ($F_{(2, 122)}$=3.26, p<0.05, ηp^2=0.16)
Psychiatric Symptoms and Disorders		
Dowlati *et al* (2010)	34 depressed patients, 87 non-depressed patients (all attending a cardiac rehabilitation center)	Mean HCC was not different between depressed and non-depressed patients (log mean±SD: 4.96±0.58 vs. 5.04±0.59, p=0.53)
Karlén *et al* (2011)	95 students (20 with serious life events)	Mean HCC were higher students who had experienced serious life events within the last 3 months vs. those who had not (mean±SD: 33.0±57.0 vs 16.3±22.5 pg/mg, p=0.045)
Steudte *et al* (2011)	10 traumatized individuals with PTSD, 16 traumatized controls without PTSD	Traumatized PTSD patients had significantly higher HCC compared with traumatized non-PTSD controls ($F_{(1,25)}$=5.35, p=0.03, ηp^2=0.18)[D]
Steudte *et al* (2011)	15 generalized anxiety disorder patients (GAD), 15 controls	HCC were significantly lower in GAD patients compared with controls for first 3-cm segment ($F_{(1,27)}$=11.80, p=0.002, ηp^2=0.304)[D] and second 3-cm segment ($F_{(1,24)}$=8.894, p=0.006, ηp^2=0.270)[D]. A non-significant trend for lower HCC in GAD patients was observed for the third segment ($F_{(1,17)}$=4.138, p=0.058, ηp^2=0.196)[D]
Dettenborn *et al* (2012)	23 depressed patients, 64 healthy controls	Depressed patients had elevated HCC in the first 3-cm (mean±SD: 26.7±20.8 vs. 18.7±11.5 pg/mg, p<0.05) and second 3-cm hair segments (mean±SD: 21.9±23.7 vs. 13.4±9.6 pg/mg, p<0.05) compared with controls
Luo *et al* (2012)	32 individuals with PTSD, 32 traumatized non-PTSD controls, 20 non-traumatized non-PTSD controls	For the hair segment corresponding to 2 months before and 1 month after the earthquake, HCC were elevated in traumatized individuals (PTSD and non-PTSD) compared with the non-traumatized non-PTSD controls (F=3.88, p=0.0499 and F=6.27, p=0.013, respectively)[D]. For the hair segment corresponding to 2 to 4 months after the earthquake, traumatized non-PTSD controls had significantly higher HCC compared with traumatized PTSD individuals (F=6.17, p=0.0137)[D] and non-traumatized non-PTSD controls (F=11.74, p=0.0007)[D]. For the period corresponding to 5 to 7 months after the

Study	Sample size for analysis	Summary of Findings
		earthquake, traumatized non-PTSD controls had higher HCC compared with traumatized individuals with PTSD (F=4.11, p=0.0438)[D]
Manenschijn *et al* (2012)	100 bipolar disorder (BD) patients, 195 healthy controls	No difference between mean HCC of BD patients and that of controls (mean(95%CI): 31.84(28.38–35.81) vs. 28.18(25.94–30.62) pg/mg, p=0.233). However, in a subsample, individuals with older age of onset (≥30 years) had higher HCC compared with those with early onset (<30 years old) (β=0.335, p=0.004)
Gerber *et al* (2013)	42 undergraduate students (22 women, 20 men)	HCC were negatively associated with depressive symptoms (as assessed using the Beck Depression Inventory (BDI)), β=−0.24, p=0.009.
Grassi-Oliveira *et al* (2012)	23 treatment seeking crack cocaine-dependent women	HCC was significantly related with negative life events exposure 90 days before hospital admission (r =0.56; p=0.007) and 30 days (r =0.42; p=0.048) prior to admission at the hospital, but not with 60 days prior to admission (r=0 .23; p =0.293)
Groeneveld *et al* (2013)	42 children (mean age, 50.1 months, SD=0.42 months, 45% boys); 21 children scored high on a fearfulness questionnaire	HCC significantly increased after the start of school, but only in children who scored high for fearfulness $F_{(1, 19)}=4.67$, p=0.04, $\eta p^2=0.20$.
Hinkelmann *et al* (2013)	43 major depressive disorder patients, 41 age- and sex-matched controls[E]	HCC was not associated with current depression, nor with atypical depression[CD]
Saleem *et al* (2013)	56 patients who completed a one-year cardiac rehabilitation program(26 of whom had normal HCC at baseline, 30 had normal HCC a baseline)[E]	The prevalence of depression did not differ between the normal HCC group (26.9%) and the high HCC group (30%) (p=0.80)
Steudte *et al* (2013)	25 PTSD patients, 25 traumatized controls, 28 non-traumatized controls	HCC was 59% lower in PTSD patients and 51% lower in traumatized controls (TC), compared to non-traumatized controls (NTC) (mean±SD HCC: PTSD 6.44±6.05 pg/mg; TC 7.78±7.01 pg/mg; NTC 15.75±14.77 pg/mg; p<0.05). No differences in HCC between PTSD and TC groups (p=0.535). HCC was not correlated with Beck Depression Index (BDI-II) scores (r= −0.127, p=0.256). HCC was inversely correlated with number of different lifetime traumatic events, frequency of traumatic experiences, time since traumatization, and severity of intrusion symptoms.
Other Medical Conditions		
Cardiovascular Disease, Cardio-Metabolic Syndrome, and Chronic Pain		
Van Uum *et al* (2008)	15 chronic pain patients receiving opioid treatments, 39 non-obese controls	Chronic pain patients had higher HCC compared with controls (mean(range): 83.1(33.0–205) pg/mg vs. 46.1(27.2–200) pg/mg, p<0.01)
Pereg *et al* (2010)	56 acute myocardial infarction patients (AMI), 56 non-AMI hospital controls	AMI patients had significantly elevated median HCC compared with non-AMI hospital controls (median (range): 295.3(105.4–809.3) vs. 224.9(76.58–949.9) ng/g, p<0.01)
Karlén *et al* (2011)	95 students (28 reported taking pharmaceuticals)	No difference in HCC according to use of pharmaceuticals (mean ±SD: 22.9±44.9 pg/mg vs 18.6±27.2 pg/mg, p=0.57)
Manenschijn *et al* (2011)	46 healthy participants	No correlation between HCC and systolic (p=0.109)[D] or diastolic blood pressure (p=0.365)[D]
O'Brien *et al* (2012)	135 adults (18–66 years, 65% female, 48% minority)	HCC was positively correlated with systolic blood pressure (r=0.25, p<0.01); however no significant correlation was observed between HCC and diastolic blood pressure (r=0.08, p>0.05)
Manenschijn *et al* (2013)	283 elderly adults (age range: 65–85 years)	HCC was significantly associated with CVD. The odds ratio (OR) was 1.9 (p=0.09) for the second, 2.0 (p=0.08) for the third and 2.7 (p=0.01) for the fourth quartile. Highest levels of HCC quartile assocated

Study	Sample size for analysis	Summary of Findings
		with type 2 diabetes mellitus (OR 3.2, p=0.04).
Saleem *et al* (2013)	56 patients who completed a one-year cardiac rehabilitation program(26 of whom had normal HCC at baseline, 30 had normal HCC a baseline)[E]	High baseline HCC (i.e. HCC ≥153.2 ng/g) predicted less improvement in verbal memory performance after one year ($F_{(1, 50)}$=5.50, p=0.02,.Number of weeks since acute coronary event, percutaneous coronary intervention, and myocardial infarction did not differ between normal and high HCC groups, however history of coronary artery bypass surgery differed. Between the normal and high HCC groups: prevalence of hypertension (53.8% vs 63.3%, p=0.47), diabetes (7.7% vs 16.7%, p=0.31), maximum heart rate (118±18 vs 121±22 bpm, p=0.55), SBP (176±24 vs 177±27 mmHg, p=0.90), DBP (76±9 vs 80±11 mm Hg, p=0.18), and maximal oxygen intake (20±5 vs 20±5, p=0.98) seemed to differ, though the p-values were not significant.
Stalder *et al* (2013)	1258 men and women[E] (about 24% had metabolic syndrome (MetS))	HCC was positively associated with metabolic syndrome (MetS): [mean(±SD), no MetS: 7.70 (±8.12) pg/mg; MetS: 10.78 (±11.37) pg/mg, p<0.0001]. Furthermore, in the adjusted partial correlations, HCC was not significantly associated with mean arterial pressure (r=0.047, p>0.05), high-density lipoprotein cholesterol (r=−0.037, p>0.05), triglycerides (r=−0.015, p>0.05), and glucose (r=0.005, p>0.05). However, HCC was associated with low-density lipoprotein cholesterol (r=−0.080, p<0.01), and glycated hemoglobin (r=0.116, p<0.001)
Adrenocorticoidal Conditions		
Thomson *et al* (2010)	6 female Cushing's syndrome patients, 32 healthy controls (21 females, 11 males)	Cushing's patients had significantly higher HCC compared with controls: (median(range): 679(279–2500) vs. 116(26–204) ng/g, p<0.001)
Gow *et al* (2011)	93 adrenal insufficiency (AI) patients on hydrocortisone replacement therapy, 62 household partners	AI patients had higher median HCC than controls (median(range): 230.7(22.7–1377) vs. 184.7(57.7–1479) ng/g, p=0.08), although the result was not significance. Hydrocortisone dose was also correlated with HCC (r=0.03, p=0.004)
Manenschijn *et al* (2011)	195 healthy controls, 9 hypercortisolemics	Hypercortisolemics had significantly elevated HCC compared with healthy controls (p<0.0001)[CD]
Manenschijn *et al* (2012)	14 patients with Cushing's syndrome (CS), 6 patients with cyclic Cushing's syndrome, 96 non-obese healthy controls	Patients with CS (excluding cyclic CS patients) had significantly elevated mean HCC compared with healthy individuals (mean(95%CI): 399.7(171.8–930.0) vs. 27.3(24.6–30.4) pg/mg, p<0.0001)
Adiposity		
Manenschijn *et al* (2011)	39 shift workers, 89 day workers	HCC positively correlated with participant BMI (b=0.262, p<0.05). HCC increased across BMI groups as follows: (mean(95%CI): (BMI<25kg/m^2: 31.26(26.79–36.48)); (BMI 25–30kg/m^2: 36.06(30.48–42.76)); (BMI >30 kg/m^2: 60.95(43.95–84.72) pg/mg, p=0.002)
Manenschijn *et al* (2011)	195 healthy people, 9 hypercortisolemic, 1 hypocortisolemic	HCC was positively correlated with waist circumference (r=0.392, p<0.05) and WHR (r=0.425, p<0.05). HCC was not significantly correlated with BMI (p=0.646)[D] or hip circumference (p=0.096)[D]
Manenschijn *et al* (2012)	Bipolar patients: 100 individuals; BMI median (IQR): 25.3(23.5–28.0 kg/m^2) Healthy controls: 195 people: BMI median (IQR): 23.7(21.7–26.5 kg/m^2)	No significant relationship between HCC and BMI in bipolar (r=0.163, p=0.11) and control individuals (r=0.042, p=0.59)
O'Brien *et al* (2012)	135 adults	HCC was not significantly correlated with waist-to-hip ratio (r=−0.03, p>0.05)
Stalder *et al* (2012)	155 adults; BMI: (mean±SD: 22.2±3.4;	HCC positively correlated with participant weight (r=0.29, p≤0.001) and BMI (r=0.33, p≤0.0001)

Study	Sample size for analysis	Summary of Findings
	range:16.5–35.8) kg/m^2	
Stalder *et al* (2012)	58 university students; BMI: (mean±SD:24.0±4.9; range:16.9–42.1) kg/m^2	HCC positively correlated with participant weight (r=0.36, p<0.05) and BMI (r=0.42, p≤0.001)
Manenschijn *et al* (2013)	283 elderly adults (65–85 years old, 66.1% female)	No significant correlation between HCC and BMI (r=0.06, p=0.36), waist circumference (r=0.11, p=0.08)
Saleem *et al* (2013)	56 patients who completed a one-year cardiac rehabilitation program(26 of whom had normal HCC at baseline, 30 had normal HCC at baseline)^E	Between the normal and high HCC groups, mean±SD of BMI did not differ (27.1±5.0 kg/m^2 vs 27.4±3.5 kg/m^2, p=0.75), neither did waist circumference (96.2±10.1 cm vs. 98.8±8.8, p=0.29)
Pregnancy		
D'Anna-Hernandez *et al* (2011)	21 non-smoking pregnant women (<17 weeks gestational age)	HCC increased across successive trimesters and then decreased during the first 2 to 3 months postpartum. HCC in third trimester was higher than in the first (t=4.1, p=0.001)^D as well as post-partum (t=2.9, p=0.004)^D
Kirschbaum *et al* (2012)	103 mothers with newborn 2–4 days of age, 19 mothers of toddlers 3 to 9 months, 20 non-pregnant nulliparous women (n=20).	HCC of mothers of newborns 2–4 days of age (their hair corresponded with the third trimester) was significantly elevated in the first 3-cm segment compared with that of control women ($t_{(1,120)}$=4.77, p<0.0001) ^{D}; however there was no significant difference in HCC of the two groups of women for segments 2 and 3
Krumbholz *et al* (2013)	1 woman (HCC over a pregnancy)	The highest HCC (13.4 pg/mg) was observed for the hair segment that reflected the last month of pregnancy and first month postpartum
Early Life Adversity		
Yamada *et al* (2007)	60 NICU infants > 25 weeks gestational age, NICU term infants, healthy term infants	HCC for NICU infants was elevated compared with HCC for healthy term infants (mean±SD: 2.06±2.05 vs. 0.11±0.42 nmol/g, p=0.004). Total number of days on the ventilator was associated with HCC (increased 0.2 nmol/g, p=0.03, on average, for each additional day). There was no difference between HCC of term infants in NICU and HCC of preterm infants in NICU
Grassi-Oliveira *et al* (2012)	23 treatment seeking crack cocaine-dependent women	No correlation between HCC and severity of early life stress ^{CD}
Hinkelmann *et al* (2013)	43 major depressive disorder patients, 41 age- and sex-matched controls^E	HCC was lower in subjects who experienced childhood maltreatment compared to controls ($F_{1,71}$=4.11, p=0.05)^C
Steudte *et al* (2013)	25 PTSD patients, 25 traumatized controls, 28 non-traumatized controls^E	No significant correlation was observed between HCC and childhood trauma scores (r=-0.144, p=0.197)
Lifestyle and Behavioral Factors		
Alcohol Use		
Stalder *et al* (2010)	23 alcoholics in acute withdrawal, 25 abstinent alcoholics, 20 controls	HCC (mean±SD) was highest in acute withdrawal alcoholics (51.99±43.30 pg/mg), compared with abstinent alcoholics (13.98±10.63 pg/mg, p<0.001) and controls (16.35±12.59 pg/mg, p<0.001). No
		difference between HCC of abstinent alcoholics and controls (p=0.91)
Manenschijn *et al* (2013)	283 elderly adults (17.4% who had never drank, 52.8% light drinkers, 26.2% moderate drinkers, 3.6% (very) excessive drinkers)	HCC was positively associated with alcohol consumption. HCC were 21.2 pg/mg hair (IQR, 15.2–29.4) in non-drinkers, 21.7 pg/mg hair (IQR, 16.9–28.9) in light drinkers, 24.5 pg/mg hair (IQR, 17.1–36.3) in moderate drinkers, and 30.4 pg/mg hair (IQR, 25.0–45.0) in excessive drinkers (p=0.05)

Study	Sample size for analysis	Summary of Findings
Stalder *et al* (2013)	1258 men and women[E] (43% reported regular consumption of ≥3 alcoholic drinks/week)	No association between HCC and self-reported alcohol consumption ($F_{1, 1208} = 0.11$, $p>0.05$)[D]; HCC was positively associated with serum γ-glutamyltransferase (γGT), ($r=0.11$, $p<0.0001$)
Cigarette Smoking		
Dettenborn *et al* (2012)	18–49 year olds[E]	No influence of smoking status on HCC ($p=0.836$)[D]
Stalder *et al* (2012)	Study I: 155 adults (17.6% smokers), Study II: 58 students at the Technical University of Dresden (27.6% smokers)	Study I: No association between smoking status and HCC ($p>0.10$)[D] Study II: No association between smoking status and HCC ($p>0.30$)[D]
Skoluda *et al* (2012)	304 amateur endurance athletes (7.1% habitual smokers); 70 controls (20% habitual smokers)	Smoking was not associated with HCC ($F_{(1, 364)}=0.02$, $p=0.88$)[D]
Hinkelmann *et al* (2013)	43 major depressive disorder patients; 41 age- and sex-matched controls[E]	HCC was not significantly associated with smoking status ($p>0.10$)[CD]
Manenschijn *et al* (2013)	283 elderly adults (38.2% never smoked; 52.7% former smokers, 8.8% current smokers)	No difference in HCC based on smoking status. HCC were 21.2 pg/mg hair (IQR, 16.6–26.9) in those who never smoked, 22.1 pg/mg hair (IQR, 17.4–33.6) in former smokers, and 26.3 pg/mg hair (IQR, 18.7-32.3) in current smokers ($p=0.22$)
Stalder *et al* (2013)	1258 men and women[E] (33.5% smokers)	HCC was not associated with smoking status ($p>0.05$)[CD]
Oral Contraceptives and Medication Intake		
Dettenborn *et al* (2012)	(16.7% of 360 individuals in the sample used oral contraceptives)[E] 252 individuals aged 18–49 years old[E] 29 elderly individuals (medication intake analysis)[E]	No influence of oral contraceptive use on HCC in 18–49 year old women ($p=0.110$)[D] No influence of overall medication intake on HCC in 18–49 year olds ($p=0.610$)[D] No influence of medication intake in elderly group ($F_{(1,29)}=0.245$, $p=0.625$)[D]
Stalder *et al* (2012)	Study I: 72 oral contraceptive users, 42 not taking oral contraceptives Study II: 16 oral contraceptive users, 23 not taking oral contraceptives	Study I: No association between oral contraceptive use and HCC ($p>0.10$)[D] Study II: No association between oral contraceptive use and HCC ($p>0.30$)[D]
Groeneveld *et al* (2013)	42 children (mean age, 50.1 months, SD=0.42 months, 45% boys)[E]	HCC of children was not related to use of corticosteroids or other medications[CD]
Hinkelmann *et al* (2013)	43 major depressive disorder patients, 41 age- and sex-matched controls[E]	HCC was not associated with antidepressant treatment ($p>0.1$)[D]
Saleem *et al* (2013)	56 patients who completed a one-year cardiac rehabilitation program(26 of whom had normal HCC at baseline, 30 had normal	Use of concomitant medications seemed to differ with specific drugs between normal and high HCC groups, however no statistically significant difference was observed; beta-blocker (80.8% vs 70%, $p=0.35$); calcium channel blocker (26.9% vs 10%, $p=0.10$); diuretics (23.1% vs 20%, $p=0.78$);
	HCC at baseline)[E]	antihypertensives (50% vs 63.3%, $p=0.32$); antidiabetics (3.8% vs 16.7%, $p=0.12$); antidepressants (3.8% vs 10%, $p=0.37$) ; anxiolytics (11.5% vs 3.3%, $p=0.23$)
Steudte *et al* (2013)	25 PTSD patients (36% used regular medication (RM)), 25 traumatized controls (20% used RM), 28 non-traumatized controls (29% used RM)[E]	No relationship between HCC and medication intake ($p>0.31$)[D]
Steudte *et al* (2013)	25 PTSD patients (96% female, 7 women used oral contraceptives (OC)), 25 traumatized controls (92% female, 4 used OC), 28 non-traumatized controls (89 female, 9 used OC)[E]	No relationship between HCC and oral contraceptive use ($p>0.31$)[D]

Study	Sample size for analysis	Summary of Findings
Physical Activity		
Skoluda *et al* (2012)	304 amateur endurance athletes, 70 controls	On average, athletes had higher HCC compared with controls (mean±SD: 18.18±9.6 vs. 12.43±6.2 pg/mg; and gender did not alter the effect of endurance sports on HCC, p=0.048). Additionally, they observed a significant correlation between HCC and training kilometers run per week (r=0.32, p<0.001), training hours per week (r=0.22, p<0.001) and number of competitions per year (r=0.29, p<0.001), but not with number of training years (r=0.008, p=0.89).
Stalder *et al* (2013)	1258 men and women[E] (physical activity score range: 3–15)	No association of HCC with light or moderate physical activity; positive association with vigorous physical activity (p>0.05)[CD]
Gerber *et al* (2013)	42 university students (vigorous physical activity: 0–213 weekly minutes; moderate physical activity: 164–775 weekly minutes)	HCC positively correlated with vigorous physical activity (β=0.33, p=0.05, ?R^2=0.106). HCC was not significantly correlated with moderate physical activity (r=−0.08, p>0.05)
Diet		
Stalder *et al* (2013)	1258 men and women[E] (self-reported daily fruit and vegetable consumption)	No association between HCC and fruit and vegetable consumption (p>0.05)[CD]

[A] We used SAS 9.2 to calculate these values

[B] We excluded two outlier values

[C] Authors did not report exact p-values

[D] Authors did not report mean HCC, median HCC, correlation or odds ratio

[E] Sample size used in analysis might be different from total sample size (and/or) authors did not specify the proportion of subjects in comparison groups