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Daily hassles, loneliness, and diurnal salivary cortisol in emerging adults

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ABSTRACT

This study used an intensive protocol to examine the effects of daily hassles and loneliness on diurnal salivary cortisol levels. Fifty Chinese undergraduates (28 females) provided six saliva samples each day for two consecutive days (at 0, 0.5, 3, 6, and 12 h after waking and at bedtime) and completed a questionnaire that included scales to measure daily hassles experienced over the previous month, trait loneliness, and depression. Cortisol data were aggregated over two days and used in subsequent analyses, focusing on the cortisol awakening response, diurnal slope, and overall cortisol output operationalized as the area under the curve with reference to the ground (AUC_G). Multiple regression analysis showed that an increase in loneliness had a significant association with an increase in the AUC_G and with a steeper diurnal slope. Loneliness also showed a significant interaction with daily hassles in that the positive association between daily hassles and AUC_G was accentuated in the participants who reported a greater degree of loneliness. Our findings demonstrate for the first time the importance of trait loneliness in modulating the association between daily hassles and diurnal cortisol levels, which has significant clinical implications. Interventions to reduce loneliness should help college students to better cope with daily stressors. Increased attention should also be paid to the health implications of an elevated cortisol level in this relatively young and healthy population.

1. Introduction

1.1. Loneliness and cortisol

It was recently documented that loneliness (or perceived social isolation) (Cacioppo et al., 2014) is an important determinant of health and morbidity (Hawkley & Cacioppo, 2007; Hawkley & Cacioppo, 2010; Rueggeberg et al., 2012). This has been attributed to the association of trait loneliness with potentiated nonspecific immunity and suppressed humoral immunity, which explains the increased susceptibility of lonely individuals to inflammation-driven and infectious diseases (Cole, 2009). Cole et al. (Cole et al., 2007) used DNA microarray analysis to show that this specific pattern of vulnerability is associated with the upregulation of genes involved in immune activation and the downregulation of genes involved in mature B lymphocyte function. The association between increased loneliness and excessive inflammation was verified in a large prospective study (Nersesian et al., 2018), and a separate study showed that a greater degree of loneliness is also associated with a poorer response to flu vaccination (Pressman et al., 2005).

As the end product of the hypothalamic-pituitary-adrenal (HPA) axis, cortisol is crucial to the regulation of both inflammation (e.g., Sudheimer et al., 2014) and B lymphocyte function (e.g., Marketon & Glaser, 2008), so its relationship to loneliness has been studied in various age groups (e.g., Adam et al., 2006; Doane & Adam, 2010). Dysregulation of the function of the HPA axis is an important mechanism mediating stress and ill health (Foley & Kirschbaum, 2010) and is a unique feature of the lonely phenotype (Cacioppo & Cacioppo, 2018). However, no consistent relationship pattern between loneliness and cortisol has emerged.

1.1.1. Loneliness and separate components of the diurnal rhythm of cortisol

Studies of loneliness and cortisol levels have focused on specific components of the diurnal rhythm of cortisol: the cortisol awakening response (CAR), the diurnal slope (DS), and the diurnal output. These three cortisol parameters provide complementary information about the diurnal rhythm of cortisol. The CAR refers to the increase in cortisol from waking to 30 to 45 min after waking. Although no agreed norms for the magnitude of the CAR have been established, the average increase in the waking cortisol value is between 50% and 160% (Clow et al., 2004). This component of the diurnal rhythm of cortisol has shown a positive association with chronic stress (Fries et al., 2009); it is

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regarded as "the first and the largest ultradian episode of the day" (Evans et al., 2019) and coincides with the onset of circadian activity (Lightman & Conway-Campbell, 2010). The DS of cortisol refers to the decline in the cortisol level that occurs between waking and bedtime. A recent meta-analytic review showed that a flatter slope is associated with a multitude of poor health outcomes (Adam et al., 2017). The diurnal output of cortisol is the overall secretion of cortisol throughout the day and can be defined as the area under the curve with reference to the ground (AUC_G) (Pruessner et al., 2003), which is a reliable index of exposure to cortisol. The AUC_G has important health and behavioural implications, including a negative association with cognitive function in middle-aged adults (Echouffo-Tcheugui et al., 2018; Franz et al., 2011) and a positive association with chronic burnout (Melamed et al., 1999), depression (Belanoff et al., 2002), and chronic stress (Bauer et al., 2000).

The findings on the association between loneliness and the CAR are inconsistent. Two studies reported a positive association (Doane & Adam, 2010; Steptoe et al., 2004), one study found a negative relationship (Lai et al., 2018), and one failed to find a significant relationship in an elderly sample (Schutter et al., 2017). A few studies have demonstrated an association between a greater degree of loneliness and a flattened DS of cortisol (e.g., Cole et al., 2007; Doane & Adam, 2010), but these results were not replicated in more recent studies (Drake et al., 2016; Lai et al., 2018). In fact, a greater degree of loneliness was found to be correlated with a steeper DS in the two recent studies. These contradictory findings are likely to be attributable to variations in several methodological factors, such as the saliva sampling protocol, the operationalization of the diurnal cortisol components, the participants' age, the measurement of loneliness, or a combination of these factors (Lai et al., 2018).

In contrast, converging evidence shows an association between loneliness and an elevated cortisol level (e.g., Cacioppo et al., 2000; Lai et al., 2018) and between loneliness and potentiated proinflammatory cytokines (e.g., Hänsel et al., 2010; Jaremka et al., 2013; Moieni et al., 2015), and provides support for the glucocorticoid resistance hypothesis (Cohen et al., 2012; Miller et al., 2002; Pace et al., 2007). Glucocorticoid resistance is marked by reduced responsiveness by the immune system to glucocorticoids due to impaired functioning of the glucocorticoid receptor. This condition is closely associated with chronic stress (Cohen et al., 2012) and major depression (Miller et al., 2009). Although glucocorticoid resistance has not been examined in relation to loneliness, the evidence above suggests that this condition could translate loneliness into a variety of disease and behavioural outcomes.

1.2. Loneliness and stress

Loneliness is not only associated with neuroendocrine function but has been suggested as one of the major psychological mechanisms that contribute to dysregulation of the HPA axis (e.g., Cacioppo et al., 2003). Compared to their non-lonely peers, it has been shown that lonely individuals have greater exposure to more chronic stressors (Hawkley & Cacioppo, 2007) and greater susceptibility to the negative health effects associated with stress (Cacioppo et al., 2003). Despite the relevance of stress to studies of loneliness and cortisol, past studies of loneliness rarely controlled for the effects of stress or examined the interactive effects of loneliness and stress on the cortisol level. The study conducted by Doane and Adam (Doane & Adam, 2010) is an exception. These investigators examined chronic stress in the interpersonal domain two years before the study via a semistructured interview. Although chronic stress has shown relevance to health outcomes in the literature, several studies have shown that the health effects of chronic stress measured by major life events were reduced drastically when the effects of cumulative stress as measured by daily hassles were taken into account (e.g., DeLongis et al., 1982; Kanner et al., 1981). As noted by several researchers (e.g., Lazarus, 1999; Zautra, 2003), the cumulative health effect of daily hassles could be greater than that of relatively infrequent traumatic events, which may explain why chronic stress did not show an association with any of the cortisol parameters examined by Doane and Adam (Doane & Adam, 2010). Furthermore, Chamberlain and Zika (Chamberlain & Zika, 1990) argued that daily hassles should replace major life events in the measurement of chronic stress because of the former's superior validity and reliability.

1.3. Our study

To address the issues reviewed above, this study examined the relationship between loneliness and cortisol in relation to the cumulative stress of daily hassles in undergraduates. We chose to examine daily hassles because minor daily stressors have greater relevance to a young healthy sample (Cacioppo et al., 2000), in addition to their potentially greater impact on health. Moreover, the challenges inherent in the higher education environment (e.g., Blanco et al., 2008) and that accompany emerging adulthood create a suitable context in which to examine how the interplay between stressors and vulnerability factors such as loneliness affects cortisol levels in undergraduates (Lai et al., 2018).

This study also addressed issues related to the effects of trait loneliness on diurnal cortisol parameters. Although recent studies that used multilevel or growth curve modeling with the time centered at waking are suitable to address the effects of the trait variables on waking cortisol levels and diurnal rhythms (e.g., Doane & Adam, 2010; Drake et al., 2016), this approach may not be the most appropriate to address the association between traits and diurnal cortisol levels. This has particular relevance to trait loneliness, as its association with elevated cortisol levels is likely a result of reduced receptor sensitivity and accentuated inflammatory processes. To overcome this limitation, we examined the association of cumulative daily stress and loneliness with multiple cortisol parameters, as adopted by Lai et al. (Lai et al., 2018). In particular, we examined the CAR, the diurnal slope (DS), and the diurnal cortisol output (AUC_G) in relation to loneliness and daily stress. Consistent with the glucocorticoid resistance hypothesis and findings previously reported by the principal author (Lai et al., 2018), we expected to find an association between higher loneliness scores and an accentuated AUC_G. With respect to the CAR and the DS, the mixed findings in the literature do not reliably suggest any specific hypotheses.

2. Methods

2.1. Participants

The 50 participants (28 female) were all ethnic Chinese undergraduates studying at a university in Hong Kong. Their ages ranged from 18 to 29 years (mean, 20.92 years; SD, 1.92 years), and most (62%) were in their second year of study. The participants had no known diagnosis of any psychiatric disorder or cardiovascular disease, were not currently taking medication that could affect cortisol levels, and did not smoke habitually. None of the female participants used oral contraceptives. The participants were recruited from an introductory psychology class to volunteer for the study. Course credits and cinema vouchers were given in return for their participation. Conduction of the study was approved by the Human Subject Ethics Sub-Committee of the University Research Committee of the City University of Hong Kong. Informed consent was obtained from all participants.

2.2. Procedure

The participants were invited to a briefing session during which they were given a detailed description of the saliva sampling procedure and a study package with written instructions, questionnaires, saliva sampling tubes (Salivette), and an electronic device (MEMS TrackCaps,

WestRock) to monitor the timing of saliva sampling. The participants were asked to collect by themselves six saliva samples each day on two consecutive weekdays at 0, 0.5, 3, 6, and 12 h after awakening and at bedtime. The 12 saliva sampling swabs were removed from the original Salivette tubes by the experimenter and put into vials covered at the top with MEMS TrackCaps. The participants were instructed to open the vial and remove one swab at each designated sampling time. They were told to gently chew and roll the swab around the mouth for 2 min until the swab was saturated with saliva and to then put the wet swab in a Salivette tube. The participants were also instructed to refrain from exercise, smoking, brushing teeth, eating, and drinking beverages that contain alcohol or caffeine before collection of the first two saliva samples and for 1 h before collecting the remaining four samples during a day. The importance of exact timing for saliva collection was stressed, and the participants were instructed on how the monitoring devices could record their saliva collection times. A window of ± 5 min was used for the 30-minute saliva samples, and a 30-minute window was used for subsequent samples (Stalder et al., 2016). At the end of the briefing session, the participants completed a questionnaire consisting of scales to measure trait loneliness, daily hassles, and depression. They were also asked to provide health and demographic information relevant to our study. The participants were instructed to store their saliva samples in their home freezers until they returned them to the experimenter. The returned samples were then stored in the laboratory at −20 °C until they were thawed for biochemical analysis.

2.3. Measures

Trait Loneliness was measured with a Chinese version of the eight-item Loneliness Scale (ULS-8) (Hays & DiMatteo, 1987) adapted via backward translation. The scale includes six items phrased in the lonely direction (e.g., “I lack companionship”) and two phrased in the non-lonely direction (e.g., “I can find companionship when I want it”). An adapted version was validated with a sample of 130 Taiwanese undergraduates by Wu and Yao (Wu & Yao, 2008). The Chinese ULS-8 used in this study was validated by the principal author in a Chinese undergraduate sample from Hong Kong (N = 273, including 207 women between 18 and 27 years of age; mean age, 20.27 years) (Lai, 2017). The scale was found to show internal consistency in the validation sample (Cronbach's α = 0.85).

The responses to the items of the ULS-8 in the validation sample were subjected to principal component analysis using the Kaiser criterion with varimax rotation (Kaiser, 1960). A single factor was extracted that accounted for 49.16% of the total variance. The one-factor solution was further supported by inspection of the scree plot, and the initial eigenvalue (3.939) was substantially greater than the remaining seven values (0.91, 0.75, 0.69, 0.56, 0.46, 0.40, and 0.29). The Kaiser-Meyer-Olkin measure was 0.86, and the result of Bartlett's test of sphericity was significant [$\chi^2(28) = 774.95$; $p < .001$]; both indicate sampling adequacy for factor analysis (Kaiser & Rice, 1974; Tabachnick & Fidell, 2013). Factor loadings for items of the ULS-8 were (1) “I lack companionship”: 0.81; (2) “There is no one I can turn to”: 0.67; (3) “I am an outgoing person” (reversed): 0.61; (4) “I feel left out”: 0.74; (5) “I feel isolation from others”: 0.79; (6) “I can find companionship when I want it” (reversed): 0.60; (7) “I am unhappy being so withdrawn”: 0.66; and (8) “People are around me but not with me”: 0.69.

To examine the concurrent validity of the Chinese ULS-8 scale, measures to examine optimism (the Chinese version of the Revised Life Orientation Test) (Lai, 2009), psychological distress (the Chinese version of the General Health Questionnaire) (Lai & Yue, 2000), and depression (the Chinese version of the Center for Epidemiological Studies Depression Scale, CES-D) (Zhang et al., 2011) were also administered to the validation sample. Consistent with past findings, a greater degree of loneliness showed a significant correlation with less optimism ($r = -0.45$; $p < .001$), a greater degree of depression ($r = 0.53$; $p < .001$), and more psychological distress ($r = 0.35$; $p < .001$). Neither gender nor age showed a significant correlation with loneliness. This correlation pattern provided support for the concurrent validity of the Chinese ULS-8.

To complete the scale, the study participants indicated how they experienced the feelings depicted in each of the eight items in general on a 4-point scale from 1 (never) to 4 (always). A trait loneliness score was computed by adding the ratings of the six lonely items and the reversed ratings of the two non-lonely items. The mean score and the Cronbach's α for trait loneliness were 15.82 (SD, 4.84) and 0.87, respectively, in our sample.

Cumulative Stress of Daily Hassles was assessed with a Chinese version of the Inventory of College Students' Recent Life Experiences (Kohn et al., 1990) adapted by the principal author (Lai & Mak, 2009). The adapted scale consists of 47 items of daily stressors commonly experienced by college students (e.g., too many things to do at once; lower grades than hoped for). The scale has been shown to be a reliable and valid measure of daily stress in Chinese undergraduates (Cronbach's α = 0.91) (Lai & Mak, 2009). To complete the scale, the participants were asked to indicate the extent to which each of the 47 stressors had been a part of their lives *in the past month* using a 4-point scale from 1 (not at all) to 4 (very much). A hassles score was computed by adding the ratings for the 47 items. The mean score and the Cronbach's α of the scale were 86.68 (SD, 13.37) and 0.88, respectively, in our sample.

Depression was measured with the CES-D (Cheung & Bagley, 1998; Zhang et al., 2011), which scale includes 20 items, with 16 phrased in a negative or depressed direction (e.g., “I felt depressed”) and four in a positive direction (e.g., “I was happy”). To complete the scale, the respondents were asked to indicate the weekly frequency with which they experienced each of the 20 items within the past month (1 = none; 2 = once or twice; 3 = three or four times; 4 = five times or more). The mean score and the Cronbach's α of the CES-D in our sample were 37.04 (SD = 8.09) and 0.86, respectively.

2.4. Cortisol assays

The cortisol concentrations were analyzed with an enzyme-linked immunosorbent assay (Enzo Life Sciences, Inc.) similar to those used in previous studies with Hong Kong Chinese participants (e.g., Lai et al., 2018). The assays were conducted in the laboratory of the Chemistry Department at the City University of Hong Kong. The saliva samples were thawed and centrifuged at 3500 rpm for 15 min at room temperature, and clear supernatants were used for analysis. The sensitivity of the assays was 0.2 nmol/L. The intra- and inter-assay coefficients of variation are lower than 12%, which is comparable to similar assays used in previous studies with Hong Kong Chinese participants (e.g., Lai et al., 2010; Lai et al., 2018).

3. Results

3.1. Cortisol data

The participants' rates of noncompliance, defined as the failure to self-collect saliva samples at one or more of the scheduled sampling times, were 30% and 38% on the first and second days, respectively. To control the effects of noncompliance on the cortisol parameters, two dummy variables were created for inclusion in subsequent analyses. The missing cortisol data were imputed using the expectation-maximization method (IBM SPSS 24) and winsorized at the low end to 0.2 nmol/L (Lai et al., 2018). The randomness of the missing data was supported by the result of the missing completely at random test: $\chi^2(56) = 51.29$; $p = .653$.

Table 1 summarizes the cortisol levels over the 2 days. The results of a 2 × 6 analysis of variance with repeated measures (*Day* × *Sampling Times*) and adjustments using the Greenhouse-Geisser correction show that the main effect of *Day* was not significant [$F(1, 49) = 0.68$; $p = .413$, $\text{eta}^2 = 0.00$], nor was the interaction effect [$F(2.288,$

Table 1
Mean (SEM) of cortisol levels (nmol/L) across two days.

Day	Saliva sampling times (hours post-awakening)					
	Waking	0.5 h	3 h	6 h	12 h	Bedtime
1	7.66 (0.98)	13.28 (1.50)	5.19 (0.67)	4.31 (0.72)	2.19 (0.53)	2.27 (0.43)
2	7.85 (0.92)	12.84 (1.63)	4.32 (0.58)	3.10 (0.50)	2.97 (0.51)	2.33 (0.43)
Combined	7.75 (0.86)	13.06 (1.38)	4.75 (0.58)	3.71 (0.56)	2.94 (0.46)	2.30 (0.37)

111.57) = 0.63; p = .533, eta^2 = 0.00]. The effect of *Sampling Times* was significant in that the cortisol levels varied significantly throughout the day: F(1.40, 68.71) = 58.64; p < .001, eta^2 = 0.27. The cortisol levels over the 2 days were correlated significantly at each of the six sampling times: waking, r = 0.63; 30 min, r = 0.57; 3 h, r = 0.70; 6 h, r = 0.68; 12 h, r = 0.55; bedtime, r = 0.48.

To examine the associations between cortisol, hassles, and loneliness, the cortisol data for the 2 days were aggregated to compute the indices of the diurnal rhythm of cortisol for subsequent analyses. Specifically, we focused on the overall diurnal output of cortisol, as operationalized by the AUC_G (Pruessner et al., 2003). The CAR was defined as the percentage increase from waking to 30 min after waking. The DS of cortisol was operationalized as the decrease in the cortisol level per hour from awakening to bedtime. The mean values (standard deviation) of the three indices were AUC_G = 72.15 (60.93); DS = −0.34 (0.29); CAR = 123.98% (243.85). An outlier was identified and removed for subsequent analyses in response to the observation of the accentuated value of the standard deviation of the CAR.

3.2. Hassles, loneliness, and diurnal cortisol

Table 2 shows the product-moment correlation coefficients among the key variables examined in this study. Gender, age, and compliance showed no correlation with the three indices of cortisol. However, the waking time was significantly correlated with the CAR (r = −0.32; p < .05). Participants who awakened later exhibited a lower CAR than their peers who woke earlier, which is in line with the results of previous studies (e.g., Kudielka & Kirschbaum, 2003). On the other hand, a greater AUC_G was associated with a greater degree of loneliness, more hassles, and greater depression, but only loneliness and depression showed negative associations with the DS and the CAR. The significant correlation between the AUC_G and depression could be attributed to a positive correlation between depression and the waking cortisol level (r = 0.45; p = .001) rather than to a negative correlation between depression and the bedtime cortisol level (r = 0.43; p = .002). The significant correlation between gender and age was attributed to two older female participants whose ages were 26 and 29 years, respectively.

The associations among hassles, loneliness, and cortisol were further examined using multiple regression analyses while controlling for the effects of covariates including gender, age, compliance, waking time, and depression. The main goals were to investigate (1) the effects of loneliness while controlling for the effects of daily stress and (2) the effects of the interaction between hassles and loneliness, on each of the three cortisol indices. Two models, with and without the aforementioned covariates, were compared. Model 1 used daily hassles, loneliness, and their interaction as predictors, whereas Model 2 included these three predictors and all covariates. Predictors and covariates were mean-centered before running the regression analysis for both models. In Model 1, daily hassles were entered into the regression equation first, followed by loneliness and finally the interaction term of these two predictors. The three predictor variables and covariates were entered hierarchically in regression equations in Model 2 in the following order: gender (male = 0, female = 1), age, waking time, compliance (compliant = 0, non-compliant = 1), depression, hassles, loneliness, and the interaction term between hassles and loneliness. Table 3 summarizes the results of these analyses. Cohen's d for one-sample t-tests is used to denote the effect size of the difference between a regression coefficient and the null value of zero in units of standard deviation (Lakens, 2013). With respect to Model 1, daily hassles showed a significant and positive association only with the AUC_G (β = 0.24; t = 2.26; p = .029, Cohen's d = 0.33). A greater degree of loneliness showed a significant association with a higher AUC_G (β = 0.55; t = 5.04; p = .000008, Cohen's d = 0.69), a steeper DS (β = −0.58; t = −4.74; p = .000021, Cohen's d = 0.71), and an attenuated CAR (β = −0.43; t = −3.04; p = .004, Cohen's d = 0.43). These findings replicate those of a recent study by the principal author (Lai et al., 2018). However, a significant interaction between hassles and loneliness was observed only in the AUC_G.

After controlling for the effects of covariates, the AUC_G or diurnal output of cortisol no longer showed a significant association with daily hassles (β = 0.22; t = 1.77; p = .085, Cohen's d = 0.25) in Model 2. However, the inclusion of covariates had no effect on the significant association between loneliness and the AUC_G. A greater degree of loneliness was associated with a larger AUC_G (β = 0.48; t = 3.29; p = .002, Cohen's d = 0.47) and a more positive association between

Table 2
Product-moment correlations between key variables and cortisol indices.

Variable	Age	Compliance1	Compliance2	Waking time	Hassles	Loneliness	Depression	AUC_G	DS	CAR
Gender	0.43**	−0.12	−0.05	−0.01	−0.14	−0.02	−0.01	−0.08	0.04	−0.14
Age		−0.20	−0.08	0.14	−0.15	−0.03	−0.18	−0.02	−0.06	−0.25
Compliance1			0.30*	−0.002	0.03	0.04	0.05	0.01	0.02	0.03
Compliance2				−0.03	0.02	−0.29*	−0.09	−0.19	0.19	0.18
Waking time					0.14	0.08	0.07	0.14	−0.11	−0.32*
Hassles						0.30*	0.40**	0.38**	−0.24	−0.24
Loneliness							0.59***	0.66***	−0.62***	−0.45**
Depression								0.48***	−0.34*	−0.35*
AUC_G									−0.59**	−0.33*
DS										0.44**
Mean	20.92	–	–	09:08	86.68	15.82	37.04	72.15	−0.34	123.98%
SD	0.27	–	–	1 h 18 min	13.37	4.84	8.09	60.93	0.29	243.85

Note. AUC_G = area under the curve with reference to ground; DS = diurnal slope; CAR = cortisol awakening response; gender: male = 0, female = 1; Compliance1 = compliance on Day 1; Compliance2 = compliance on Day 2; compliance: compliant = 0, non-compliant = 1.
After the outlier has been removed, the mean and SD of CAR = 90.98%, 71.61, respectively. Correlations of CAR with other variables were computed without the outlier (n = 49).
* p < .05.
** p < .01.
*** p < .001.

Table 3
Linear regression models predicting cortisol indices.

Predictor	AUC_G			DS			CAR		
	β	*t*	*p*	*β*	*t*	*p*	*β*	*t*	*p*
Model 1									
Hassles	0.24	2.26	0.029	−0.08	−0.63	0.531	−0.10	−0.69	0.496
Loneliness	0.55	5.04	0.000008	−0.58	−4.74	0.000021	−0.43	−3.04	0.004
Hassles × loneliness	0.25	2.43	0.019	−0.09	−0.73	0.471	−0.07	−0.47	0.641
Model 2									
Hassles	0.22	1.77	0.085	−0.09	−0.62	0.536	−0.09	−0.59	0.557
Loneliness	0.48	3.29	0.002	−0.60	−3.64	0.001	−0.30	−1.69	0.099
Hassles × loneliness	0.26	2.32	0.026	−0.08	−0.66	0.516	0.05	0.36	0.723

Note. AUC_G = area under the curve with reference to ground; DS = diurnal slope; CAR = cortisol awakening response; gender: male = 0, female = 1; compliance: compliant = 0, non-compliant = 1. Gender, age, waking time, compliance and depression were included as covariates in Model 2. Regression analyses of CAR were run after removing the single outlier.

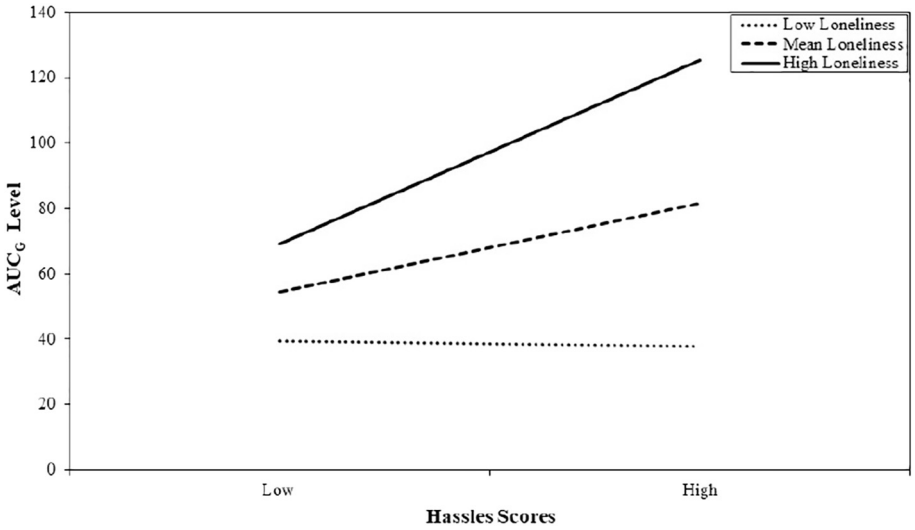

Fig. 1. Regression lines predicting AUC_G from hassles and loneliness. AUC_G = area under the curve with reference to ground; Low Hassles = −1 SD; High Hassles = +1 SD; Low Loneliness = −1 SD; High Loneliness = +1 SD.

hassles and the AUC_G, as indicated by a significant interaction between hassles and loneliness ($\beta = 0.26$; $t = 2.32$; $p = .026$, Cohen's d = 0.33). This significant interaction was probed with the simple slope technique (Aiken & West, 1991). Fig. 1 illustrates the nature of this interaction: the positive correlation between hassles and the diurnal cortisol level became stronger as the loneliness scores increased from −1 SD through the centered mean to +1 SD. For the diurnal decline in cortisol, no significant association was found between hassles and the DS ($\beta = -0.09$; $t = -0.62$; $p = .536$, Cohen's d = 0.10), and no significant interaction was found between hassles and loneliness ($\beta = -0.08$; $t = -0.66$; $p = .516$, Cohen's d = 0.01). However, a greater degree of loneliness was associated with a steeper DS ($\beta = -0.60$; $t = -3.64$; $p = .001$, Cohen's d = 0.51), which was driven by higher waking cortisol levels ($r = 0.74$; $p < .001$) rather than lower bedtime levels ($r = 0.62$; $p < .001$) in the lonely participants. Loneliness also showed a significant correlation with the remaining four sampling times (30 min, $r = 0.58$; 3 h, $r = 0.62$; 6 h, $r = 0.53$; 12 h, $r = 0.59$). Finally, the significant association between loneliness and the CAR disappeared when the effects of the covariates were considered ($\beta = -0.30$; $t = -1.96$; $p = .099$, Cohen's d = 0.24). However, an attenuated CAR was associated with a later waking time.

Because the significant association between loneliness and the CAR reported by the principal author and his associates in an earlier study was not replicated in Model 2, additional multiple regression analyses were performed to examine possible variations in the association between hassles, loneliness, and the CAR across the two sampling days in this study, with all covariates included in the analyses. Loneliness showed a significant association with the CAR on day 1 ($\beta = -0.39$; $t = -2.23$; $p = .031$, Cohen's d = 0.32) but not on day 2 ($\beta = 0.03$; $t = 0.19$; $p = .853$, Cohen's d = 0.03), which could be attributed to the absence of a correlation in the CAR across the 2 days ($r = -0.003$, $p = .981$) in comparison with the DS ($r = 0.30$, $p = .036$) and the AUC_G ($r = 0.80$, $p = .000$). The reason for this pattern of correlation is not immediately apparent but could be attributed to the greater sensitivity of the CAR to state factors (Hellhammer et al., 2007).

4. Discussion

The findings of this study partially replicate those of an earlier study conducted by the principal author (Lai et al., 2018) in which a greater degree of loneliness was associated with a higher AUC_G and a steeper DS. Moreover, these findings are also consistent with that reported previously regarding loneliness and diurnal cortisol levels (Cacioppo et al., 2000) and that for loneliness and diurnal slope (Drake et al., 2016). The overall accentuation of diurnal output in lonely participants lends support to the glucocorticoid resistance hypothesis. In contrast, the significant association between greater loneliness and the attenuated CAR reported by Lai et al. (Lai et al., 2018) was not observed in this study, possibly because of the enhanced effects of state factors on cortisol based on saliva samples collected in a single day (Hellhammer et al., 2007) in the study of Lai et al. (Lai et al., 2018).

The significant moderating effect of loneliness on the link between

hassles and diurnal cortisol output demonstrates how loneliness influences endocrine outcomes and has important health implications in the context of stress. Cacioppo et al. (2003) stated that the relationships among stress, loneliness, and cortisol could be subsumed under three hypotheses. First, the "added-stress hypothesis" postulates that the frequent social rejection and isolation experienced by lonely individuals produces negative feelings/affects that combine with the intensity of stress experienced by lonely individuals to contribute to elevated cortisol levels. In other words, stress could mediate the relationship between loneliness and cortisol. Second, according to the "differential-exposure hypothesis," lonely individuals are exposed to stressful events more frequently than their non-lonely peers, and the higher level of stress experienced by lonely individuals could thus lead to greater cortisol output. This hypothesis implies that loneliness could mediate the relationship between stress and cortisol. Third, the "differential-stress-buffering hypothesis" posits that although the intensity and frequency of the stressors experienced by lonely versus non-lonely individuals are similar, the former still exhibit higher cortisol levels because they cannot cope with stressors as effectively as their non-lonely peers. The effect of the significant interaction between hassles and loneliness on the AUC_G observed in our study provides support for the "differential-stress-buffering hypothesis." This also explains the mixed findings in previous studies (e.g., Doane & Adam, 2010; van Eck et al., 1996; Pruessner et al., 2003) because the strength of the relationship between life stress (or hassles) and cortisol is determined by a moderator variable such as loneliness. Our findings should not be attributed to the use of a lonelier sample because the mean loneliness score of our sample (15.82) was slightly lower than that of a similar group of Taiwanese undergraduates (17.12) examined in a previous study (Wu & Yao, 2008).

Although Doane and Adam (2010) demonstrated a significant effect of the interaction between chronic life stress and loneliness on momentary increases in cortisol, it was based on "momentary" (or state) loneliness rather than trait loneliness. These investigators showed that chronic stress enhances the positive association between state loneliness and daily cortisol levels, which differs from the enduring effects of trait loneliness on the relationship between stress and cortisol demonstrated in our study. The significant moderating effect of trait loneliness on the relationship between hassles and cortisol has both theoretical and clinical implications. As mentioned above, this finding provides clear support for the "differential-stress-buffering hypothesis" (Cacioppo et al., 2003) and clarifies the mixed findings in the literature concerning the relationship between life stress and cortisol levels. The significant moderating effect of loneliness also has important clinical implications by pinpointing appropriate targets for intervention. Fig. 1 illustrates that the effects of loneliness on cortisol were stronger in the participants who reported a higher level of stress, which implies that loneliness is an appropriate target of intervention, because a reduction in the experience of loneliness would attenuate the effects of stress on cortisol. The viability of this intervention is supported by ample evidence of the effectiveness of interventions to reduce loneliness (Masi et al., 2011). Although loneliness is not particularly prevalent in the age group that generally includes college undergraduates (Schultz & Moore, 1988), our findings show that a reduction in loneliness should also benefit this young and healthy population. Moreover, a recent randomized trial showed that an 8-week mindfulness-based stress reduction program significantly reduced the expression of proinflammatory genes in older adults (Creswell et al., 2012), so extending the research to young populations provides additional support to the glucocorticoid resistance hypothesis and the generalizability of the effectiveness of the mindfulness-based stress reduction program. In addition, our findings also suggest that training in stress management skills could be an effective mode of intervention to reduce stress and thus cortisol levels.

Despite the significance of our findings, their impact is moderated by several limitations and issues that may not have been addressed adequately. First, the relatively smaller sample size ($N = 50$) than those used in previous studies, noncompliance, and missing data may limit the generality of our findings, although noncompliance had no relationship with cortisol levels. Second, the use of self-reports of waking time may undesirably affect the assessment of waking time and thus the CAR. Third, compliance with the prohibition of eating, smoking, and other behaviors before saliva sampling should have been monitored. Fourth, our findings deviate significantly from those reported in two previous studies that used equally vigorous methods (Doane & Adam, 2010; Drake et al., 2016). The major finding of these two studies was an association between higher loneliness scores and flatter diurnal slopes, which was not observed in our study. As reported above, the main finding of our study is a positive association between loneliness and cortisol levels throughout the day. Although this discrepancy could be explained by methodological factors, such as variations in the analysis strategies and operationalizations of the construct of loneliness, the influence of contextual or sociocultural factors should not be overlooked. Finally, our study does not illuminate the physiologic mechanisms that translate loneliness into ill health via glucocorticoid resistance because proinflammatory cytokines and objective health data were not examined. Although lonely participants produce more proinflammatory cytokines in response to acute laboratory stressors (e.g., Jaremka et al., 2013), the manner in which loneliness promotes proinflammatory cytokines via activation of the HPA axis induced by life stress is not completely understood. It has been suggested that sensitization of the inflammatory response to repeated exposure to stressors may contribute to the long-term development of diseases in overly stressed or vulnerable individuals (Rohleder, 2014). Evidently, further research is needed to better explain the role played by cortisol in this process.

5. Conclusions

Our findings demonstrate the importance of the construct of loneliness in studies of cortisol. This is the first study to illustrate that loneliness is not only associated with an increase in cortisol output but also accentuates the positive association between daily hassles and cortisol output. Although the detrimental effects of chronic exposure to a higher level of cortisol have been well documented, evidence to show that the glucocorticoid resistance hypothesis contributes to the association between loneliness and ill health remains elusive. The ontogenesis of loneliness is also poorly understood, and a systematic study is needed to illuminate the link among loneliness, an elevated cortisol level, and objective health outcomes with vigorous designs informed by developmental theories. Increased emphasis should be placed on the mechanisms through which early experiences such as childhood adversities are biologically embedded and lead to the lonely phenotype.

Declaration of Competing Interest

There are no competing interests to declare.

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