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Marital status as a predictor of diurnal salivary cortisol levels and slopes in a community sample of healthy adults

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Abstract

Married people tend to be healthier than both the previously (bereaved, divorced, and separated) and never married, but the mechanisms through which this occurs remain unclear. To this end, research has increasingly focused on how psychological stress experienced by unmarried versus married individuals may differentially impact physiological systems related to health. One key system that is modulated by stress is the hypothalamic-pituitary-adrenal (HPA) axis, of which cortisol is a key hormonal product. Increased cortisol production and disruption of cortisol's daily rhythm have been linked to poorer health outcomes. This study examined the association between current marital status and these two indices of cortisol in a community sample of 572 healthy men and women aged 21–55. It also tested whether marriage buffers against the effect of stress (perceived stress by marital status interaction) on cortisol production. Participants provided salivary cortisol samples during waking hours on three nonconsecutive separate days to calculate diurnal cortisol levels and slopes. Married individuals had lower cortisol levels than either their never married or previously married counterparts. Differences in cortisol levels were due at least in part to currently married individuals having a more rapid decline in cortisol through the afternoon hours compared to individuals who were never married (but not those who were previously married). Furthermore, there was an interaction between perceived stress and marital status in predicting cortisol levels. Specifically, higher stress was associated with higher cortisol levels for previously married individuals but not for the married or never married. The results of this study support cortisol as one candidate mechanism accounting for the association of marital status and health.

Keywords

salivary cortisol; marital status; stress buffering; diurnal slopes

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Conflict of interest statement

All authors declare there are no conflicts of interest.

1. Introduction

Married people tend to be healthier than unmarried people (Burman & Margolin, 1992; Kiecolt-Glaser & Newton, 2001). Indeed, compared to those who are married, numerous studies document higher rates of morbidity and mortality among previously (divorced, separated, widowed; e.g., Matthews & Gump, 2002; Carey et al., 2014; Dahl et al., 2015) and never married individuals (e.g., Johnson et al., 2000; Murray, 2000; Idler et al., 2012). Previously married individuals experience increased social isolation (Stafford et al., 2013), a loss of social support (Cohen & Wills, 1985), and stigma related to their separation (Gerstel, 1987). Individuals who are never married also experience stigma and discrimination based on their non-normative marital status (Byrne & Carr, 2005; DePaulo & Morris, 2005; Morris et al., 2007). Recent interpretations of this literature suggest that the health benefits of marriage are partly or wholly attributable to better health in the happily married (e.g., Robles et al., 2014). However, there are reasons that married people might be healthier irrespective of the quality of their relationships such as their access to health insurance, contact with a broader social network, and more regular routines. Either way, this literature suggests that unmarried individuals are subject to experiencing multiple sources of stress that might put them at risk for disease and death.

How might the stress associated with previous marital dissolution or being unmarried impact health? To address this question, it is necessary to consider how stressful experiences may disturb physiological systems (Cohen et al., 2016). One system that is often implicated in models linking stress and disease is the hypothalamic-pituitary-adrenocortical (HPA) axis. The HPA axis produces the hormone cortisol, which plays an important regulatory role for numerous immunological and metabolic processes related to health (Sapolsky et al., 2000). Cortisol levels follow a diurnal pattern, consisting of a rapid rise immediately after waking followed by a decline throughout the afternoon and evening. This rhythm is referred to as the diurnal cortisol slope, and steeper negative cortisol slopes across the day are generally associated with better health outcomes (Adam & Kumari, 2009). Meta-analytic evidence suggests that interpersonal stressors, including experiences of shame or loss, are potent triggers of increased cortisol levels as well as flatter diurnal cortisol slopes (Dickerson & Kemeny, 2004; Miller et al., 2007). In turn, heightened cortisol levels and flatter cortisol slopes are associated with adverse health outcomes such as metabolic syndrome (e.g., Brunner et al., 2002; Anagnostis et al., 2009), coronary atherosclerosis (e.g., Matthews et al., 2006; Dekker et al., 2008), and hastened cancer mortality (e.g., Sephton et al., 2000). As previously discussed, unmarried individuals are more likely to experience ongoing feelings of stigma, social isolation, and discrimination. Insofar as these experiences threaten social well-being, unmarried individuals may be at increased risk for maladaptive changes in cortisol activity as well as for their potential downstream implications for health.

The primary aim of this study was to examine the association between current marital status and daily cortisol levels and slopes. Based on previous literature showing that being currently married is associated with better health and less interpersonal stress, we hypothesized that married individuals would show lower daily cortisol levels and steeper negative cortisol slopes compared to previously married and never married people. We also explored the possibility that lower levels of cortisol among married individuals may stem

from their marital status providing protection (stress-buffering) from the consequences of life stress (Cohen & Wills, 1985; Burman & Margolin, 1992; Hostinar et al., 2014). Models of stress buffering contend that close social relationships provide an individual with psychological and material resources that improve coping (Thoits, 1986; Cohen, 2004). To examine this hypothesis, we tested whether perceived stress was associated with less HPA activation among the married than among the previously and never married (perceived stress by marriage status interaction).

To evaluate our hypotheses, we collected multiple measures of salivary cortisol across the waking period of each of three non-consecutive days and compared married, previously married, and never married participants on average area under the curve and steepness of diurnal slopes across the three sampling days. We controlled for demographics, body mass index (BMI), seasons of the year the samples were collected, as well as extraversion, neuroticism, and agreeableness, personality characteristics that may be associated with both selection into marriage as well as cortisol outcomes (Miller et al., 1999).

2. Methods

2.1 Participants

We examined archival data combined from three viral-challenge studies. Pittsburgh Cold Study 2 (PCS2) was conducted from 1997–2001, Pittsburgh Mind-Body Center Study (PMBC) from 2000–2004, and Pittsburgh Cold Study 3 (PCS3) from 2007–2011. All three studies were conducted by our laboratory. Full descriptions and data from each study can be found at www.commoncoldproject.com.

In total, 740 participants completed the studies. We excluded those younger than 21 ($n = 109$) because one of the studies (PMBC) was limited to those 21 or older, and because of the very low rate of marriage (6.4% vs 28% in remainder of sample) in participants under 21 years. We also excluded participants from analyses if they were missing relevant covariates ($n = 1$) or primary outcome data ($n = 58$). The final sample consisted of 572 healthy adults between the ages of 21 and 55 years ($M = 33.7$, $SD = 10.2$) recruited from the Pittsburgh, PA area. The sample was 48% female, and 63% white, 32% African-American, and 5% other ethnicities. All participants provided informed consent and received financial compensation for their participation. The institutional review boards at both the University of Pittsburgh School of Medicine and Carnegie Mellon University approved each study.

2.2 Procedures

In each of the three studies, participants completed both a telephone screening and a physical examination administered by the study physician to determine that they were in good health and did not meet any of the study exclusion criteria: history of chronic illness, regular use of prohibited medication (including but not limited to antidepressants, sleeping pills, and tranquilizers), recent psychiatric hospitalization, recent psychiatric diagnosis, HIV seropositivity, abnormal clinical profiles as determined by urinalysis, complete blood count, and blood chemistry, current pregnancy or lactation, recent participation in another psychological study, having cold or flu-like symptoms within the previous 30 days from

baseline, previous flu-related hospitalization, and/or use of a steroid or immunosuppressant within the previous three months. The three parent viral-challenge studies all included baseline data collected before participants were exposed to a virus and all data reported here were collected during that baseline period.

2.3 Measures

2.3.1 Salivary Cortisol—Multiple measures of salivary cortisol were collected across the waking period of each of three non-consecutive days (see suggestions in Kraemer et al., 2006; Saxbe, 2008). To collect saliva samples for quantifying cortisol, participants were provided with rolls of cotton inside of a plastic collection tube (Salivettes®; Sarstedt AG & Co, Nümbrecht, Germany). They were instructed to place the cotton in their mouth, allow it to become saturated with saliva, spit the cotton back into the tube, and then reseal it. Both detailed written instructions and either a pre-programmed wristwatch (PCS2) or handheld computer (PCS3 and PMBC) were provided to signal participants at each collection time. In addition, the signaling device also provided a unique alphanumeric code for each collection. Participants were instructed to write the code as well as the exact time and date of collection on each tube right after it was sealed and then store the tubes in their refrigerator. Participants then brought the tubes with them to their baseline study session where they were collected by staff.

Two of the three cortisol samples were collected on non-consecutive days in participant's natural environments (home, work, etc.) 1–6 weeks prior to the viral-challenge; the third was collected on the baseline day in quarantine (prior to the viral-challenge). In PMBC and PCS3, salivary cortisol was assessed seven times daily (1, 2, 4, 6, 8, 12, and 14 hours post-waking) on each of the pre-quarantine days, and eight times during the first 24 hours of quarantine (0, 1, 2, 4, 5, 7, 9, and 14 hours post-waking). In PCS2, cortisol was collected 11 times daily on each of two pre-quarantine days and 14 times during the first 24 hours of quarantine before viral-challenge. In order to minimize between study method variance (establish approximate equivalency across studies) while still accurately capturing the diurnal rhythm of cortisol, we used 7 samples from pre-quarantine days (assessed at 1, 2, 4, 7, 9, 11, and 14 hours post-waking) and 8 samples from the baseline day of quarantine (0, 1, 4.25, 6.25, 7.25, 9.25, 12.75, and 16.75 hours post-waking) when analyzing data from the PCS2 participants (cf. Janicki-Deverts et al., 2016; <http://www.cmu.edu/common-cold-project/combining-the-5-studies/variable-modifications.html>).

The laboratory of Dr. Clemens Kirschbaum in Dresden, Germany assayed cortisol for PCS2 and PCS3. Cortisol concentrations were determined using time-resolved fluorescence immunoassays with a cortisol-biotin conjugate as a tracer (Dressendörfer et al., 1992). Intra- and inter-assay variables were both less than 12%. The Immunologic Monitoring and Cellular Products Laboratory at the University of Pittsburgh Cancer Institute assayed cortisol for PMBC. Here, cortisol concentrations were determined via enzyme-linked immunosorbent assays (Salimetrics, State College, PA); average deviation between individual pairs of replicates was 4%. Previous research has shown these two assay procedures provide very similar results ($r = .97$; Dressendörfer et al., 1992). Nonetheless, to

control for any potential differences in methodology among the studies, we included study as a covariate.

In all cases, samples were only included for analysis if they were collected ± 45 minutes of the scheduled collection time. This was based on our earlier work indicating we could maintain 95% or more of the data using this range and at the same time retain the normal diurnal rhythm (e.g., Janicki-Deverts et al., 2016; also, see <http://www.cmu.edu/common-cold-project/combining-the-5-studies/variable-modifications.html>). Samples collected outside of this window were treated as missing. The actual rather than the expected (signaled) time the participant provided each cortisol sample was used in the calculation of both AUC and slopes. To calculate a measure of average daily cortisol levels, the area under the curve (AUC) each day was computed for individuals with sufficient data following methods established by Pruessner et al. (2003). Sufficient data was defined as not missing any of the first three samples of the day (when diurnal rhythm is steep) or more than two of the day's remaining samples (when diurnal rhythm flattens). Five hundred seventy two (90.8%) of the 630 potential participants (ages 21–55; not missing any covariates) had sufficient data to calculate cortisol AUC. Next, average total cortisol levels were calculated for participants who had data for at least two of the three collection days. This was done by averaging total adjusted concentrations from all days with sufficient data. Finally, due to non-normality of the data, the average total concentrations of cortisol were log-10 transformed.

We also used these cortisol samples across the three study days to model participants' typical daily cortisol slopes. For this, the raw cortisol values were log-10 transformed, and slopes were modeled directly using multilevel modeling methods (see Data Analysis section below). These methods accommodate missing data and hence use all available data. Again, we used the actual time each sample was collected in model. Among the 572 participants in the sample with sufficient data, there were 12,584 potential cortisol measurement opportunities (572 participants $\times$ 22 possible observations per person) for use in analyses of diurnal slopes, and cortisol data was available for 96.3% ($n = 12,117$) of these collection times.

2.3.2 Marital Status—An item from the Social Network Index (SNI; Cohen et al., 1997) asked participants to report if they were (a) currently married and living together, or living with someone in a marital-like relationship, (b) never married and never had lived with someone in a marital-like relationship, (c) separated, (d) divorced or formerly had lived with someone in a marital-like relationship, or (e) widowed. Due to a low percentage of participants identifying as separated, divorced, or widowed, these three categories were collapsed into a single category for previously married. Of the 572 participants, 160 were currently married (CM; 28%), 292 had never been married (NM; 51%), and 120 had been previously married (PM; 80 divorced, 34 separated, and 6 widowed; 21%). See Table 1 for demographic information within marital categories.

2.3.3 Perceived Stress—Perceived stress was measured using the 10-item Perceived Stress Scale (PSS-10; Cohen & Williamson, 1988). The PSS-10 asks participants to rate how often they find their lives to be unpredictable, uncontrollable, and overloaded on a five-point

Likert scale ranging from 0 = “*Never*” to 4 = “*Very Often*.” Reversed items were recoded (e.g., 4=0) and responses for each item were summed to create an index of total perceived stress. Internal consistency across the three samples was satisfactory (Cronbach’s $\alpha = .87$).

2.3.4 Personality—In PCS2 and PMBC, the personality traits of extraversion, neuroticism, and agreeableness were measured using a modified version of Goldberg’s Adjective Scale (Goldberg, 1992) which asked participants to rate the extent to which several trait-like adjectives accurately described them compared to other individuals of the same sex and age. In PCS3, extraversion, neuroticism, and agreeableness were measured using the International Personality Item Pool (IPIP; Goldberg et al., 2006) which asked participants to rate the extent to which several self-referent descriptive phrases describe them compared to others of their sex and age. Correlations between the adjective subscales and the IPIP have been reported as being between .72 and .84 for extraversion and neuroticism, and between .54 and .66 for agreeableness (Saucier & Goldberg, 2002). To establish inter-study equivalency, we converted the raw values for each subscale to *z*-scores before inclusion in analyses.

2.3.5 Primary Control Variables—All the primary control variables were selected *a priori* because of their potential associations with both marital status and cortisol (e.g., Aughinbaugh et al., 2013; Averett et al., 2008; Champaneri et al., 2013; Cohen et al., 2006; Van Cauter et al., 1996). The seven primary covariates were assessed at screening as part of a demographics questionnaire: age (years), sex (male or female), race (dichotomized as white or non-white due to the small number of non-black racial groups represented), and education (years). BMI (weight [kg]/height [m]²) was computed from objective measures of height and weight taken on the first day of quarantine. Season of the year of the trial was determined from the date of the last day of cortisol sampling and was indexed using three dummy codes with spring being the reference category. Finally, because of differences between the studies’ cortisol assays and personality questionnaires, two dummy variables indicating which of the three studies the participant had been in (using PCS3 as the reference category) were included as covariates to adjust for any potential effects of these differences. All seven of these variables were included in all of the analyses. These are the same primary control variables used in our earlier analyses predicting cortisol response in cold study data (e.g., Janicki-Deverts et al., 2016).

2.4 Data Analysis

2.4.1 Waking Day Cortisol Levels—All analyses with cortisol AUC as the outcome were performed using SPSS (Version 23; IBM Corporation). We used hierarchical multiple linear regression to examine the association between marital status and cortisol levels as well as the interaction between perceived stress and marital status on cortisol levels. In the first step, we entered the primary control variables (age, sex, race, BMI, education, season of the year, and study). Then, in the second step, we added dummy codes for marital status (currently married individuals served as the reference group). Next, in the third step, we added the continuous perceived stress variable. Finally, in the fourth step, we entered the marital status by perceived stress interaction terms. In other words, the model testing for the association between marital status and cortisol did not include interaction terms but the

model testing for interactions included both main effects as well as the interaction terms (Cohen et al., 2013). For significant interactions, we tested the decomposed individual regression slopes (i.e., “simple slopes”) for each marital category using the PROCESS macro for SPSS (Version 2.16.1; Hayes, 2013). For these analyses, we report unstandardized regression coefficients (b), p -values, and 95% CIs for the b -values. To provide an index of effect size, we also provide standardized effect size estimates (d), for group differences in cortisol levels.

2.4.2 Waking Day Cortisol Slopes—We used multilevel modeling to assess whether daily cortisol slopes differed as a function of marital status, as well as whether perceived stress moderated this association. Specifically, due to the amount of variability in cortisol that can be attributed to day-to-day fluctuations ($ICC = 0.31$), we evaluated 3-level models (individual cortisol measurements nested within sampling days nested within people) using the software HLM (Version 6.08; Raudenbush et al., 2004). At Level-1, log-transformed cortisol values were predicted as a function of time since the first sample of the day. For the time variable, we used the actual times (in hours) that participants recorded on their Salivette® plastic collection tubes. We also included the quadratic term for time, which allowed us to examine possible differences in the acceleration of the decline in cortisol across the day as a function of marital status. For two of the three sampling days for all three studies, the first cortisol measure was taken at 1-hour post waking; on the third day it was taken at waking. As such, to better reflect the majority of our data in the test for the intercept, we transformed the time variable by subtracting 1 from each value so that 0 represented the estimate of cortisol at 1-hour post waking. At Level-2, the Level-1 intercept and slopes were modeled by intercept terms for the three sampling days. At Level-3, the Level-2 intercepts were predicted as a function of individual differences in marital status. For this, we once again used two binary dummy codes to capture the three categories of marital status, with currently married individuals being the reference group. In the model testing for the interaction between perceived stress and marital status, we also added the perceived stress variable along with the two interaction terms between perceived stress and the dummy variables for marital status at Level-3 (currently married individuals were the reference group). Models were estimated using an unstructured covariance matrix, and we modeled the random effects for all Level-1 and Level-2 intercept and slope terms (for full model results including the random effects, please refer to the Supplementary Online Tables).

We included several person-level covariates at Level-3. Specifically, we adjusted for individual differences in age, sex, race, years of education, study, BMI, and season. We centered these covariates around their respective means prior to analysis. While mean centering binary variables such as sex and race may at first seem unintuitive, it is an accepted statistical practice that allows for examining the nature of intercepts and regression slopes regardless of individual differences in the various binary variables (for further discussions about the rationale for centering binary variables in multilevel modeling, see Raudenbush & Bryk, 2002; Hox et al., 2010). Here, we report the multilevel model regression coefficients (γ), p -values, and 95% CIs for the γ -values. Additionally, we report

χ^2 tests for the contrasts testing whether two (or more) regression slopes differ from each other.

3. Results

3.1 Marital Status and Waking Day Cortisol Levels

Adjusting for the control variables, we found an association between marital status and total levels of cortisol. Specifically, relative to currently married individuals, we found higher cortisol levels among those who were either never married, $b = .05$, $p = .01$, 95% CI = [.01, .09], $d = .25$, or previously married, $b = .06$, $p = .01$, 95% CI = [.01, .10], $d = .28$ (see Figure 1). These findings were not attenuated by further adjusting the model simultaneously for individual differences in extraversion, neuroticism, and agreeableness.

3.2 Interaction of Perceived Stress with Marital Status on Cortisol Levels

Adjusting for the control variables, marital status groups did not differ in their average PSS-10 scores, $F(2,559) = .77$, $p = .47$, nor was there an overall association between perceived stress and cortisol levels, $b = -0.0001$, $p = .94$, 95% CI = [-.003, .002]. However, stress moderated the association between marital status and cortisol levels, $R^2 = .01$, $F(2,556) = 3.28$, $p = .04$. To evaluate the nature of this interaction, we tested the simple slopes for each of the three marital status categories (see Figure 2). There was no association between perceived stress and cortisol levels among individuals who were either currently married, $b = -.001$, $p = .55$, 95% CI = [-.006, .003], or never married, $b = -.002$, $p = .31$, 95% CI = [-.005, .002]. Conversely, there was an association between perceived stress and cortisol levels among previously married individuals, with increases in stress predicting higher daily cortisol levels, $b = .006$, $p = .02$, 95% CI = [.001, .012].

3.3 Marital Status and Daily Cortisol Slopes

We used multilevel modeling to test if daily cortisol rhythms differed as a function of marital status. There were no differences in cortisol between the three marital status categories at 1-hour post-waking (i.e., the intercept coefficients), $\chi^2(2) = 0.79$, $p > .50$, and all three groups showed a normative decline in cortisol over the day, $\gamma_{CM} = -0.06$, $p_{CM} < .001$, 95% CI = [-0.07, -0.05]; $\gamma_{NM} = -0.04$, $p_{NM} < .001$, 95% CI = [-0.05, -0.03]; and $\gamma_{PM} = -0.05$, $p_{PM} < .001$, 95% CI = [-0.06, -0.04]. There was also an interaction between marital status and time, $\chi^2(2) = 8.96$, $p = .01$ (see top panel of Figure 3), such that individuals who were currently married had a steeper daily decline in cortisol slopes than individuals who were never married, $\gamma = -0.02$, $p = .004$, 95% CI = [-0.03, -0.01]. While currently married individuals in our sample also showed apparently steeper slopes than previously married individuals, the difference was marginal, $\gamma = -0.01$, $p = .13$, 95% CI = [-0.022, 0.003].

When testing the association between the quadratic term for time and cortisol (i.e., whether the quadratic term for each marital status group was different from 0), currently married individuals showed evidence for a curvilinear association between cortisol and time, $\gamma_{CM} = 0.0011$, $p_{CM} < .001$, 95% CI = [0.0005, 0.0017]. Conversely, we did not find curvilinear associations between cortisol and time among never or previously married individuals, $\gamma_{NM} = 0.0001$, $p_{NM} = .61$, 95% CI = [-0.0003, 0.0006], and $\gamma_{PM} = 0.0007$, $p_{PM} = .08$, 95% CI =

[−0.0001, 0.0014]. Next, we tested whether there was an interaction between marital status and the quadratic term for time. We found evidence that marital status moderated the association between the quadratic term for time and cortisol, $\chi^2(2) = 5.44$, $p = .06$ (see bottom panel of Figure 3), such that individuals who were currently married showed a more rapidly accelerating decline in cortisol through the afternoon before flattening in the evening compared to individuals who were never married, $\gamma = -0.0009$, $p = .02$, 95% CI = [−0.0018, −0.0001]. In contrast, while currently married individuals in our sample also showed apparently stronger curvilinear cortisol responses than previously married individuals, the difference in the magnitude of the association was not statistically distinguishable, $\gamma = -0.0004$, $p = .33$, 95% CI = [−0.0012, 0.0004]. Consistent with the analyses of cortisol levels, further adjusting the model simultaneously for individual differences in the personality traits extraversion, neuroticism, and agreeableness did not alter either the linear or the quadratic findings.

Finally, we tested whether perceived stress moderated the association between marital status and cortisol slopes (i.e., perceived stress $\times$ marital status dummy codes $\times$ time). For this, we entered the variable for perceived stress along with interaction terms for perceived stress and marital status at Level-3 of the model. We did not find evidence that perceived stress moderated the association between marital status and cortisol slopes, $\chi^2(6) = 5.38$, $p > .50$.

4. Discussion

Both cross-sectional and prospective studies of the association of marital status and health suggest that married individuals are healthier than either individuals who were never or previously married (divorced, separated, or widowed). These effects are often attributed to higher levels of interpersonal stress experienced by unmarried people. Elevated levels of cortisol, a potential biological consequence of interpersonal stress, is one candidate mechanism accounting for the association of marital status and health. Here we tested cortisol levels assessed throughout the day on three non-consecutive days to evaluate daily cortisol levels and slopes. We found that married individuals had lower diurnal cortisol levels than either their never married or previously married counterparts. Furthermore, individuals who were currently married showed steeper daytime cortisol slopes than individuals who had never or previously (marginal difference) married.

Differences in daily cortisol levels between married and unmarried individuals were not due to general differences in individuals' starting values for cortisol at the beginning of the day. Rather, overall differences in cortisol levels were due at least in part to currently married individuals having shown a more rapidly accelerating decline in cortisol through the afternoon hours (before flattening off in the evening hours) compared to individuals who were never married (though not individuals who had been previously married). These data are consistent with evidence that individuals who face persistent threats to their social well-being tend to have higher afternoon cortisol than those who do not (Miller et al., 2007).

Marriage may be associated with lower cortisol and steeper slopes because most married people are relatively satisfied with their marriages (Davis et al., 2006), and hence lack much of the interpersonal stress associated with being in a poor marriage or being unmarried.

However, a recent meta-analysis found no relationship between marital quality and diurnal cortisol slopes, although this conclusion was tempered by inadequate numbers of sampling days, and small sample sizes (Robles et al., 2014). (This study was unable to test the role of marital quality because a validated marital quality measure was not available across the three studies). Alternatively, married persons may have lower cortisol levels and steeper slopes irrespective of the quality of their relationships because they have better access to healthcare due to the availability of insurance, more economic support, or because marriage encourages regular routines, positive health practices and less risky behavior.

We did not find differences in overall levels of perceived stress as assessed by the PSS among the three categories of marital status. This was contrary to our hypothesis that unmarried individuals would have higher levels of cortisol resulting from heightened psychological stress. One possible explanation for this comes from the literature discussed earlier suggesting that unmarried individuals experience more threats to the social-self (e.g., shame and loss), and that these social stressors trigger cortisol dysregulation (Dickerson & Kemeny, 2004; Miller et al., 2007). However, the stress instrument we employed, the PSS, evaluates broad, global feelings of stress. As such, a more targeted assessment of interpersonal stress may have been more sensitive for detecting relevant differences.

We did find partial support for the stress-buffering effect of marriage (Cohen & Wills, 1985; Burman & Margolis, 1992). Specifically, while individuals who were currently or never married did not show differential cortisol levels as a function of perceived stress, previously married individuals who reported more stress had higher daily cortisol AUC values. This could reflect greater coping resources among the currently and never married (though exploratory analyses did not find evidence for any moderating, mediating, or mediated moderating effects of social support). Alternatively, the elevated levels of interpersonal stress associated with being previously married may exacerbate the consequences of global life stress on cortisol (Gove & Shin, 1989). Conversely, there was no stress-buffering effect of marriage for daily cortisol slopes. One possible explanation for this is that while cortisol levels may be sensitive to general perceptions of stress, diurnal cortisol slopes may be affected only by severe and enduring stressors (e.g., Cohen et al., 2006).

Our results differ from two earlier studies that did not find differences in cortisol levels based on marital status (Luecken et al. 1997; Englert et al., 2008). However, these studies varied from ours in a number of ways. First, while both studies included health-related covariates based on either self-reported health status (Englert et al., 2008) or medication use (Luecken et al., 1997), participants in these samples were not (like in the current study) specifically selected because they were in good health as indicated by a thorough medical exam. Second, both of these other studies included participants over 55 years old (our maximum). Older individuals are more likely to suffer from maladies that might influence cortisol regulation, which may in turn increase variability in observed cortisol responses making it more difficult to find differences attributable to marital status. Third, both of these studies were limited to women. In studies of the association of marital status with morbidity and mortality, the benefits of marriage are generally found to be stronger for men than women (Kiecolt-Glaser & Newton, 2001). Hence studies of marital status and cortisol that are restricted to women may provide a constrained test of the hypothesis. Finally, both

studies assessed cortisol during only one day (Luecken et al., 1997 used 24 hr urines and Englert et al., 2008 overnight urines). The 3 waking day assessments we collected over several weeks provides a more reliable indicator of cortisol levels.

There are several limitations to the current research that warrant consideration. First, the observational nature of this study prevents us from drawing causal inferences about the direction of the association between marital status and cortisol. However, it seems unlikely that differences in cortisol influence who becomes or remains married, especially in a sample selected to be in excellent health. To our knowledge, elevated levels (within normal ranges) of cortisol themselves are not associated with any behavioral manifestations that select people into a marital status. Furthermore, we adjusted our analyses for personality characteristics that might have confounded our results (extraversion, neuroticism and agreeableness). Doing so did not result in any attenuation in the strength of the association between marital status and cortisol. Of course, it is always possible that some other unmeasured third variable might account for our findings. Second, this research examined a relatively young sample selected for good health and thus the current results may not be generalizable to older or less healthy populations. It is certainly possible that various classifications of marital status may have very different meanings among younger versus older individuals. For example, being divorced, separated or bereaved may be more acceptable (and normative) for a 60-year-old than a 30-year-old. Third, it is possible that the use of three days of cortisol collection was not sufficient to optimize the reliability of the cortisol measures (Segerstrom et al., 2014; see alternative views in Kraemer et al., 2006; Saxbe, 2008). If this is the case, we have underestimated the effect sizes reported here.

Finally, an assumption of this study is that cortisol may be an important mediator of the association between marital status and health outcomes. Although not reported here, marital status was *not* related to risk of objectively catching a cold in the parent studies. Consequently, we were unable to test cortisol as a mediator of the association between marital status and disease. However, there is increasing evidence that elevated levels of cortisol and flatter cortisol rhythms may play a role in other important health outcomes including cardiovascular risk (Matthews et al., 2006) and cancer mortality (Sephton et al., 2000).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Married have lower cortisol levels and steeper slopes than the unmarried.
- Differences in cortisol output between groups were greatest during the afternoon.
- Higher stress is related to higher cortisol only for the previously married.

Figure 1.

Daily cortisol levels (expressed as area under the curve, AUC) averaged across the three days as a function of marital status, adjusted for the control variables (error bars represent 95% confidence intervals for the means).

Figure 2.

Average cortisol levels across study days (expressed as area under the curve, AUC) as a function of the interaction between marital status and perceived stress, adjusted for the control variables. Lines represent the simple slopes obtained from the regression analysis for the three marital status categories.

Figure 3.

Linear (top panel) and quadratic (bottom panel) regression lines for the multilevel model predicting diurnal cortisol slopes as a function of marital status, adjusted for the control variables. The intercept value of 0 (lighter vertical dashed line) represents the cortisol sample taken at 1-hour post-waking.

Table 1

Demographic Information by Marital Status (N = 572)

	Married (n = 160)	Never Married (n = 292)	Previously Married (n = 120)
Sex			
Male	77 (48.1%)	167 (57.2%)	56 (46.7%)
Female	83 (51.9%)	125 (42.8%)	64 (53.3%)
BMI	28.54 (6.6)	27.37 (6.4)	27.90 (6.2)
Education	13.95 (2.1)	13.98 (1.8)	13.78 (2.1)
Age	36.56 (9.9)	29.49 (8.7)	40.33 (9.3)
Race			
White	107 (66.9%)	184 (63.0%)	70 (58.3%)
Non-White	53 (33.1%)	108 (37.0%)	50 (41.7%)

Notes.

BMI = body mass index. For binary variables (i.e., sex and race), numbers inside parentheses represent percentage of sample. For continuous variables (BMI, education, and age), numbers inside parentheses represent standard deviations. Marital status differed as a function of age, $F(2,569) = 70.31, p < .001$ but was not significantly associated with any of the other demographic variables.