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Salivary Cortisol and Cold Pain Sensitivity in Female Twins

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Abstract

Background—There is a dearth of knowledge about the link between cortisol and pain sensitivity.

Purpose—We examined the association of salivary cortisol with indices of cold pain sensitivity in 198 female twins and explored the role of familial confounding.

Methods—Three-day saliva samples were collected for cortisol levels and a cold pressor test was used to collect pain ratings and time to threshold and tolerance. Linear regression modeling with generalized estimating equations examined the overall and within-pair associations.

Results—Lower diurnal variation of cortisol was associated with higher pain ratings at threshold ($p = 0.02$) and tolerance ($p < 0.01$). The relationship of diurnal variation with pain ratings at threshold and tolerance was minimally influenced by familial factors (i.e., genetics and common environment).

Conclusions—Understanding the genetic and non-genetic mechanisms underlying the link between HPA axis dysregulation and pain sensitivity may help to prevent chronic pain development and maintenance.

Keywords

cortisol; pain; cold pressor; genetics; twins

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INTRODUCTION

Chronic pain is a serious public health problem that reduces quality of life and productivity (1, 2). The annual cost of chronic pain in the U.S. is estimated at $560-$635 billion, including medical expenses as well as productivity losses (1). Despite the high prevalence and heavy burden of chronic pain, the etiology and pathophysiology of numerous chronic pain conditions remain largely unknown. Theories across many disciplines point to a complex network of biological, psychological, and social elements working in bidirectional pathways to generate the experience of chronic pain (1). Notably, research has implicated psychological stress in the development and maintenance of many chronic pain conditions (3), raising important questions about how stress “gets under the skin” (4).

The hypothalamus-pituitary-adrenal (HPA) axis, which connects sensory information with the central nervous system, may be one pathway by which stress influences the perception of painful stimuli (5). In response to perceived stressors, the HPA axis initiates a cascade of hormones that affect numerous physiological functions (6). Cortisol is one product of this hormonal cascade that has been widely studied because of its influence on cognition, metabolism, and immune function, and its role in linking stress and disease (6). Cortisol levels naturally fluctuate during the day and are elevated in response to acute stressors (7). However, unlike the adaptive HPA response to acute stress, chronic stress can alter or dysregulate HPA axis function, resulting in overproduction of cortisol (hypercortisolism), underproduction of cortisol (hypocortisolism), a flattened cortisol awakening response (CAR), or a flattened change from morning to evening cortisol levels (diurnal variation) (6-8). This HPA axis dysregulation is associated with numerous pain conditions, although the mechanisms that link HPA axis function and chronic pain are not well understood (9-11).

Previous studies have examined HPA axis dysregulation in chronic pain populations as well as the relationship between cortisol and pain sensitivity in these individuals. Chronic pain conditions such as fibromyalgia (FM), chronic widespread pain (CWP), and chronic fatigue syndrome are associated with changes in HPA axis function (8, 10, 12-14). Findings from a prospective population-based study suggest that participants who later developed CWP were more likely to have higher evening, lower morning, and higher post-suppression test cortisol levels than those who did not. Although these results have not been independently replicated, they suggest that HPA axis dysregulation resulting in flattened diurnal variation may be a risk factor in developing CWP or other chronic pain conditions (15). In individuals with chronic pain, HPA axis function has also been associated with the experience of pain symptoms or pain sensitivity. In FM populations, cortisol levels are associated with pain ratings at waking and within one hour of waking, but not at other times of day (16). One intervention study supports a link between high evening cortisol and pain sensitivity, insofar as treatment lowered evening cortisol levels and pain scores while increasing pressure pain threshold in people with FM (17). Other studies of provide evidence that HPA axis dysregulation through a flattened CAR and reduced diurnal variation is associated with increased pain in individuals with chronic pain conditions (18, 19).

In addition to clinical pain research, the mechanisms behind the cortisol-pain relationship have been examined by experimental studies. In a recent laboratory study, healthy individuals with a flattened CAR reported significantly elevated ratings of pain intensity and unpleasantness during a cold pressor test (20). Sensory amplification, or increased sensory responsiveness, was related to elevated pain ratings in healthy volunteers with induced hypocortisolism (21). Collectively, these findings support the theory that HPA axis dysregulation influences the perception of painful stimuli by modifying processes of sensory

responsiveness, but more research is needed to understand how HPA axis function is related to experimental pain sensitivity.

Despite evidence of HPA axis dysregulation in individuals with chronic pain and a relationship between HPA axis function and pain symptoms in clinical populations, little is known about HPA axis dysregulation and pain sensitivity in individuals without chronic pain. If cortisol function in pain-free individuals is indicative of later development of chronic pain conditions, will HPA axis dysregulation in this population be associated with increased pain sensitivity? If so, the link between experimental pain sensitivity and cortisol function could serve as an early indicator of developing pain conditions before they become chronic (22, 23). Therefore, studying how cortisol relates to pain sensitivity in individuals without chronic pain conditions may provide insight into how HPA axis dysregulation is associated with the onset of increased pain sensitivity, including the genetic and familial influences, before increased pain sensitivity is maintained and perpetuated as in chronic pain populations.

Further, to our knowledge, no research to date has examined the role of genetic and common environmental influences (i.e., familial factors) in the relationship between HPA axis function and experimental pain sensitivity. Genetics and common environmental factors both influence HPA axis function (24), and genetic factors have been found to impact experimental pain sensitivity (25). Further, genetic factors may also contribute to the HPA-pain relationship, as genes related to HPA axis function have been linked to musculoskeletal pain and comorbid symptoms such as poor sleep quality and depression (26). Therefore, genetic and familial factors may be implicated in the cortisol-pain sensitivity relationship either as potential risk or protective factors for increased pain sensitivity and later development of chronic pain.

The aims of the present study were a) to examine the association of basal morning and evening salivary cortisol, and diurnal variation (i.e., changes in cortisol from morning to evening), with indices of cold pain sensitivity in relatively pain-free individuals, and b) to explore the role of familial confounding in those associations. We hypothesized that lower basal morning, higher basal evening, and lower diurnal variation would be associated with increased pain sensitivity. Due to the novel examination of genetic and familial influence on the cortisol-pain sensitivity relationship, we did not have any hypotheses about how these factors would contribute to the associations.

METHODS

Participants

Participants were 198 female twins (99 pairs) drawn from the community-based University of Washington Twin Registry (UWTR), which uses data from the Washington State Department of Licensing to identify twins (27, 28). Participants were enrolled in a study addressing psychological, behavioral, and physiological risk factors for medically unexplained chronic pain in women (29). Recruitment occurred between 2006 and 2010. Potential participants were randomly selected from the UWTR and included both pain-free twin pairs and pairs discordant for medically unexplained pain. Because the study focused on medically unexplained chronic pain, potential participants were excluded if they had any medical conditions that could explain their pain (e.g., cancer, autoimmune diseases) or that might affect data collection (e.g., uncontrolled endocrine and allergy conditions, cardiopulmonary problems, sleep disorders). Further exclusionary criteria included current smoking, positive test on a screen for drugs of abuse, a body mass index (BMI) of less than 20 or more than 30 kg/m^2, current or planned pregnancy during the study period, limb amputation, and complete deafness or blindness. All participants provided written informed

consent before commencing any study activities. The study protocol received approval from the Institutional Review Boards of the University of Washington, the University of California, San Diego, and the University of Kentucky.

General Procedures

Once enrolled in the study, participants were asked to stop taking all medications that might affect pain, sleep, the HPA axis, or the autonomic nervous system during the study period and for two weeks before the study procedures. Participants were permitted to take over-the-counter pain medication (e.g., Tylenol) as needed during the study period, but no participants reported taking pain relievers during the two-day laboratory portion of the study. Restrictions were also placed on the consumption of alcohol (two drinks per week) and caffeine (one cup of coffee per day) for the study period. Adherence to these restrictions was confirmed using urine drug tests performed at the lab visit with no subjects showing non-adherence.

The study began with an at-home protocol including basal salivary cortisol collection for three days and a questionnaire packet. The questionnaire packet measured basic sociodemographic data, including age, ethnicity, level of education, and marital status. To determine zygosity, we used questions about childhood similarity that have been shown to classify zygosity with an accuracy of 95%-98% compared with biological indicators (30-33). Approximately two weeks after the at-home protocol, participants completed a two-day visit to the University of Washington General Clinical Research Center. The laboratory protocol included a medical history and physical examination, laboratory and exercise testing, pain sensitivity tasks, and Holter monitoring. Both twins in each pair visited the laboratory during the same two-day period but underwent all study procedures separately.

Cortisol

Participants collected salivary cortisol at home for three days in the morning within 30 minutes of waking up (AM) and in the evening within 30 minutes of going to bed, but no later than 11:30 PM (PM). Participants were instructed to collect saliva by chewing or placing an absorbent swab (Salimetrics, State College, PA) under the tongue and then sealing it in a salivate tube. During this period, participants had daily contact with research staff to troubleshoot any saliva collection problems, and kept a daily log to report any problems encountered during the collection process. A sleep log was used to capture the time participants woke up and went to bed each day during the at-home collection period. Saliva samples were stored in home freezers and brought to the Seattle research center on dry ice during the laboratory visit.

Thereafter, samples were stored in a −80°C freezer and batch shipped to the University of Kentucky General Center for Clinical and Translational Science on dry ice for batch processing. Samples were processed by centrifugation, and cortisol concentrations were determined with High Sensitivity Salivary Cortisol Enzyme Immunoassay Kits (Salimetrics, State College, PA) following manufacturer's instructions. Sensitivity for cortisol assays is < 0.003 ug/dL. Inter-assay variability was ascertained from internal control samples in every assay. Samples were analyzed by using a KC4 uQuant Plate Reader (BioTek Instruments, Inc., Winooski, VT). Average values for the three daily AM and three daily PM samples were calculated; log transformed values were used in the statistical models, given non-normal distributions. As the pattern of change in cortisol from morning to evening can be indicative of HPA axis dysregulation, a cortisol change variable, diurnal variation, was calculated by subtracting basal evening cortisol levels from basal morning cortisol levels and averaging these difference scores across the three days of collection. This is the most basic method of calculating diurnal variation in cortisol as only two time points, morning

and evening, are required (34). Previous research has used morning and evening basal levels to draw conclusions about the diurnal variation of cortisol (15). Diurnal variation values were left on their original, non-log transformed scale. Altogether, three cortisol indicators were used in the models: log average AM cortisol, log average PM cortisol, and average diurnal variation.

Experimental Pain Sensitivity

The cold pressor test was used to measure experimental pain sensitivity. For this test, a large container of water was kept at 1-2°C with ice cubes and a submerged pump (35). Participants immersed their dominant hands and forearms in the water and indicated when the sensation became painful (threshold) and when they could no longer stand the pain (tolerance). Participants removed their arms from the water at tolerance or at 5 minutes, whichever came first. Two pain latency variables were captured from this test: the time from test start when the participant put his/her hand and forearm in water to the threshold point (time to threshold) and the time from test start to the tolerance point (time to tolerance). Participants were also instructed to rate their overall pain intensity level “at this moment” on a visual analog scale (VAS), anchored at “no pain” (score of 0) and “worst pain ever” (score of 100). VAS ratings were taken immediately before the start of the procedure (baseline), at threshold, and at tolerance. As a result, we had four indicators of experimental pain sensitivity: pain latency measures of time in seconds to threshold and to tolerance, and pain intensity ratings at threshold and at tolerance.

Statistical Analyses

We first calculated means and standard deviations (*SD*) for the continuous variables (age, cortisol measures, and pain sensitivity measures) and percentages for the categorical variables (ethnicity, twin status, education, and marital status). To investigate the overall associations between the three cortisol indicators and four experimental pain indicators, all individual twins were included in separate linear regression models to assess the cross-sectional relationships between cortisol and pain sensitivity. Initial models showed no association between the cortisol indicators and pain latency (i.e., time to threshold and tolerance), so we present results for pain intensity ratings only. We fit generalized estimating equations (GEE) regression models with robust standard errors to account for the lack of independence of members of twin pairs. Models were adjusted for age, baseline pain intensity rating, and pain latency. Time to threshold was used as the pain latency covariate for the threshold pain rating model, and time to tolerance was used for tolerance pain rating. To facilitate interpretation of the parameter estimates for log-transformed exposure variables (AM and PM cortisol), we present results as the change in pain rating for a 10% change in the independent variable. Results for diurnal variation, which was not log-transformed, are presented with the typical beta-coefficient interpretation: change in pain rating for a 1-unit change in the independent variable.

As the overall models do not take genetic or familial factors into account, we estimated within-pair models that are inherently adjusted for familial factors such as genetic and common environmental influences in twin pairs. These within-pair analyses are based on the assumptions that monozygotic (MZ) pairs share 100% of their DNA; dizygotic (DZ) pairs share, on average, 50% of their DNA; and twin pairs are typically raised in the same household. Therefore, within-pair associations control for both genetic and common environmental factors that may influence the relationship between two traits. If the associations in the within-pair models are attenuated compared to the overall associations, we can conclude that familial factors contribute to these relationships. Alternately, a within-pair association that is not attenuated compared to the association in the overall model provides evidence that familial factors do not play a prime role in that relationship.

To calculate within-pair effects, GEE linear regression models were fit such that the exposure of interest was the deviation of the individual cortisol measurement from the pair-level mean cortisol (36). Within-pair effects were estimated only for associations that were significant in the overall analyses. Within-pair models were also adjusted for age, baseline pain rating, and pain latency. The significance level for all analyses was set at $p < 0.05$. Data were analyzed by using Stata/SE 12.1 for Windows (StataCorp LP, College Station, TX, 2012).

RESULTS

Participant Characteristics

Table 1 displays the demographic and clinical characteristics of the sample. Of the 198 twins who participated in the study, four (2%) were excluded because their zygosity was indeterminate and eighteen (9%) were excluded because their cortisol measurements were missing. All participants were female, with 77% identified as MZ. The average age of the sample was 29 years (*SD* = 10); 85% self-identified as White, 52% had a Bachelor's degree or higher, and 34% were married or cohabitating. As expected, the sample exhibited a pattern of higher AM cortisol levels and lower PM cortisol levels. Participants required an average of 18.3 seconds to reach pain threshold (*SD* = 19.9) and 65.8 seconds to reach pain tolerance (*SD* = 73.9). Average pain ratings on a scale from 0 to 100 were 7.8 (*SD* = 12.1) at baseline, confirming that the sample was relatively pain-free. Average threshold and tolerance pain ratings on the 0-100 VAS scale were 43.9 (*SD* = 19.3) and 66.9 (*SD* = 18.9), respectively.

AM and PM Cortisol and Experimental Pain Sensitivity

Threshold and tolerance pain intensity ratings were not significantly correlated with AM cortisol or PM cortisol in the overall models (Table 2). Baseline pain rating was the only covariate significantly associated with pain rating at threshold or tolerance; this finding was consistent for both AM and PM cortisol models.

Diurnal Variation in Cortisol and Experimental Pain Sensitivity

In the overall analyses, diurnal variation was negatively associated with pain intensity ratings at both threshold ($p = 0.02$) and tolerance ($p < 0.01$) (Table 3). Baseline pain rating was also significantly associated with pain ratings at threshold and tolerance. Within-pair analyses for diurnal variation showed effects of similar magnitude compared to the overall analyses ($B = -12.7$ versus -11.9 for threshold and $B = -13.1$ versus -14.7 for tolerance pain rating), but only the association between diurnal variation and pain rating at tolerance remained significant ($p = 0.04$). Baseline pain rating remained the only covariate significantly associated with pain ratings at threshold ($p = 0.03$) or tolerance ($p = 0.04$) in within-pair analyses.

DISCUSSION

To our knowledge, this is the first study to examine the relationship between basal salivary cortisol and experimental pain sensitivity in a twin sample in which familial confounding could be examined. Using the cold pressor test, we found that lower diurnal variation of cortisol was significantly associated with higher pain intensity ratings at both threshold and tolerance, even after controlling for age, baseline pain intensity rating, and pain latency. In within-pair analyses, we found that the magnitude of the associations remained relatively robust after controlling for shared familial factors, even though the significance levels for the association of diurnal variation with pain ratings at threshold and tolerance were reduced (and rendered non-significant for threshold). These findings suggest that familial factors

may play only a partial role in the relationship between diurnal variation in cortisol and experimental pain sensitivity. As expected, we found that baseline pain ratings, even in this relatively pain-free sample, were significantly associated with pain ratings at threshold and tolerance in both the overall and with-pair models (37). We also found no significant associations between any of the cortisol indicators and the experimental pain latency measures, nor were there any associations between AM or PM cortisol levels and pain intensity ratings at threshold and tolerance.

Cortisol and Pain Sensitivity

Cortisol is a measure of HPA axis functioning, which is a component of the autonomic nervous system that responds to psychological or physiological stress. Levels of cortisol naturally fluctuate during the day, such that high morning levels taper into lower evening levels, with minor changes in response to acute stressors. Although research supports large individual differences in basal cortisol levels and diurnal variation of cortisol (38), chronic stress is associated with dysregulation of the HPA axis, including flattened CAR and lower levels of morning cortisol that do not diminish over the course of the day, producing a flattened diurnal variation (5, 10). However, the association between stress and HPA axis function in the cortisol awakening response may depend on the type of stressor (39). Overall, limited studies in clinical and experimental settings tend to support the theory that heightened pain sensitivity is related to HPA axis dysreguation through either a flattened CAR (20) or reduced diurnal variation (10, 17), but the findings and exact associations are inconsistent across studies (6).

This study cannot speak to the association of CAR and pain sensitivity. However, it is the first study to report the association between decreased diurnal variation in cortisol and increased experimental pain sensitivity. In line with studies conducted in individuals with chronic pain, our findings support that HPA dysregulation resulting in a flattened cortisol diurnal rhythm is associated with increased pain sensitivity (18, 19). Our findings are consistent with the one study demonstrating flattened diurnal variation as a predictor of later development of CWP (15). Additional studies are needed to further examine the association of absolute AM or PM cortisol versus diurnal changes in cortisol with pain sensitivity and to determine through prospective studies if HPA axis dysregulation is a reliable predictor of both increased pain sensitivity and chronic pain development.

Our findings that lower diurnal variation is associated with higher pain ratings at threshold and tolerance also expand upon the existing literature on cortisol dysregulation and pain sensitivity. This finding suggests that absolute levels of cortisol in the AM or PM may not be as important as changes in cortisol levels from morning to evening. In other words, diurnal variation may capture intra-individual differences in cortisol throughout the day that may be more strongly associated with pain sensitivity than are inter-individual differences in absolute cortisol levels in the morning or evening.

Unlike the limited previous literature, we did not find associations between basal AM or PM cortisol levels and pain sensitivity. This divergence may result from differing methodologies. The current study treated cortisol as a continuous variable, whereas other studies (16, 20) have compared pain sensitivity across groups determined by clinical pain status or normal/abnormal CAR. These grouping methods may reduce inter-individual variance by creating groups with more homogeneous cortisol levels. It should be noted that basal AM and PM cortisol levels did contribute to the diurnal variation variable in this study. However, it is not clear if the dysregulation of the diurnal variation was due to low AM levels, high PM levels, or the combination of the two. Although neither basal AM nor basal PM cortisol levels were significantly associated with pain ratings in this study, the association between high PM cortisol and high pain ratings was stronger than the

relationship between low AM cortisol and pain ratings (Table 2). If these associations were significant, they could provide support that the relationship between diurnal variation and pain sensitivity may be more influenced by higher PM cortisol than low AM cortisol in this sample.

Diurnal variation in cortisol was associated only with pain intensity and not with pain latency. This asymmetry might indicate that the two sets of pain sensitivity measures used in our protocol actually assess separate pain constructs. Following Melzack's model (40), pain ratings might be more closely related to the affective component of pain perception, whereas pain latency could be tapping into action programs associated with the physical sensation of pain. The HPA axis might have a stronger relationship with the affective experience of pain than with behavioral response during the pain experience; such a relationship might also explain the close ties between chronic pain and affective conditions (41). The difference in findings between pain intensity ratings and pain latency measures also highlights the difficulty in measuring pain and related behaviors in humans to elucidate psychological, biological, and neurological mechanisms.

Genetic and Familial Influences

Co-twin studies that examine within-pair differences are one of the most sensitive approaches to assessing the association of subtle clinical measurements, such as salivary cortisol and pain sensitivity. Although we found that the magnitude of the association between diurnal variation and pain intensity ratings at threshold changed minimally, the association became non-significant in the within-pair analysis. In contrast, the association of diurnal variation and pain intensity ratings at tolerance remained significant in the within-pair analysis. On one hand, these differing results for pain at threshold and at tolerance suggest that the experience of pain at threshold and the experience of pain at tolerance may be separate constructs, with distinct mechanisms related to HPA axis function. On the other hand, the similarly robust magnitude of these associations, despite dissimilar levels of statistical significance, argues against such an interpretation.

In lieu of experimental designs that may be impossible or unethical, co-twin studies constitute a natural experiment that can help to distinguish potentially causal relationships from relationships that stem from shared genetic or common environmental factors (42, 43). For example, if the association between two traits is not causal but results from genetic and environmental factors that affect both traits, we would expect little association between the two traits in a within-pair design that adjusts for the confounding effects of genetics and environment. Alternately, if the association between two traits is causal, then we would expect a robust association even within our closely matched twin pairs.

We found indications that familial factors play only a small role in the link between diurnal variation in cortisol and the experience of pain at both threshold and tolerance. The relative lack of familial confounding is consistent with the possibility that diurnal variations in cortisol and pain response have causal dynamics (42). Additional research with larger MZ and DZ twin samples is necessary to validate these findings and further explicate the role of genetic and familial factors in the association between HPA axis functioning and pain sensitivity. In addition, prospective studies are needed to better understand these relationships, especially to examine whether perturbations in diurnal variation in cortisol predispose a person to heightened pain sensitivity, or conversely, whether a heightened pain response distorts the natural rhythm of cortisol. Lastly, more research is needed to determine if the influence of genetic and familial factors on the cortisol-pain sensitivity relationship is also involved in the development and maintenance of chronic pain conditions.

Implications

The present study is unique insofar as our sample included healthy, relatively pain-free participants and our methods did not involve experimental manipulation of HPA axis functioning. Our findings have both theoretical and practical implications. Because of the role stress may play in precipitating or predisposing individuals to chronic pain conditions (13), HPA-associated physiological changes should be investigated as factors that might lead to heightened pain sensitivity, even before the appearance of perpetuating factors (e.g., central sensitization, chronic inflammation, or neurological changes) that may turn increased pain sensitivity into a chronic pain condition (44, 45). Clinically, daily cortisol patterns might serve as a biomarker of increased pain sensitivity that would alert healthcare professionals to the potential development of chronic pain. If the directionality between HPA axis function and pain sensitivity is established, it may be that other factors implicated in pain sensitivity (e.g., inflammatory cytokines or lipid messengers) exist as mediators in this pathway (46-48). Although findings from the current study support the association of HPA axis dysregulation with increased pain sensitivity, cortisol may serve as one of many possible biomarkers of chronic pain development in addition to the possible mediators of the stress-pain relationship.

Limitations

This study has several limitations. First, our sample consisted of young adult female twin pairs who were mostly pain-free at the time of examination. As a result, our results can be generalized only with great caution to men, to elderly populations, or to people with chronic pain. Second, we did not collect cortisol at multiple morning times, unlike other investigations of the link between cortisol and pain (20, 49). Therefore, we could not examine the CAR, determine diurnal slope based on multiple time points during the day, or perform an area-under-the-curve analysis for total cortisol output throughout the day. We chose to collect salivary cortisol only twice a day to reduce burden on participants and increase compliance with the study protocol. Replication studies with improved sampling strategies (i.e., more daily collections over more days of collection) may provide more reliable estimates of the relationship between diurnal variation and pain sensitivity. Third, our experimental procedure captured only cold pain sensitivity, so our findings may not be relevant to other modalities such as heat, pressure, or mechanical pain. Finally, this was a cross-sectional study. Although some of our findings suggest potentially causal relationships, prospective designs are necessary to further investigate these findings and fully understand the direction of association between HPA axis functioning and pain sensitivity and to further examine potential mediators.

Conclusions

In conclusion, we found that reduced diurnal variation in cortisol was related to higher pain intensity ratings at threshold and at tolerance in a cold pressor task. Familial factors appeared to contribute minimally to the link between diurnal variation in cortisol and these pain sensitivity measures. Collectively, our findings provide support for cortisol as a potential biomarker of pain in research settings. The mechanisms underlying the link between HPA axis dysregulation, like flattened diurnal variation, and pain sensitivity are likely complex and multifactorial. Future research should further examine genetic, familial, and other mechanisms to illuminate factors that might prevent the development and persistence of chronic pain.

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REFERENCES

1. Turk DC, Okifuji A. Psychological factors in chronic pain: Evolution and revolution. J Consult Clin Psych. 2002; 70:678–690.
2. Stewart WF, Ricci JA, Chee E, Morganstein D, Lipton R. Lost productive time and cost due to common pain conditions in the US workforce. JAMA. 2003; 290:2443–2454. [PubMed: 14612481]
3. Gatchel RJ, Peng YB, Peters ML, Fuchs PN, Turk DC. The biopsychosocial approach to chronic pain: Scientific advances and future directions. Psychol Bull. 2007; 133:581–624. [PubMed: 17592957]
4. Hassett AL, Clauw DJ. Does psychological stress cause chronic pain? Psychiat Clin N Am. 2011; 34:579–594.
5. Kudielka BM, Wüst S. Human models in acute and chronic stress: Assessing determinants of individual hypothalamus-pituitary-adrenal axis activity and reactivity. Stress. 2010; 13:1–14. [PubMed: 20105052]
6. Miller GE, Chen E, Zhou ES. If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenocortical axis in humans. Psychol Bull. 2007; 133:25–45. [PubMed: 17201569]
7. Fries E, Dettenborn L, Kirschbaum C. The cortisol awakening response (CAR): Facts and future directions. Int J Psychophysiol. 2009; 72:67–73. [PubMed: 18854200]
8. Fries E, Hesse J, Hellhammer J, Hellhammer DH. A new view on hypocortisolism. Psychoneuroendocrinology. 2005; 30:1010–1016. [PubMed: 15950390]
9. Cohen S, Janicki-Deverts D, Miller GE. Psychological stress and disease. JAMA. 2007; 298:1685–1687. [PubMed: 17925521]
10. Maletic V, Raison CL. Neurobiology of depression, fibromyalgia and neuropathic pain. Front Biosci. 2009; 14:5291–5338.
11. Raison CL, Miller AH. When not enough is yoo much: The role of insufficient glucocorticoid signaling in the pathophysiology of stress-related disorders. Am J Psychiat. 2003; 160:1554–1565. [PubMed: 12944327]
12. Riva R, Mork PJ, Westgaard RH, Magne R, Lundberg U. Fibromyalgia syndrome is associated with hypocortisolism. Int J Behav Med. 2010; 17:223–233. [PubMed: 20458566]
13. Van Houdenhove B, Luyten P, Egle UT. Stress as a key concept in chronic widespread pain and fatigue disorders. J Musculoskelet Pain. 2009; 17:390–399.
14. McBeth J, Chiu YH, Silman AJ, et al. Hypothalamic-pituitary-adrenal stress axis function and the relationship with chronic widespread pain and its antecedents. Arthritis Res Ther. 2005; 7:R992–R1000. [PubMed: 16207340]
15. McBeth J, Silman AJ, Gupta A, et al. Moderation of psychosocial risk factors through dysfunction of the hypothalamic-pituitary-adrenal stress axis in the onset of chronic widespread musculoskeletal pain: findings of a population-based prospective cohort study. Arthritis Rheum. 2007; 56:360–371. [PubMed: 17195240]
16. McLean SA, Williams DA, Harris RE, et al. Momentary relationship between cortisol secretion and symptoms in patients with fibromyalgia. Arthritis Rheum. 2005; 52:3660–3669. [PubMed: 16258904]
17. Bonifazi M, Suman AL, Cambiaggi C, et al. Changes in salivary cortisol and corticosteroid receptor-alpha mRNA expression following a 3-week multidisciplinary treatment program in

patients with fibromyalgia. Psychoneuroendocrinology. 2006; 31:1076–1086. [PubMed: 16962248]

18. Park JY, Ahn RS. Hypothalamic-pituitary-adrenal axis function in patients with complex regional pain syndrome type 1. Psychoneuroendocrinology. 2012; 37:1557–1568. [PubMed: 22445364]
19. Johansson AC, Gunnarsson LG, Linton SJ, et al. Pain, disability and coping reflected in the diurnal cortisol variability in patients scheduled for lumbar disc surgery. European Journal of Pain. 2008; 12:633–640. [PubMed: 18077197]
20. Mayes LA, McGuire L, Page GG, et al. The association of the cortisol awakening response with experimental pain ratings. Psychoneuroendocrinology. 2009; 34:1247–1251. [PubMed: 19375866]
21. Kuehl LK, Michaux GP, Richter S, Schächinger H, Anton F. Increased basal mechanical pain sensitivity but decreased perceptual wind-up in a human model of relative hypocortisolism. Pain. 2010; 149:539–546. [PubMed: 20381248]
22. Dadabhoy D, Crofford LJ, Spaeth M, Russell IJ, Clauw DJ. Biology and therapy of fibromyalgia: Evidence-based biomarkers for fibromyalgia syndrome. Arthritis Res Ther. 2008; 10:211. [PubMed: 18768089]
23. Edwards RR. Individual differences in endogenous pain modulation as a risk factor for chronic pain. Neurology. 2005; 65:437–443. [PubMed: 16087910]
24. Ouellet-Morin I, Dionne G, Perusse D, et al. Daytime cortisol secretion in 6-month-old twins: genetic and environmental contributions as a function of early familial adversity. Biol Psychiatry. 2009; 65:409–416. [PubMed: 19013558]
25. Kim H, Mittal DP, Iadarola MJ, Dionne RA. Genetic predictors for acute experimental cold and heat pain sensitivity in humans. Journal of Medical Genetics. 2006; 43
26. Holliday KL, Nicholl BI, Macfarlane GJ, et al. Genetic variation in the hypothalamic-pituitary-adrenal stress axis influences susceptibility to musculoskeletal pain: Results from the EPIFUND study. Ann Rheum Dis. 2010; 69:556–560. [PubMed: 19723618]
27. Afari N, Noonan C, Goldberg J, et al. University of Washington Twin Registry: Construction and characteristics of a community-based twin registry. Twin Res Hum Genet. 2006; 9:1023–1029. [PubMed: 17254446]
28. Strachan E, Hunt C, Afari N, et al. University of Washington Twin Registry: Poised for the Next Generation of Twin Research. Twin research and human genetics: the official journal of the International Society for Twin Studies. 2012:1–8. [PubMed: 22784447]
29. Afari N, Mostoufi S, Noonan C, et al. C-Reactive Protein and Pain Sensitivity: Findings from Female Twins. Ann Behave Med. 2011; 42:277–283.
30. Eisen S, Neuman R, Goldberg J, Rice J, True W. Determining zygosity in the Vietnam Era Twin Registry: an approach using questionnaires. Clin Genet. 1989; 35:423–432. [PubMed: 2736790]
31. Magnus P, Berg K, Nance WE. Predicting zygosity in Norwegian twin pairs born 1915-1960. Clin Genet. 1983; 24:103–112. [PubMed: 6577993]
32. Reed T, Plassman BL, Tanner CM, et al. Verification of self-report of zygosity determined via DNA testing in a subset of the NAS-NRC twin registry 40 years later. Twin Res Hum Genet. 2005; 8:362–367. [PubMed: 16176721]
33. Torgersen S. The determination of twin zygosity by means of a mailed questionnaire. Acta Genet Med Gemellol (Roma). 1979; 28:225–236. [PubMed: 297423]
34. Nicolson, NA. Measurement of Cortisol. In: Gallo, LC.; Luecken, LJ., editors. Handbook of Physiological Research Methods in Health Psychology. Sage Publications, Inc.; Thousand Oaks, CA: 2008. p. 37-74.
35. Turk, DC.; Meichenbaum, D.; Genest, M. Pain and behavioral medicine: A cognitive-behavioral perspective. Guilford; New York: 1983.
36. Begg MD, Parides MK. Separation of individual-level and cluster-level covariate effects in regression analysis of correlated data. Statistics in medicine. 2003; 22:2591–2602. [PubMed: 12898546]
37. Fillingim RB, Edwards RR, Powell T. The relationship of sex and clinical pain to experimental pain responses. Pain. 1999; 83:419–425. [PubMed: 10568849]

38. Stone AA, Schwartz JE, Smyth J, et al. Individual differences in the diurnal cycle of salivary free cortisol: a replication of flattened cycles for some individuals. Psychoneuroendocrinology. 2001; 26:295–306. [PubMed: 11166492]
39. Chida Y, Steptoe A. Cortisol awakening response and psychosocial factors: a systematic review and meta-analysis. Biol Psychol. 2009; 80:265–278. [PubMed: 19022335]
40. Melzack R. Pain and the neuromatrix in the brain. J Dent Educ. 2001; 65:1378–1382. [PubMed: 11780656]
41. Blackburn-Munro G. Hypothalamo-pituitary-adrenal axis dysfunction as a contributory factor to chronic pain and depression. Curr Pain Headache Rep. 2004; 8:116–124. [PubMed: 14980146]
42. McGue M, Osler M, Christensen K. Causal Inference and Observational Research: The Utility of Twins. Perspect Psychol Sci. 2010; 5:546–556. [PubMed: 21593989]
43. Kendler KS, Campbell J. Interventionist causal models in psychiatry: repositioning the mind-body problem. Psychological medicine. 2009; 39:881–887. [PubMed: 18845010]
44. Basbaum AI, Bautista DM, Scherrer G, Julius D. Cellular and molecular mechanisms of pain. Cell. 2009; 139:267–284. [PubMed: 19837031]
45. Voscopoulos C, Lema M. When does acute pain become chronic? Br J Anaesth. 2010; 105(Suppl 1):i69–85. [PubMed: 21148657]
46. De Jongh RF, Vissers KC, Meert TF, et al. The role of interleukin-6 in nociception and pain. Anesth Analg. 2003; 96:1096–1103. table of contents. [PubMed: 12651667]
47. Marchand F, Perretti M, McMahon SB. Role of the immune system in chronic pain. Nat Rev Neurosci. 2005; 6:521–532. [PubMed: 15995723]
48. Malan TP Jr. Porreca F. Lipid mediators regulating pain sensitivity. Prostaglandins Other Lipid Mediat. 2005; 77:123–130. [PubMed: 16099397]
49. Roberts ADL, Wessely S, Chalder T, Papadopoulos A, Cleare AJ. Salivary cortisol response to awakening in chronic fatigue syndrome. Brit J Psychiat. 2004; 184:136–141.

Table 1

Demographic and clinical characteristics of female twins

Characteristic	**(*n* = 176)**
Demographics	
Age, *mean years (SD)*	28.6 (9.7)
White, %	85.2
Monozygotic, %	76.7
Bachelor's degree or higher, %	52.3
Married or cohabitating, %	34.1
Cortisol (μg/dL)	
Average AM cortisol	0.38 (0.22)
Average PM cortisol	0.10 (0.16)
Average diurnal variation	0.28 (0.24)
Experimental Pain Sensitivity	
Pain Latency (seconds)	
Time to threshold, *mean (SD)*	18.3 (19.9)
Time to tolerance, *mean (SD)*	65.8 (73.9)
Pain Rating (0-100)	
Pain rating at baseline, *mean (SD)*	7.80 (12.1)
Pain rating at threshold, *mean (SD)*	43.9 (19.3)
Pain rating at tolerance, *mean (SD)*	66.9 (18.9)

Table 2

Overall associations for cortisol and experimental pain sensitivity at pain threshold and tolerance

	Threshold pain rating			Tolerance pain rating		
Independent variable	***B*** [a]	**(95% CI)**	***P***	***B*** [a]	**(95% CI)**	***P***
AM Cortisol						
Cortisol	−0.088	(−0.47, 0.30)	0.66	−0.167	(−0.579, 0.245)	0.43
Age	0.004	(−0.037, 0.045)	0.85	0.000	(−0.033, 0.034)	0.99
Baseline pain	0.027	(0.004, 0.050)	0.02	0.015	(0.002, 0.028)	0.03
Pain latency [b]	−0.010	(−0.022, 0.003)	0.13	−0.004	(−0.009, 0.001)	0.09
PM Cortisol						
Cortisol	0.134	(−0.195, 0.463)	0.42	0.214	(−0.086, 0.514)	0.16
Age	0.003	(−0.035, 0.041)	0.89	−0.002	(−0.032, 0.029)	0.92
Baseline pain	0.028	(0.005, 0.050)	0.02	0.015	(0.002, 0.028)	0.03
Pain latency [b]	−0.010	(−0.022, 0.003)	0.12	−0.004	(−0.009, 0.001)	0.08

[a] *B* = change in pain rating for a 10% change in the independent variable;

[b] Threshold (tolerance) models adjust for time to pain threshold (tolerance); CI = confidence interval.

Table 3

Overall and within-pair associations for average diurnal variation in cortisol and experimental pain sensitivity measures

	Threshold pain rating			Tolerance pain rating		
Independent variable	***B*** [a]	**(95% CI)**	***P***	***B*** [a]	**(95% CI)**	***P***
Overall model						
Diurnal variation	−11.9	(−21.7, −2.04)	0.02	−14.7	(−23.2, −6.13)	<0.01
Age	0.022	(−0.380, 0.423)	0.92	−0.022	(−0.357, 0.313)	0.90
Baseline pain	0.298	(0.057, 0.538)	0.02	0.165	(0.032, 0.299)	0.02
Pain latency [b]	−0.101	(−0.232, 0.031)	0.14	−0.040	(−0.089, 0.009)	0.11
Within-pair model						
Diurnal variation	−12.7	(−27.7, 2.33)	0.10	−13.1	(−25.6, −0.544)	0.04
Age	0.037	(−0.383, 0.458)	0.86	−0.002	(−0.342, 0.338)	0.99
Baseline pain	0.278	(0.033, 0.522)	0.03	0.140	(0.004, 0.276)	0.04
Pain latency [b]	−0.090	(−0.233, 0.053)	0.22	−0.039	(−0.088, 0.010)	0.12

[a] *B* = change in pain rating for a 1-unit change in the independent variable;

[b] Threshold (tolerance) models adjust for time to pain threshold (tolerance); CI = confidence interval.