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The Cortisol Awakening Response Mediates the Relationship Between Acculturative Stress and Self-Reported Health in Mexican Americans

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Abstract

Background—The assessment of acculturative stress as synonymous with acculturation level overlooks the dynamic, interactive and developmental nature of the acculturation process. An individual's unique perception and response to a range of stressors at each stage of the dynamic process of acculturation may be associated with stress-induced alterations in important biological response systems that mediate health outcomes. Evidence suggests the Cortisol Awakening Response (CAR) is a promising pre-clinical biomarker of stress exposure that may link acculturative stress to self-reported health in Mexican Americans.

Purpose—The aim of the current study was to examine whether alterations in the CAR mediate the relationship between acculturative stress and self-reported health in Mexican Americans.

Methods—Salivary cortisol samples were collected at awakening, 30, 45, and 60 minutes thereafter, on two consecutive weekdays from a sample of adult Mexican Americans. Acculturative stress and self-reported health were assessed. Data were aggregated and analyzed (n=89) using a mixed effects regression model and path analysis.

Results—Poorer self-reported health was associated with attenuated CAR profiles (primarily due to a diminished post-awakening rise in cortisol) predicted by both moderate and high levels of exposure to acculturative stress. Stress-induced alterations in the CAR mediated the relationship between exposure to acculturative stressors and self-reported health.

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Compliance with Ethical Standards

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional review committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. (See statement regarding IRB approval in the manuscript in a separate section before References).

Informed Consent: Informed consent was obtained from all individual participants included in the study (See statement regarding consent in the first paragraph of the manuscript under the section entitled, "Results").

Statement of Conflicts of Interest: The authors declare that they have no conflict of interest.

Conclusions—Findings demonstrate that different levels of acculturative stress are associated with distinct CAR profiles and suggest the CAR is one possible biological pathway through which exposure to culturally unique stressors may be linked to health disparities.

Keywords

Acculturative stress; Health; HPA axis; Cortisol; Mexican Americans

Introduction

There are inconsistent conceptualizations of acculturation in cultural research. Traditionally, acculturation has been conceptualized as the process of acquiring the cultural characteristics of the host culture following entry into a host culture (1). At the individual level, intercultural contact involves a linear trajectory from unacculturated to acculturated, as a new cultural identity replaces an old one affecting a range of behaviors, perceptions, attitudes, language, values, relationships, and cultural orientation (2, 3). However, intercultural contact can also involve a bi-dimensional process involving reciprocal changes in both the old and new host cultures (4). Inconsistencies in conceptualization have led to significant differences in the measurement of acculturation in much of the research focused on understanding the relationship between level of acculturation and risk of health problems (5, 6). These inconsistencies highlight the contention that acculturation is an all encompassing, descriptive construct that does not necessarily increase or decrease risk for negative health outcomes (5). It is more likely that individuals with varying levels of acculturation are differentially exposed to risk factors that predict greater vulnerability for negative health outcomes (7). It has been suggested that acculturative stress, defined as the stress associated with adjusting to a new host culture (8), may be a more direct, proximal measure of the risk for negative health outcomes associated with the process of acculturation (9, 10).

The notion that acculturation and acculturative stress involve empirically distinct processes that differentially influence health outcomes is supported by numerous findings. While some findings suggest that lower levels of acculturation are associated with higher stress and poorer health (11,12), other findings support the contention that lower levels of acculturation are often associated with lower acculturative stress and better health outcomes (13, 14,15). Thus, the assessment of acculturative stress as synonymous with acculturation level, assumes that a particular placement on a linear trajectory predicts level of stress, and ignores the dynamic, interactive and developmental nature of the acculturation process, the potential chronic nature of acculturative stress and individual risk and resilience factors. These inconsistencies highlight the importance of isolating the unique effects of acculturation from acculturative stress (16, 10). To help clarify these relationships it is recommended that future investigations examining the relationship between acculturation, acculturative stress and health outcomes refrain from relying solely on self-report measures of acculturative stress, and employ evidence-based measures of the biological stress response (10). This recommendation is founded on the possibility that an individual's unique perception and response to a range of stressors at each stage of the dynamic process of acculturation are associated with stress-induced alterations in important biological stress response systems

that may mediate health outcomes. Some support for this contention is provided by evidence suggesting that acculturative stressors are associated with altered biological responses (17).

Assessment of alterations in the dynamics of the HPA axis, an important biological stress response system, is a commonly used biological marker of stress. However, to date, few studies have assessed the HPA axis to examine the relationship between acculturation, acculturative stress and health. The HPA axis regulates the neuroendocrine stress response and compelling evidence suggests the pathway between stress and disease is mediated by prolonged activation of the axis (18). Numerous studies have examined the Cortisol Awakening Response (CAR) as one indicator of stress-induced alterations in HPA axis dynamics (19, 20). The majority of studies examining the effects of a wide range of chronic stressors on the CAR have provided evidence in support of an attenuated CAR (21, 22, 23), although some studies have reported an enhanced CAR (24, 25). Some insight into this discrepancy was provided by a meta analyses suggesting that while the CAR may be elevated soon after the onset of a stressor, over time, chronic stress exposure may lead to HPA hypoactivity and blunting of the axis (19, 26). In line with this contention, findings from two studies, conducted in our laboratory, examining the relationship between acculturation (defining acculturation as predominately language mastery) and the CAR in Mexican Americans, showed that higher levels of acculturation are associated with attenuation of the cortisol awakening response (CAR) (27,28).

A second line of evidence provides support for a link between alterations in the CAR and health outcomes (19, 21). Several studies have found that subjects reporting higher levels of chronic stress with an attenuated CAR have poorer health compared to their low stress counterparts with a more robust CAR and better health (29, 30). Consistent with these findings, attenuation of the CAR has been associated with a range of different immune related disorders including metabolic and cardiovascular risk profiles (31, 32), hypertension (33), type 2 diabetes (34), and chronic fatigue syndrome (35). Indeed the CAR has been described as a promising early biomarker of psychosocial risk and negative health outcomes (36). Although the precise mechanisms underlying the relationship between alterations in the CAR and negative health outcomes remains to be fully elucidated, there is abundant evidence suggesting the HPA axis may mediate associations between glucocorticoid resistance and inflammation following chronic psychosocial stress, leading to immune related disorders (91). Indeed, population based studies have demonstrated an association between proinflammatory cytokine levels, including increased levels of interleukin-6 (IL-6) and tumor necrosis factor (TNF- α) and attenuation of the CAR (91). Based on these merging lines of evidence, we propose that the CAR may be one biological pathway that links exposure to acculturative stressors to negative health outcomes in Mexican Americans.

The specific aims of the present study were threefold. Our first specific aim was to characterize the effects of acculturative stress on the CAR in a sample of Mexican American young adults. Our second specific aim was to examine the relationship between the post awakening change in CAR and self-reported physical health. Our third specific aim was to determine whether the post-awakening change in CAR mediated the relationship between acculturative stress and self-reported health. Because acculturative stress and acculturation are distinct constructs, we hypothesized that higher levels of acculturative stress would be

associated with an attenuated post-awakening rise in CAR, after controlling for acculturation, socioeconomic status, and other potential confounds. We further hypothesized that an attenuated post-awakening rise in CAR would predict poorer self-reported health. Finally, we hypothesized that the CAR would significantly mediate the relationship between acculturative stress and self-reported physical health.

Methods

Participants and Study Design

Informed consent was obtained from all participants following protocol approval by the institutional review board. Participants of Mexican descent ($N = 105$), aged 18–38, were recruited in the San Antonio, Texas metropolitan area, through advertisements posted on a local college campus. During an initial visit to the laboratory, an in-person screening interview and a battery of self-report assessments were administered to screen and exclude participants with factors known to potentially affect the HPA axis. Hispanic participants other than of Mexican descent (e.g., Cuban, Puerto Rican, South or Central American, etc.) were excluded from participation in the study. Additional exclusion criterion included severe obesity (defined as a body mass index of $> 30.0 \text{ kg/m}^2$), abnormal sleeping patterns, current use of medications known to affect the HPA axis and use of oral contraceptives. In addition, participants were screened and excluded for a reported lifetime diagnosis of a mood disorder, reported history of psychiatric hospitalization, current antidepressant use, assessed risk for current major depressive disorder and for current alcohol and other drug use disorders. Detailed screening procedures and preliminary analysis of a portion of the current sample are reported elsewhere (27).

Psychometric assessment

The SPSS Statistics 22 software (37) was used to calculate internal consistency point estimates (Cronbach's α) for all study measures in the current sample. In addition, bootstrapping with 2000 replicates was implemented using ZumaStat 4.0 for SPSS to generate 95% confidence intervals for Cronbach's α .

Demographics—Demographic variables included as covariates were age, sex, body mass index (BMI), and generational status. Household income was included as a measure of socioeconomic status.

Assessment of major depressive disorder—Given the well documented effects of major depressive disorder on HPA axis dynamics and perceived health, participants were screened for current depression using the Hamilton Depression Inventory Short Form (HDI-SF, 38), and excluded from participation based on a score of 10 or greater, indicating a strong likelihood of current depressive disorder (38). Estimated internal consistency reliability of the HDI-SF in the current sample was acceptable, $\alpha = .79$, 95% CI [.58, .92]. Trait related proneness to subclinical depressive symptoms was assessed using the depression facet of the NEO-PI-R, and included as a covariate.

Assessment of trait related proneness to negative affect—Given the potential effects of negative affect on HPA axis dynamics and perceived health, negative affect (trait related proneness toward anger, hostility, frustration and bitterness) was assessed using the anger/hostility facet of the NEO-PI-R and included as a covariate.

Substance abuse/dependence—Participants were screened for potential risk of alcohol and other substance use disorders using the World Health Organization Alcohol, Smoking and Substance Involvement Screening Test (ASSIST; 39). The ASSIST has strong psychometric properties and reliability for the current sample was acceptable $\alpha = .80$, 95% CI [.69, .90].

Assessment of ethnicity/nativity—Participants were determined to be of Mexican descent if the family origin of both biological parents was Mexico (as opposed to Puerto Rico, or South or Central America). Generational status was characterized as whether individual participants of Mexican descent were first, second or third generation Mexican Americans where first generation (subject immigrated to U.S. as a child or as an adult), second generation (subject was born in the U.S. and has U.S. born parents), and third generation (subject was born in the U.S. and has U.S. born parents and grandparents).

Health—Perceived health was assessed with the five-item General Health (GH) subscale of the RAND 36-Item Health Survey (RAND-36, 40). Questions regarding perceived health are scaled from 1 to 5, with achievable scores range from 0 to 100 with a lower score indicating poorer perceived health and a demonstrated association with diagnosable medical conditions (41). The GH subscale showed good internal consistency reliability in the current sample, $\alpha = .77$, 95% CI [.70, .83], and good reliability in Mexican American samples (42).

Acculturation—The revised Acculturation Rating Scale for Mexican Americans (ARSMA-II, 43) was used to assess acculturation by measuring subjects' orientation towards Anglo-American and Mexican culture. The ARSMA-II has strong psychometric properties, with acceptable internal consistency reliability in the current sample. The internal consistency estimates of the AOS and the MOS were $\alpha = .85$, 95% CI [.79, .90], and $\alpha = .74$, 95% CI [.46, .91], respectively.

Acculturative stress—The Hispanic Stress Inventory (HSI, 44) was used to assess acculturative stress. The HSI measures a broad spectrum of culturally relevant psychosocial stressors experienced by U.S. born Hispanics. The HSI yields a total acculturative stress score and scores for four subscales: Occupational/Economic Stress, Parental Stress, Marital Stress, and Family/Culture Stress. Higher scores indicate a greater amount of stress. The HSI has good psychometric properties (44). The HSI total score demonstrated strong internal consistency in the current sample, $\alpha = .96$, 95% CI [.94, .97].

Measurement of the CAR—Complete details of the salivary collection procedure can be found elsewhere (28). Participants were free to wake up as usual (using an alarm or spontaneously) and instructed to start sampling immediately at awakening, 30, 45 and 60 min thereafter. Participants collected samples on two consecutive weekdays and logged wake-up times, along with sampling times, for comparison with electronic monitoring data

(Medication Event Monitoring System [MEMS], AARDEX; Zug, Switzerland, 45) to maximize adherence to the sampling protocol.

Hormonal assays—Participants stored the samples in their home freezers until they were returned to the laboratory the next day and stored at -80°C until analyzed, when they were thawed at room temperature and centrifuged at $2500 \times g$ for 15 min at -4°C . Salivary cortisol levels were assayed in duplicate using high sensitivity enzyme immunoassays (Salimetrics, State College PA), with a mean lower sensitivity limit of $0.11\text{ }\mu\text{g/dL}$, standard curve range from 0.1 to $10\text{ }\mu\text{g/dL}$. The intra-assay and inter-assay coefficients of variation were less than 5% at all levels of the calibrator curve. The concentration of cortisol in saliva was expressed as $\mu\text{g/dL}$. To minimize the potential effects of exposure to stressful events during the sampling period, participants who were currently students were not sampled the week prior to scheduled class examinations. In addition, participants indicating daily hassles or exposure to stressful daily events or protocol noncompliance during sampling periods (teethbrushing, etc.) were excluded from the final analyses.

Data Analyses

The primary statistical design was mixed effects regression with repeated measures at four time points: awakening (T_0), T_{30} , T_{45} , and T_{60} . Fixed design effects were acculturative stress, measured as a continuous, dimensional variable (HSI scores ranged from 21 to 113); time elapsed after awakening (measured in min) and their interaction. The stress $\times$ time interaction was the primary focus of our investigation, since we wanted to test the association between level of acculturative stress and changes in salivary cortisol over time. Significant interactions were probed using a tertiary split of participants based on acculturative stress scores. This statistical procedure yielded fixed predicted values (estimated marginal means) of salivary cortisol at each time point (i.e., CAR profiles) for each of the acculturative stress groups, shown in Table 1. Due to multiple within- and between-subjects comparisons, Bonferroni-type corrections were applied to control the family-wise error rate at $\alpha = .05$. The association between the predicted CAR profiles and general health was tested using ordinary least squares (OLS) regression, with the predicted cortisol values as the independent variable and scores on the RAND-36 GH scale as the dependent variable. Finally, a test of mediation with bias-corrected bootstrapping (46) was conducted within a path model, with acculturative stress as the independent variable, repeated cortisol measurements as a mediator, and subjective general health as the dependent variable. All statistical analyses were conducted using SPSS Statistics 22. The mediation model was evaluated using Version 2.13 of the PROCESS macro (47) for SPSS.

Summary cortisol statistics are presented separately for days 1 and 2 (Table 1), but for the main analyses we used average values. To account for non-independence of residual error and heteroscedasticity, we selected a block diagonal matrix for the residual error covariance, where each block is a first-order heterogeneous autoregressive (ARH[1]) covariance matrix. Finally, because results of Shapiro–Wilk W tests indicated non-normality of the study variables, all analyses were repeated with log-transformed variables. Log transformation did not notably improve the analysis, thus we report results based on untransformed scores.

Results

Participant Characteristics

A total of 105 participants were initially recruited for enrollment. Eleven participants were excluded following screening procedures based on the following exclusion criteria: ethnicity (2); use of oral contraceptives (1), current use of medications known to affect the HPA axis (1), BMI > 30.0 kg/m² (1), an HDI score >10 (4), an ASSIST score of >4 (1) and current antidepressant use (1), resulting in 94 participants completing the study. Another 5 participants were excluded from the final data analyses following electronic verification of non-adherence to the sampling protocol. There were no significant differences between participants excluded and those completing the study on key demographic variables with the exception of greater depressive symptomatology reported by those excluded. Table 2 characterizes demographics for the final sample used in the analyses (N=89). Participants were predominantly bilingual, adult males (48.3%) and females (51.7%), with a mean score on the General Health scale ($M=77.88$, $SD=17.85$) comparable to healthy, young adults ($M=77.60$, $SD=20.10$; 48), and adequate variability in scores (20 – 100).

Acculturative stress and health

There was a strong negative association between acculturative stress and perceived health ($F=8.68$, $df=1, 88$, $b=-.28$, $\beta=-.30$, $t=-2.95$, $p=.004$). The relationship remained significant ($b=-.24$, $\beta=-.25$, $t=-2.11$, $p=.038$) even after controlling for acculturation ($t=-0.26$, $p=.792$), generational status ($t=-0.14$, $p=.886$), age ($t=-0.08$, $p=.936$), sex ($t=-0.11$, $p=.914$), and household income ($t=1.05$, $p=.297$) as covariates. Adding BMI as a covariate caused the relationship between acculturative stress and perceived health to fall slightly below statistical significance ($b=-.22$, $\beta=-.23$, $t=-1.91$, $p=.06$). However, BMI was not significantly associated with perceived health ($t=-1.02$, $p=.312$), accounting for all other variables in the model.

The HSI subscales primarily contributing to the observed association with perceived health were Family/Cultural Stress ($r=-.41$, $p<.001$), and Occupational/Economic Stress ($r=-.27$, $p=.009$). As expected, Marital/Partner Stress ($r=.06$, $p=.619$) and parental stress ($r=.92$, $p=.260$) were not associated with perceived health ($r=.06$, $p=.619$), given that the majority of participants were unmarried (97.1%) and childless (96.6%).

Acculturative stress and the cortisol awakening response

Findings from the mixed effects regression analysis, the primary analysis of interest, indicated a significant acculturative stress $\times$ time interaction ($F=6.47$, $df=1, 220.04$, $p=.012$) when the composite HSI score was included as a continuous variable. Even after adding all covariates including trait related subclinical depressive symptoms and negative affect, the acculturative stress $\times$ time interaction remained statistically significant ($F=2.29$, $df=6, 255$, $p=.036$), suggesting that different levels of acculturative stress are associated with unique CAR profiles.

For ease of interpretation of CAR profiles, and probe interactions, we reanalyzed the data by trichotomizing the acculturative stress variable based on total HSI score: 21 to 50 (Low, $n=$

31); 51 to 65 (Moderate, $n = 29$); and 66 to 113 (High, $n = 29$). This grouping (34.8%, 32.6%, and 32.6% of the sample, respectively) was the closest to equal possible, given the scaling of the items and the distribution of scores. The linear mixed effects regression using this tertiary split as a fixed factor in place of the continuous scores yielded a significant stress $\times$ time interaction ($F = 2.48$, $df = 6$, 189.92, $p = .025$). Moreover, the stress $\times$ time interaction remained significant ($F = 2.12$, $df = 6$, 169.08, $p = .054$) after controlling for each covariate.

Figure 1 plots the predicted cortisol values (estimated marginal means, unadjusted for covariates, shown in Table 1) for each acculturative stress group over time. Consistent with findings from previous studies, a large variation in cortisol levels between participants was observed (49, 50, 51). Of particular interest, the groups defined by level of acculturative stress showed attenuated CAR in direct relation to the level of acculturative stress. We then conducted corrected pairwise within-subjects contrasts, comparing the predicted cortisol values among all pairs of time points within each of the three groups. Univariate tests examining comparisons among the marginal means showed significant changes in cortisol over time in the low stress group ($F = 27.29$, $df = 3$, 193.83, $p < .001$), moderate stress group ($F = 11.70$, $df = 3$, 193.82, $p < .001$), and high stress group ($F = 7.06$, $df = 3$, 193.82, $p < .001$). Comparisons among the marginal means between the three groups at each time point showed significant differences in cortisol values across groups starting at T_{45} ($F = 4.07$, $df = 2$, 104.60, $p = .020$), and continuing to T_{60} ($F = 4.62$, $df = 2$, 113.23, $p < .001$), even after a correction was applied.

Planned contrasts revealed significant differences in cortisol level averaged over time, between the high and low stress groups ($t = -2.28$, $df = 2$, 91.34, $p = .025$), and between the low stress group compared with the average of the moderate and high stress groups ($t = 2.01$, $df = 2$, 91.34, $p = .048$). There was no difference in cortisol response, averaged over time, between the moderate and high stress groups ($t = -1.09$, $df = 2$, 91.34, $p = .278$). Custom contrasts revealed significant differences in cortisol between the low stress group and the average of the moderate and high stress groups at T_{45} ($F = 5.81$, $df = 1$, 86, $p = .018$) and T_{60} ($F = 4.07$, $df = 1$, 86, $p = .047$). The low stress group had significantly higher cortisol values compared to the average of the moderate and high stress groups, with mean differences of $.284\mu\text{g/dL}$ (T_{45}) and $.224\mu\text{g/dL}$ (T_{60}). Between-group comparisons suggested that the key difference in CAR was attenuation of the post-awakening rise in cortisol (delta (); calculated as peak cortisol $- T_0$) in the moderate and high acculturative stress groups. Indeed, a follow-up contrast showed that the average $-$ cortisol of the moderate and high stress groups was significantly smaller than the $-$ cortisol of the low stress group ($F = 5.03$, $df = 1$, 77, $p = .028$), with a mean difference of $-.272\mu\text{g/dL}$.

Cortisol awakening response and health

In order to examine the relationship between the stress-related CAR profiles and general health, we estimated a bivariate regression model with scores on the RAND-36 GPH scale as the dependent variable. The predicted cortisol values for each individual, based on level of acculturative stress, were entered as the independent variable. As expected, general health was positively associated with the predicted CAR profiles ($F = 5.67$, $df = 1$, 355, $p = .018$).

Individuals with a more robust CAR profile reported higher levels of general health ($b = 11.75$, $\beta = .13$, $t = 1.99$, $p = .018$), even after controlling for each covariate, with the exception of household income which caused the association between CAR and general health to fall below statistical significance ($b = 8.55$, $\beta = .09$, $t = 1.63$, $p = .105$). However, accounting for all other variables in the model, household income was not a significant predictor of perceived health ($b = 1.42$, $\beta = .11$, $t = 1.83$, $p = .068$).

Mediation model

Consistent with results of mixed effects and OLS regression, higher levels of acculturative stress (tertiary split) were associated with lower cortisol values ($b = -0.11$, $\beta = -.19$, $t = -3.56$, $p < .001$), while higher cortisol values were associated with better health, when accounting for level of acculturative stress ($b = 6.64$, $\beta = .18$, $t = 3.57$, $p < .001$). Both the total effect (acculturative stress predicting subjective health) and the direct effect (acculturative stress predicting subjective health, accounting for cortisol) of acculturative stress were significant and negative ($b = -5.68$, $\beta = -.26$, $t = -5.12$, $p < .001$, and $b = -4.94$, $\beta = -.23$, $t = -4.45$, $p < .001$, respectively). The indirect effect of acculturative stress through cortisol was significant and negative ($b = -.74$, 95% CI $[-1.31, -.30]$, $\beta = -.03$, $p = .013$). The ratio of the indirect effect to the total effect indicated that a statistically significant proportion (13.0%, 95% CI $[5.4\%, 27.0\%]$) of the relationship between level of acculturative stress and appraised health was mediated by CAR.

Discussion

This is the first examination of the extent to which alterations in the CAR mediate the relationship between acculturative stress and self-reported health in young, adult Mexican Americans. Findings from the current study extend a one dimensional conceptualization of acculturative stress, measured as self-report (10), to include measurement of the CAR to assess a biological dimension of stress (52, 53, 54). Measurement of the CAR as a biological marker of acculturative stress allows for an important distinction between the acculturation process and acculturative stressors with a specific, stress-induced biological outcome. Current findings extend our previous work (27, 28) showing that different levels of exposure to acculturative stress, when distinguished from the effects of acculturation, predict corresponding alterations in the post-awakening rise in cortisol resulting in distinct CAR profiles. Consistent with our prediction, apparently anything more than minimal exposure to acculturative stressors is associated with an attenuated CAR profile, primarily due to a significantly smaller post-awakening rise in cortisol. Exposure to both moderate and high levels of acculturative stress, particularly stressors associated with family conflict and economic/occupational problems were associated with statistically equivalent attenuated CAR profiles. Only the group with low exposure to acculturative stressors demonstrated a healthy, robust CAR. Moreover, as expected, poorer self-reported health was associated with the attenuated CAR profiles predicted by both moderate and high levels of exposure to acculturative stress. In contrast, better self-reported health was associated with the more robust CAR profile predicted by minimal exposure to acculturative stress, after controlling for acculturation, generational status, age, sex, household income and BMI.

Our findings have an additional important novel observation. A test of mediation showed a statistically significant proportion of the relationship between level of exposure to acculturative stress and self-reported health was attributable to the CAR, suggesting that alterations in the CAR may be one possible biological pathway that mediates the relationship between acculturative stress and perceived health in our sample of young, adult Mexican Americans.

Several strengths of the study increase our confidence in our findings. There is mounting agreement that studies should include biological measures of stress in minority health research to better distinguish the process of acculturation from its' psychosocial correlates and biological outcomes (10). Therefore, we measured the CAR with carefully employed statistical controls to isolate the potential effects of acculturative stress from other potential sources of variance (acculturation level, household income, BMI, sex, generational status, and age) on the CAR and perceived health. Furthermore, the study was designed to precisely control major methodological sources of variability in the CAR. For example, participants were carefully screened and excluded to minimize the influence of factors known to affect the HPA axis. Furthermore, sampling error was minimized through the use of daily logs and electronic monitoring devices to maximize adherence to the sampling schedule (55, 56). Saliva sampling was performed with strict adherence to commencement of sampling within 5 minutes of awakening, followed by sampling within 10 minutes of each subsequent time point (57, 58). Participants were carefully screened and excluded for a history of sleep disturbances, and data from those participants logging daily hassles or sleep disturbances during sampling days were excluded from analyses. To reduce the effect of state-related factors on the CAR, saliva sampling was restricted to weekdays (52, 59), and data was aggregated across two separate, consecutive sampling sessions (30, 60). Further increasing our confidence in our findings, results of a generalized linear mixed model power analysis (GLIMMPSE; 90) showed that our model (including fixed covariates) could detect statistically significant ($p = .05$) main effects and interactions at $\pi = .92$, with a sample size of 89, considerably exceeding the conventional cutoff of .80.

Our finding that greater exposure to acculturative stress is associated with attenuation of the CAR is in line with previous findings demonstrating an association between chronic stress related disorders and an attenuated CAR (52, 54), including posttraumatic stress disorder (61, 62), chronic fatigue syndrome (35, 63), chronic burnout (64), long-term care giving (22, 56), chronic exam stress (21) and chronic job stress (66, 67). However, some studies have reported an enhanced CAR associated with exposure to chronic stressors including chronic work overload (59), exam stress (68), and economic stress (24). Contradictory findings are likely due to different approaches to the quantification of the CAR (36), or the frequency and timing of cortisol sampling (58). Moreover, contradictory findings may be attributable to the demonstrated effects of a number of potential moderating factors including differences in day to day state variations versus trait factors (69, 70), and individual differences in proneness to negative affect and depressive symptoms affecting the CAR, stress and health (27, 71). However, it is unlikely that our finding that acculturative stress is associated with distinct CAR profiles is attributable to major depressive disorder, subclinical depressive symptoms, or negative affect given that we carefully assessed, and excluded participants with current depression, and included measures of negative affect and proneness to

subclinical depressive symptoms as covariates in our mixed effects model, resulting in a significant acculturative stress $\times$ time interaction ($F=2.29$, $df= 6, 255$, $p= .036$).

Consistent with our hypothesis, the attenuated CAR profiles predicted by moderate and high levels of acculturative stress were associated with poorer self-reported health. In contrast, the more robust CAR profile predicted by the lowest levels of acculturative stress was associated with better self-reported health. Our findings are in line with previous evidence suggesting an association between attenuation of the CAR and a wide range of physical symptoms including: same day and later day fatigue (71, 72), insomnia (73), greater post-operative pain (74), hypertension (33), atherosclerosis (75), and gastrointestinal disorders (76). However, findings have been mixed, with some studies reporting an association between an enhanced CAR and coronary artery disease (77), upper respiratory illness (78), obesity (24, 79) and slow wound healing (80). While distinct, regulatory components of the CAR are implicated in the expression of different symptom clusters (36), discrepancies may also be due to the potential confounding effects of individual differences in trait related negative affect and proneness to subclinical depressive symptomatology (84). Negative affect is likely not a confound affecting the relationship between cortisol and perceived health in the current sample given that it did not mediate the relationship ($Z= 1.39$, $p= .177$). However, proneness to subclinical depressive symptoms mediated the relationship between CAR and perceived health ($Z= 2.83$, $p= .005$), suggesting that subclinical depressive symptoms are an important factor affecting CAR and perceived health.

Our finding that a statistically significant proportion of the relationship between level of exposure to acculturative stress and perceived health was attributable to the CAR supports the contention that stress-induced alterations in the CAR may be one possible biological pathway through which exposure to acculturative stressors affect self-reported health in Mexican Americans. This finding is in line with previous studies suggesting the CAR may have a direct effect on the perception of physical symptoms (71, 72), and that exposure to stressors appraised as challenging leads to attenuation of the post-awakening rise in cortisol coupled with the perception of more frequent and severe physical symptoms the following morning (71). Taken together, our findings support a potential link between stressful socio-cultural contexts, modulatory stress pathways and risk for the development of health disparities.

Determination of the precise underlying mechanisms through which the CAR mediates the relationship between exposure to acculturative stress and perceived health in Mexican Americans is beyond the scope of the current study. However, one possible mechanism underlying the attenuated rise in CAR observed in the moderate and high acculturative stress groups is suggested by evidence demonstrating that higher stress related brain activity in the perigenual anterior cingulate cortex (pACC), a region modulating the HPA axis response to stress, is associated with attenuation of the initial post-awakening rise in cortisol (85). In our study it is possible that this modulatory pathway was affected by chronic exposure to both moderate and high levels of acculturative stress resulting in attenuation of the post-awakening rise in cortisol.

There is also evidence suggesting the CAR is a complex phenomenon regulated, in part, by two HPA axis independent mechanisms involving pre-awakening and post-awakening processes (36). Evidence suggests that cortisol levels at the first waking sample (T_0) represents a measure of pre-awakening cortisol secretion, whereas the dynamic rise in cortisol levels after awakening may be regulated by pre-awakening processes. Pre-awakening processes are potentially influenced by the inhibitory control of the hippocampus and suprachiasmatic nucleus (SCN) extra-pituitary pathway, while post-awakening processes are proposed to reflect activation of the HPA axis and increased adrenal sensitivity to ACTH (36). We performed a univariate analysis of variance (ANOVA) at T_0 with *post hoc* Bonferroni contrasts to explore the possible influence of acculturative stress in pre-awakening processes, and found no significant differences in mean cortisol levels between acculturative stress groups. An omnibus test was nonsignificant ($p = .46$), with no difference between the low and moderate stress groups ($p = 1.0$), between the low and high stress groups ($p = .70$), or between the moderate and high stress groups (mean difference = $-.152$ $\mu\text{g/dL}$, $p = .96$). The absence of between-group differences in the level of cortisol at first waking sample (T_0) suggests that level of acculturative stress has little effect on pre-awakening processes. Alternatively, it is possible that the observed attenuation of the post-awakening rise in cortisol predicted by moderate and high levels of acculturative stress is due to stress-induced alterations in post-awakening processes linked to negative health outcomes (36). Indeed, alterations in post-awakening processes have been implicated in switching between night and day immune activity (86, 87), associated with negative health outcomes (88, 72).

There are some limitations of the study worth noting. Given that our model tests mediation in a cross-sectional framework, an alternative model where perceived health may also affect the CAR is possible and would be consistent with a bidirectional mediating effect of CAR. It has been suggested that the CAR may play a role in initiation of post-awakening alertness and cognitive function (89), aimed at preparing an individual for challenges anticipated in the day ahead (19, 72). Whereas, the majority of findings suggest that anticipated challenge in the same or next day are associated with state-related increases in the CAR (72), a few studies have reported a diminished morning rise in cortisol following prior-day stress (69, 71). Due to limitations posed by the cross-sectional design, the assessment of day to day state-related variations in the CAR over multiple days and the intra-individual relationship between the CAR and previous or next day ratings of acculturative stress and self-reported health were not assessed in the current study. Therefore we cannot rule out the possibility that attenuation of the morning rise in cortisol in participants reporting greater acculturative stress with poorer self-reported health is due to state-related, preparatory processes in anticipation of same or next day challenges rather than more persistent alterations due to chronic exposure to acculturative stressors. It is possible that the attenuation of the post-awakening rise in cortisol observed in the low and moderate stress groups is due to state-related variations attributable to challenges anticipated the next day. However, we minimized the influence of state-related factors on the CAR by measuring cortisol on two consecutive non-weekend days and aggregating data across two separate, consecutive sampling sessions. Finally, results from a mixed effects analyses assessing day-to-day variability, including Day as an additional design factor, did not reveal any significant effects involving Day ($p > .10$),

indicating the unlikely influence of state-related factors in the attenuation of morning rise in the moderate and high stress groups.

Extending previous findings from our laboratory, the current study provides evidence for a potential biological pathway through which acculturative stressors may influence health in Mexican Americans. Graded increases in exposure to economic and familial sources of acculturative stress predicted distinct CAR profiles even after controlling for potential confounds. The CAR significantly mediated the relationship between acculturative stress and self-reported health.

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Figure 1.
The effect of acculturative stress on the cortisol awakening response (CAR)

Table 1

Raw and predicted cortisol values($\mu\text{g/dL}$).

Summary statistics (raw values)				
Day 1	Min	Max	M	SD
T ₀	0.029	1.560	0.524	0.339
T ₃₀	0.063	2.483	0.932	0.518
T ₄₅	0.097	3.057	0.946	0.588
T ₆₀	0.181	4.011	0.871	0.599
Day 2				
T ₀	0.050	2.238	0.549	0.397
T ₃₀	0.026	3.455	0.922	0.607
T ₄₅	0.023	3.888	0.903	0.664
T ₆₀	0.036	3.329	0.819	0.583
Predicted values (estimated marginal means) by level of acculturative stress				
	Low	SE	Moderate	SE
T ₀	0.534	0.063	0.548	0.065
T ₃₀	1.027	0.090	0.890	0.094
T ₄₅	1.098	0.085	0.872	0.088
T ₆₀	0.986	0.079	0.876	0.081

Table 2

Sample demographics for Mexican American adults, males and females.

Sample demographic characteristics of immigrant and U.S. born Mexican American adults. Participants (N=89) were primarily single, bilingual, healthy, young adult males and females with a minimum of one year of college education.

	Overall sample	Females	Males
Gender	(N = 89)	46 (51.7%)	43 (48.3%)
Age (median and range)	20 (18–38)	19 (18–38)	21 (18–37)
Body mass index (<i>M</i> and <i>SD</i>)	24.82 ± 4.79	24.48 ± 4.32	25.17 ± 5.26
General health score (<i>M</i> and <i>SD</i>)	77.88 ± 17.85	77.17 ± 15.48	78.63 ± 20.25
Parental income (<i>n</i> = 83)			
< 40K	47 (56.6%)	28 (68.3%)	19 (45.2%)
40K to 80K	24 (28.9%)	10 (24.4%)	14 (33.3%)
80K	12 (14.5%)	3 (7.3%)	9 (21.4%)
Participant education level (<i>n</i> = 85)			
13 years	30 (35.3%)	16 (36.4%)	14 (34.1%)
14 years	21 (24.7%)	14 (31.8%)	7 (17.1%)
15 years	12 (14.1%)	4 (9.1%)	8 (19.5%)
16 years	22 (25.9%)	10 (22.7%)	12 (29.3%)
Generational status ^a (<i>n</i> = 86)			
1st generation	23 (26.7%)	14 (32.6%)	9 (20.9%)
2nd generation	22 (25.6%)	9 (20.9%)	13 (30.2%)
3rd generation	41 (47.7%)	20 (46.5%)	21 (48.9%)

^aParticipants who immigrated from Mexico to the U.S. either as adults or children are grouped together as “first generation”.