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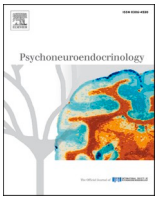

Endocrine and immunomodulatory effects of social isolation and loneliness across adulthood

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ABSTRACT

Experimental and observational evidence agreed on two interconnected biological mechanisms responsible for the links between social isolation/loneliness and health: alterations in the activity of the hypothalamic-pituitary-adrenal (HPA) axis and compromised functioning of the innate immune system. However, most existing studies did not consider the simultaneous impact of social isolation and loneliness on biological outcomes. Further, they only assessed one biological outcome at a time and did not test any moderation by age, despite empirical and theoretical evidence supporting the plausibility of this hypothesis. To address these gaps in the literature, we tested the associations between two indicators of social isolation (living status and frequency of social contacts) and loneliness and daily cortisol secretion and two markers of systemic inflammation (C-reactive protein [CRP] and interleukin-6 [IL-6]) in a sample of adults aged between 25 and 75 years old. Data were drawn from the Midlife in the United States (MIDUS) Refresher study ($N = 314$). We found that, above and beyond loneliness, living alone was associated with a flattened diurnal cortisol slope (i.e., reduced changes in cortisol levels during waking hours that are indicative of a dysregulated HPA axis) and higher CRP levels. On the other hand, higher loneliness was associated with higher IL-6 levels, above and beyond our measures of social isolation. Loneliness did not mediate any of the effects of social isolation on either cortisol or CRP, and age did not moderate any of the relationships reported above. Our findings support the idea that social isolation and loneliness have unique and independent endocrine and immune effects despite being linked to each other. Understanding the specific biological pathways through which these aspects of social well-being exert their effects on health across the lifespan has critical consequences for both intervention development and public health policies.

1. Introduction

Many countries' social distancing regulations in response to the COVID-19 pandemic left hundreds of millions of people around the world socially isolated. Fig. 1 shows the popularity of the queries "social isolation" (blue) and "loneliness" (red) in Google Search in the US from January 1, 2020, to June 1, 2020. In mid-March 2020, social isolation temporarily reached the same level of popularity of loneliness. Although social isolation has recently been brought to the forefront of the public health discourse, it needs to be considered in tandem with the emotional discomfort often associated with it (i.e., loneliness). Despite being an intuitive concept, the idea that social isolation and loneliness should be considered together has been often overlooked in empirical investigations. This is particularly true about studies interested in unraveling the biological processes through which social isolation and loneliness impact health. Accordingly, the current study aimed to test the unique contribution of social isolation above and beyond loneliness, and vice versa, on the activity of the hypothalamic-pituitary-adrenal (HPA) axis and systemic inflammation, which previous theoretical and empirical literature has identified as robust processes affected by social well-being (Quadt et al., 2020; Smith et al., 2020).

Social isolation has been defined as the objective condition of having few and infrequent social contacts, while loneliness refers to the negative affect associated with one's perception of social isolation. Social isolation and loneliness have been associated with several subjective and objective clinical endpoints, from depression (Cacioppo et al., 2006) and poor self-reported physical health (Nummela et al., 2011) to cardiovascular disease (Sorkin et al., 2002; but see Hegeman et al., 2018), premature cognitive decline (Shankar et al., 2013), and, potentially, even certain forms of cancer (Fox et al., 1994). Not surprisingly, lonely

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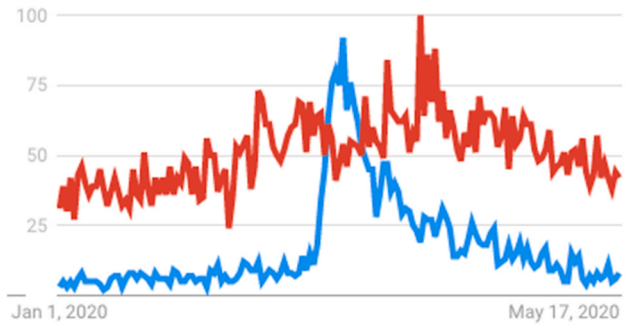

Fig. 1. Levels of popularity of the queries "social isolation" (blue) and "loneliness" (red) in Google Search in the US from January 1, 2020, to June 1, 2020. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
Data source: Google Trends (https://www.google.com/trends).

and socially isolated individuals have also been found to die earlier than their more socially integrated counterparts (Holt-Lunstad et al., 2015). Alterations in the HPA axis activity and compromised functioning of the innate immune system have been suggested to play a crucial role in mediating the effect of loneliness and social isolation on poor health outcomes (Cacioppo et al., 2014).

Human studies on the endocrine sequelae of loneliness and social isolation have focused primarily on the activity of the HPA axis and considered both individual differences in diurnal cortisol secretion (Lai et al., 2019; Pressman et al., 2005; Zilioli et al., 2019) and cortisol reactivity to acute stressors (Hackett et al., 2012). Germane to the current study, research among adults found that unmarried young and middle-aged adults (a proxy for social isolation) had higher levels of daily cortisol and flatter cortisol slopes than married individuals (Chin et al., 2017). Higher levels of loneliness in older adults have been associated with higher cortisol levels late in the day (Montoliu et al., 2019), which are often indicative of a flattened diurnal cortisol rhythm. In a sample of middle-aged and older African Americans, Zilioli et al. (2019) found that more participation in social activities, assessed through ecological momentary assessments over a week, was associated with steeper daily cortisol slopes, while living status was not associated with daily cortisol. Lastly, in one of the most recent contributions to this field, Johar et al. (2020) found that higher levels of loneliness were associated with flatter cortisol slopes in a large sample of middle-aged and older married adults. Overall, these findings seem to suggest that social isolation and loneliness are associated with alterations in the daily cortisol rhythm, specifically in the form of flattened cortisol slopes. Results concerning other daily cortisol parameters, such as the cortisol awakening response (CAR), are mixed (e.g., Adam et al., 2006).

Compared to the neuroendocrinology literature, the psychoneuroimmunology literature focused mostly on middle-aged and older adults (Smith et al., 2020; but see Matthews et al., 2019). Findings from this relatively small body of work are mixed and seem to vary depending on the biomarker of inflammation under investigation. Germane to the current study, which focuses on C-reactive protein (CRP) and interleukin-6 (IL-6), a recent review concluded that significant associations exist between loneliness and IL-6 and social isolation and CRP (Smith et al., 2020). Empirical studies that found the most robust links between loneliness and IL-6 focused on middle-aged adults and controlled in their analyses for social isolation (Cho et al., 2015; Nersesian et al., 2018). Empirical studies that found the most robust links between social isolation and CRP focused on both middle-aged and older adults and, with one exception (Shankar et al., 2011), did not control for loneliness in their analyses (Ford et al., 2006; Loucks et al., 2006; Mezuk et al., 2010). In most of these studies, indexes of social isolation were created by combining factors related to one's social network (e.g., number of yearly or monthly contacts (Ford et al., 2006; Shankar et al., 2011), living (Shankar et al., 2011) or marital status (Loucks et al., 2006), and social participation (Ford et al., 2006; Loucks et al., 2006).

Based on this literature review, our research hypotheses were that both social isolation (frequency of social contacts and living status considered separately) and loneliness would be associated with flatter cortisol slopes, social isolation would be associated with higher CRP levels, and higher levels of loneliness would be associated with higher IL-6 levels. We also tested the novel hypothesis that loneliness would mediate some of the effects of social isolation on daily cortisol slope and inflammation (Steptoe et al., 2013). Lastly, we explored whether age moderated the association between social isolation, loneliness, and biological risk. The Socioemotional Selectivity Theory (Carstensen et al., 1999) suggests that older individuals invest more effort towards emotion-related goals than younger individuals. One implication of this preference is that older adults become more selective in their social bonds and interactions, preferring to spend more time with familiar and emotionally close others. Thus, it could be hypothesized that feelings of loneliness might negatively impact the physiological health of older adults more than the physiological health of young and middle-aged adults. Somewhat surprisingly, the little empirical data available on any potential moderating role of age seem to suggest the opposite. In a recent meta-analysis of 70 empirical studies, Holt-Lunstad and colleagues (2015) found that social isolation and loneliness were stronger risk factors for early mortality among adults aged 65 and younger. In our analyses, we tested the moderating effect of age; however, we did not make any specific predictions about the directionality of this effect.

2. Methods

2.1. Participants and procedure

Data for this study were drawn from a subsample ($N = 314$) of the Midlife in the United States (MIDUS) Refresher study. Participants from this subsample completed both the biomarker project and salivary cortisol assessment component of the daily diary project. The MIDUS Refresher (2011–2014), which was designed to replenish the original MIDUS cohort, consisted of a national sample of 3577 noninstitutionalized adults aged 25–75 years living in the US. Participants who completed the phone interview and self-administered questionnaire were eligible to participate in the biomarker project ($N = 863$, 42.0% response rate) and the daily diary project ($N = 781$, 63.2% response rate). Data for the biomarker project were collected during participants' 24-h stay at three General Clinical Research Centers: University of Wisconsin, Madison; University of California, Los Angeles; and Georgetown University, Washington, DC. The daily diary project included salivary cortisol assessment over 4 days and daily phone interviews over 8 days. The sample mean age in the current study was 47.70 years ($SD = 11.85$), with 53.5% of the sample being female, and 84.7% of the sample being White.

2.2. Measures

2.2.1. Social isolation

Social isolation has been measured using various indicators (Victor et al., 2000) and scales (e.g., Greenfield et al., 2002), including measures of constructs theoretically distinct from it, such as *perceived* social support (e.g., Matthews et al., 2019). A discussion of the proper conceptual demarcations of social isolation is beyond the scope of this paper (Victor et al., 2000); however, when defined as the objective condition of being alone, the frequency of social contacts (from now on referred to as social contact) and living status (e.g., living alone) have been commonly used as direct indicators of social isolation (Shankar et al., 2013, 2011; Steptoe et al., 2013; Victor et al., 2000). In light of established theoretical recommendations (Victor et al., 2000), we adopted these two indicators of social isolation and investigated them separately to quantify their unique contribution.

Social contact was assessed using three items: (1) the frequency of contacts with any family members, on an 8-point scale (*from 1 = several times a day to 8 = never or hardly ever)*; (2) the frequency of contacts with friends, on an 8-point scale (*from 1 = several times a day to 8 = never or hardly ever)*; and, (3) the frequency of contacts with neighbors, on a 6-point scale (*from 1 = almost every day to 6 = never or hardly ever)*. Three participants had missing data on at least one of the three items. The social contact index for these three participants was treated as missing. Responses on these three items were z-scored and added to create a composite index of social contact, with higher scores reflecting less frequent social contacts. Living status was coded as 1 = living alone (i.e., participants were the only people currently living in the household) and 0 = not living alone (i.e., participants reported more than one individual living in the household).

2.2.2. Loneliness

Loneliness was assessed using the 7-item UCLA Loneliness Scale (Russell, 1996). Participants were asked to rate how often each of the seven statements (e.g., I feel isolated from others) was descriptive of them on a 4-point scale (*from 1 = never to 4 = often*). All participants answered all seven items. Responses on the seven items were added to calculate a composite score of loneliness, with higher scores reflecting higher levels of loneliness. Cronbach's alpha for this scale was 0.88 in the current study.

2.2.3. Cortisol

Salivary cortisol was collected using Salivettes (Sarstedt, Rommelsdorft, Germany) four times a day across 4 days. The daily collection time points occurred immediately upon waking, 30 min later, before lunch, and at bedtime. Cortisol concentrations were determined with a commercially available luminescence immunoassay (IBL, Hamburg, Germany). Intra- and inter-assay coefficients of variability (CVs) were less than 5%. Collection compliance was monitored with night phone interviews and paper-and-pencil logs. Raw cortisol values were log-transformed to correct for positive skew in the distribution. A constant of 1 was added before the transformation to ensure that all transformed scores were positive.

2.2.4. Systemic inflammation (CRP and IL-6)

IL-6 was assayed using the Quantikine high-sensitivity ELISA kit (R&D Systems, Minneapolis, MN), with intra- and inter-assay CVs of 3.7% and 15.7%, respectively. CRP was assayed using the BNII nephelometer (Dade Behring, Inc., Deerfield, IL), with intra- and inter-assay CVs ranging from 1.1% to 4.4%. To correct for skewed distributions, IL-6 and CRP were log-transformed.

2.2.5. Covariates

Key sociodemographic, health, and behavioral covariates were included in the analyses, as done in previous studies on salivary cortisol and inflammation (Friedman and Herd, 2010). Sociodemographic covariates included sex (0 = male, 1 = female), race (0 = non-White, 1 = White), age, and socioeconomic status (SES). SES was assessed using participants' household-adjusted income and highest education attainment on a 12-point scale, ranging from *1 = no school/some grade school to 12 = any type of doctorate*. A composite score of SES was calculated by averaging z-scored household-adjusted income and education. Average wakeup time was also included as a covariate in the analyses for diurnal cortisol. Health covariates included presence of any chronic health conditions and waist-hip ratio. The presence of at least one chronic health condition was assessed using a self-report checklist of 30 possible chronic conditions (e.g., stroke, asthma) during the past 12 months (0 = no, 1 = yes). The waist-hip ratio was included as a covariate to control for adiposity (Panagiotakos et al., 2005). Behavioral covariates included smoking (0 = never smoker/past smoker, 1 = current smoker), alcohol use (0 = no-regular alcohol use [< 3 days per week], 1 = regular alcohol use [≥ 3 days per week]), and regular physical activity for 20 min or more at least three times a week (0 = no, 1 = yes). Depressive symptoms were also included as a key covariate due to their overlap with social isolation and loneliness. Depressive symptoms were assessed using the 20-item Center for Epidemiological Studies Depression scale (C-ESD, Roberts and Vernon, 1983). Participants answered each item using a 4-point scale, ranging from *0 = rarely or none of the time to 3 = most or all of the time*. Following previous studies, one item on loneliness was dropped from the CES-D scale to avoid direct overlap with loneliness (Steptoe et al., 2013). A composite score for depressive symptoms was calculated by summing responses across the 19 items, with higher scores reflecting more severe depressive symptoms. One participant did not answer one item. Response on that item was replaced with the average score of the remaining 18 completed items. Cronbach's alpha for this scale was 0.88 in the current study.

2.3. Statistical analyses

Multivariate regression analyses were employed to test the effects of social isolation and loneliness on inflammation (i.e., IL-6, CRP). Multilevel modeling (MLM) was employed to test the effects of social isolation and loneliness on diurnal cortisol, which allowed us to estimate multiple cortisol parameters (e.g., cortisol at awakening, CAR, diurnal slope). Following previous studies (Adam et al., 2006), time since waking, time since waking-squared, and CAR (dummy coded as 1 = sample at 30-min after wakeup, 0 = other) were included as Level 1 predictors. CAR samples that deviated by 10 min or more from the requested 30-min interval were dropped from the analyses (i.e., 10.8% of the total CAR samples). At Level 2, we tested the effects of social isolation and loneliness on cortisol parameters. Cortisol intercept, CAR, and slope were treated as random effects at Level 2, while time since waking-squared was treated as a fixed effect. Continuous variables at Level 2 were grand-mean centered.

Following the procedure outlined by previous studies testing the independent and unique effects of social isolation and loneliness on health outcomes (Kobayashi and Steptoe, 2018), a series of MLM and multivariate regression models were run. Specifically, Model 1 did not adjust for covariates except for average wakeup time for the cortisol analyses, Model 2 adjusted for sociodemographic covariates (i.e., sex, race, age, SES), Model 3 additionally adjusted for health covariates (i.e., chronic health condition status and waist-hip ratio), and Model 4 additionally adjusted for behavioral covariates (i.e., smoking, alcohol use, physical activity). In Model 5, social isolation and loneliness were both added to the model, and Model 6 additionally adjusted for depressive symptoms.

To test the potential moderating role of age, interaction terms (i.e., age by social contact, age by living status, and age by loneliness) were included. These analyses were run separately for social isolation (age by social contact, age by living status) and loneliness (age by loneliness). Continuous variables were mean-centered before the interaction term was computed. Path analyses were then performed to test the potential mediating role of loneliness in the associations between social isolation and diurnal cortisol and between social isolation and inflammation. Moderation and mediation analyses were first performed without covariates and then adjusted for sociodemographic covariates, health covariates, behavioral covariates, and depressive symptoms.

Lastly, sensitivity analyses were run to test the effects of social isolation and loneliness on diurnal cortisol adopting more stringent exclusion criteria used by recent MIDUS studies on cortisol (e.g., Karlamangla et al., 2019). Specifically, cortisol values > 60 nmol/L or collected on days when participants woke before 4:00 am or after 11:00 am were deleted from the analyses. Sensitivity analyses were also run to test the associations between social isolation and loneliness with

Table 1
Means, standard deviations, and bivariate correlations among variables.

Variables	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1 Living alone	-																	
2 Social contact	.03	-																
3 Loneliness	.12*	.29***	-															
4 CRP	.16*	.04	.11*	-														
5 IL-6	.12*	.01	.15**	.63***	-													
6 Cortisol at awakening	-.06	.09	.00	-.08	-.16**	-												
7 CAR	-.04	.04	-.06	-.07	-.12*	.32***	-											
8 Diurnal slope	.12*	-.04	.13*	.21***	.21***	-.31***	-.39***	-										
9 Female	-.04	-.16**	.05	.12*	.01	-.08	.12*	.03	-									
10 White	.01	-.04	-.23***	-.03	-.14*	.00	.12*	-.11*	-.07	-								
11 Age	.22***	-.05	-.04	.14*	.38***	-.30***	-.06	.07	.03	.05	-							
12 SES	.17**	-.01	-.04	-.22***	-.12*	-.03	.09	-.10	-.11	.16**	.10	-						
13 Chronic condition	.05	.05	.15**	.08	.17**	-.05	-.03	.04	.08	-.04	.14*	-.06	-					
14 Waist-hip ratio	.11*	.19**	.01	.18**	.31**	.00	-.09	.06	-.61***	.03	.24***	-.04	.02	-				
15 Smoke	.04	.01	.05	-.01	-.05	.01	.05	.04	.04	-.02	-.01	-.17**	-.02	.01	-			
16 Alcohol use	-.07	-.10	.05	-.09	-.02	.02	.00	-.04	-.09	.09	.09	.16**	-.01	.00	.12*	-		
17 Physical activity	-.15**	-.03	-.05	-.17**	-.15**	-.08	.01	.01	.08	.02	-.04	.12*	-.04	-.22***	-.04	.06	-	
18 Depressive symptoms	-.02	.15**	.60***	.15**	.15**	-.05	-.12*	.10	.14*	-.18**	-.08	-.10	.19**	-.02	-.03	.02	-.06	-
M	53[a]	0.00	12.71	2.77[b]	2.46[c]	3.09[d]	0.34[d]	-0.10[d]	168[a]	266[a]	47.70	0.00	235[a]	0.89	23[a]	69[a]	236[a]	8.90
SD	16.9	1.93	4.49	6.29	2.30	0.26	0.07	0.02	53.5	85.5	11.85	0.81	75.8	0.11	7.3	22.0	75.2	7.49

Note. CRP = C-reactive protein; IL-6 = interleukin-6; CAR = cortisol awakening response; SES = socioeconomic status.
[a] Presented as *N* and SD below as %
[b] Unit ug/mL
[c] Unit pg/mL
[d] Average cortisol parameters across participants
* $p \leq .05$;
** $p \leq .01$;
*** $p \leq .001$.

Table 2
Effects of social isolation on diurnal cortisol profile and inflammation.

Model	Diurnal cortisol profile			Inflammation	
	CAA	CAR	Diurnal slope	IL-6	CRP
Model 1: Without covariates[a]					
Living alone	-.074(0.053)	-.010(0.047)	.011(0.005)*	.274(0.122)*	.529(0.196)**
Social contact	.018(0.010)	-.002(0.008)	-.001(0.001)	.002(0.024)	.022(0.038)
Model 2: Model 1 + sociodemographic[b]					
Living alone	-.012(0.051)	-.022(0.046)	.010(0.005)*	.136(0.117)	.595(0.184)**
Social contact	.011(0.010)	.004(0.008)	-.001(0.001)	.009(0.023)	.034(0.036)
Model 3: Model 2 + health[c]					
Living alone	-.013(0.050)	-.023(0.046)	.010(0.005)*	.092(0.117)	.538(0.182)**
Social contact	.011(0.010)	.003(0.008)	-.001(0.001)	-.010(0.023)	.011(0.036)
Model 4: Model 3 + behavioral[d]					
Living alone	-.022(0.051)	-.028(0.045)	.010(0.005)*	.084(0.112)	.503(0.178)**
Social contact	.012(0.010)	.002(0.008)	-.001(0.001)	-.009(0.022)	.011(0.036)
Model 5: Model 4 + loneliness					
Living alone	-.016(0.051)	-.026(0.046)	.009(0.005)	.043(0.118)	.465(0.184)*
Social contact	.014(0.010)	.004(0.008)	-.001(0.001)	-.026(0.025)	-.006(0.038)
Model 6: Model 5 + depressive symptoms					
Living alone	-.020(0.053)	-.033(0.047)	.009(0.005)*	.055(0.119)	.486(0.184)**
Social contact	.014(0.010)	.003(0.008)	-.001(0.001)	-.026(0.025)	-.006(0.038)

Note. Unstandardized coefficients (standard errors) were presented. CAA = cortisol at awakening; CAR = cortisol awakening response; IL-6 = interleukin-6; CRP = C-reactive protein.
[a] Wakeup time was included as a covariate for diurnal cortisol.
[b] Sociodemographic covariates included sex, race, age, and socioeconomic status.
[c] Health covariates included chronic health condition and waist-hip ratio.
[d] Behavioral covariates included smoking, alcohol use, and physical activity.
* $p \leq .05$;
** $p \leq .01$.

CRP, with participants with CRP values greater than 10 mg/L ($N = 14$) being removed from the analyses (Pearson et al., 2003).

The mean incidence of missing data for Level 2 predictors was about 0.3%, and the expectation-maximization algorithm was used to impute missing data on continuous variables. Mode imputation was used for categorical variables. Previous studies suggest that the expectation-maximization algorithm yields less biased estimates than ad hoc methods (e.g., listwise deletion; Schafer and Graham, 2002). All analyses were run in Mplus 7.0 using maximum likelihood estimation with robust standard errors.

3. Results

Table 1 displays descriptive statistics and bivariate correlations between study variables. Both living alone and loneliness correlated with IL-6, CRP, and diurnal cortisol slope ($ps < .05$) but not cortisol at awakening or CAR ($ps > .20$). Social contact did not correlate with any inflammatory markers or diurnal cortisol parameters ($ps > .10$).

3.1. Effects on social isolation and loneliness on diurnal cortisol and inflammation

Table 2 displays the effects of social isolation on diurnal cortisol parameters and inflammation. Results showed that living alone, but not social contact, was associated with a flatter diurnal cortisol slope ($\beta_{21} = 0.011$, $p = .015$; $\beta_{22} = -0.001$, $p = .40$; respectively). Neither living alone nor social contact was associated with cortisol at awakening or CAR ($ps > .05$). The strength of the association between living alone and diurnal cortisol slope only changed slightly after adjusting for sociodemographic covariates, health covariates, behavioral covariates, loneliness, and depressive symptoms ($\beta_{21} = 0.009$, $p = .050$). Living alone was also positively associated with IL-6 and CRP ($b = 0.274$, $p = .025$; $b = 0.529$, $p = .007$; respectively). The association between living alone and IL-6 became nonsignificant after adjusting for sociodemographic covariates ($b = 0.136$, $p = .25$). In contrast, the association between living alone and CRP remained significant after controlling for sociodemographic covariates, health covariates, behavioral

Table 3
Effects of loneliness on diurnal cortisol profile and inflammation.

Model	Diurnal cortisol profile			Inflammation	
	CAA	CAR	Diurnal slope	IL-6	CRP
Model 1: Without covariates[a]	.000(0.005)	-.002(0.003)	.001(0.000)	.029(0.011)*	.032(0.015)*
Model 2: Model 1 + sociodemographic[b]	-.001(0.005)	-.002(0.003)	.001(0.000)	.026(0.010)*	.032(0.014)*
Model 3: Model 2 + health[c]	-.001(0.005)	-.002(0.004)	.001(0.000)	.021(0.010)*	.027(0.013)*
Model 4: Model 3 + behavioral[d]	-.002(0.005)	-.002(0.004)	.001(0.000)	.022(0.010)*	.028(0.013)*
Model 5: Model 4 + social isolation	-.004(0.005)	-.002(0.004)	.001(0.000)	.025(0.011)*	.023(0.015)
Model 6: Model 5 + depressive symptoms	-.001(0.006)	.002(0.005)	.001(0.001)	.017(0.013)	.010(0.018)

Note. Unstandardized coefficients (standard errors) were presented. CAA = cortisol at awakening; CAR = cortisol awakening response; IL-6 = interleukin-6; CRP = C-reactive protein.
[a] Wakeup time was included as a covariate for diurnal cortisol.
[b] Sociodemographic covariates included sex, race, age, and socioeconomic status.
[c] Health covariates included chronic health condition and waist-hip ratio.
[d] Behavioral covariates included smoking, alcohol use, and physical activity.
* $p \leq .05$.

covariates, loneliness, and depressive symptoms ($b = 0.486$, $p = .008$). Social contact was not associated with IL-6 or CRP ($ps > .30$).

Table 3 displays the effects of loneliness on diurnal cortisol profile and inflammation. Loneliness was not associated with any cortisol parameters ($ps > .05$). Loneliness, however, was associated with IL-6 and CRP ($b = 0.029$, $p = .011$; $b = 0.032$, $p = .033$; respectively). The associations between loneliness and IL-6 and CRP remained significant after adjusting for sociodemographic, health, and behavioral covariates ($b = 0.022$, $p = .024$; $b = 0.028$, $p = .036$; respectively). Loneliness remained associated with IL-6 after further adjusting for social isolation ($b = 0.025$, $p = .024$), whereas the association between loneliness and CRP became nonsignificant after controlling for social isolation ($b = 0.023$, $p = .12$). When further adjusting for depressive symptoms, there were no associations between loneliness and IL-6 or CRP ($ps > .10$).

3.2. Age differences

Moderation analyses showed that living alone did not interact with age in predicting cortisol at awakening, CAR, or diurnal cortisol slope ($\beta_{04} = -0.002$, $p = .63$; $\beta_{14} = 0.001$, $p = .81$; $\beta_{24} = 0.000$, $p > .99$; respectively). Similarly, there were no interactive effects between social contact and age in predicting cortisol at awakening, CAR, or diurnal cortisol slope ($\beta_{05} = 0.000$, $p = .93$; $\beta_{14} = 0.000$, $p = .79$; $\beta_{24} = 0.000$, $p = .27$; respectively). Age also did not moderate the associations between living alone and systemic inflammation ($b = -0.005$, $p = .52$, for IL-6; $b = -0.025$, $p = .13$, for CRP) and between social contact and systemic inflammation ($b = 0.000$, $p = .96$, for IL-6; $b = 0.001$, $p = .80$, for CRP). After adjusting for sociodemographic covariates, health covariates, behavioral covariates, and depressive symptoms, all two-way interaction terms continued to remain nonsignificant ($ps > .10$). There were no interactive effects between loneliness and age on cortisol at awakening, CAR, or diurnal cortisol slope ($\beta_{03} = 0.000$, $p = .81$; $\beta_{13} = 0.000$, $p = .23$; $\beta_{24} = 0.000$, $p = .80$; respectively). Loneliness also did not interact with age in predicting IL-6 or CRP ($b = -0.001$, $p = .34$; $b = 0.000$, $p = .77$; respectively). These results did not change after adjusting for sociodemographic covariates, health covariates, behavioral covariates, and depressive symptoms ($ps > .10$).

3.3. The mediating role of loneliness

Given that there were no effects of loneliness on cortisol parameters, we only tested the mediating effects of loneliness for the association between social isolation and biomarkers of systemic inflammation. The mediation model showed that both living alone and social contact were associated with loneliness ($b = 1.379$, $p = .038$; $b = 0.683$, $p < .001$; respectively), which, in turn, was associated with IL-6, but not CRP ($b = 0.028$, $p = .021$; $b = 0.026$, $p = .11$; respectively). There was a direct effect of living alone on CRP but not IL-6 ($b = 0.492$, $p = .014$; $b = 0.234$, $p = .067$; respectively). Social contact was not associated with IL-6 or CRP ($b = -0.018$, $p = .50$; $b = 0.004$, $p = .93$; respectively). The indirect effects, which were tested using the bootstrapping method (i.e., confidence interval [CI] obtained from 1,000 resamples), indicated significant indirect effects of living alone and social contact on IL-6 through loneliness (effect = 0.039, 95% CI [0.003, 0.112]; effect = 0.019, 95% CI [0.004, 0.043]; respectively). However, after adjusting for sociodemographic covariates, health covariates, behavioral covariates, and depressive symptoms, these indirect effects became nonsignificant (effect = 0.028, 95% CI [− 0.011, 0.098] for living alone; effect = 0.009, 95% CI [− 0.005, 0.025] for social contact).

3.4. Sensitivity analyses

Sensitivity analyses for diurnal cortisol showed similar results to what reported above, with the exception that social contact was now significantly associated with cortisol at awakening ($\beta_{02} = 0.024$, $p = .019$). Living alone remained associated with diurnal cortisol slope ($\beta_{21} = 0.010$, $p = .036$). There were no other associations between social isolation and diurnal cortisol parameters ($ps > .10$). Loneliness was not associated with any diurnal cortisol parameters ($ps > .10$). The effect of living alone on diurnal cortisol slope remained of similar magnitude but fell short of statistical significance after controlling for sociodemographic covariates (Model 2, $\beta_{21} = 0.008$, $p = .081$), additionally adjusting for health and behavioral covariates (Model 4, $\beta_{21} = 0.009$, $p = .054$), and further controlling for loneliness and depressive symptoms (Model 6, $\beta_{21} = 0.008$, $p = .090$).

Sensitivity analyses for CRP showed similar results to the ones reported above. Living alone, but not social contact, was associated with higher CRP levels ($b = 0.362$, $p = .048$; $b = 0.017$, $p = .61$; respectively). The association between living alone and CRP remained significant after adjusting for sociodemographic and health covariates (Model 3, $b = 0.327$, $p = .044$) but did not reach statistical significance after further controlling for behavioral covariates (Model 4, $b = 0.294$, $p = .068$), and additionally including loneliness and depressive symptoms as covariates (Model 6, $b = 0.275$, $p = .094$). Loneliness was associated with CRP ($b = 0.027$, $p = .049$), and this association remained significant after controlling for sociodemographic covariates (Model 2, $b = 0.027$, $p = .037$). However, the association between loneliness and CRP became nonsignificant when further adjusting for health covariates (Model 3, $b = 0.022$, $p = .082$) and further including behavioral covariates, social isolation, and depressive symptoms in the model (Model 6, $b = 0.012$, $p = .48$).

4. Discussion

Several findings emerged from this study. Our results indicated that living alone, but not social contact, was associated with a flattened diurnal cortisol slope and higher CRP levels. These effects were robust to the inclusion of sociodemographic and health-related covariates. Notably, they also remained significant after including loneliness and depressive symptoms in the model, suggesting that living alone impacted diurnal cortisol and CRP above and beyond psychological constructs tightly related to this objective condition. Our second measure of social isolation, social contact, was not related to any of the endocrine and immune outcomes considered. Although loneliness was not associated with any daily cortisol parameters, people reporting higher levels of loneliness had higher systemic inflammation, indexed by higher levels of both CRP and IL-6. However, these associations, which were robust to the inclusion of sociodemographic and health-related covariates, changed when social isolation (indexed by both living alone and social contact) and depressive symptoms were included in the model. Specifically, loneliness continued to be associated with IL-6 but ceased to be associated with CRP when social isolation variables were included in the model. No associations between loneliness and systemic inflammation emerged when the frequency of depressive symptoms was further added as a covariate in the model. Lastly, we did not find support for the hypothesis that loneliness might mediate some of the effects of social isolation on daily cortisol and systemic inflammation. Similarly, no evidence was found supporting the idea that age might moderate some of the associations between social isolation and loneliness and the biological processes investigated.

Consistent with prior studies (Chin et al., 2017; Loucks et al., 2006; Mezuk et al., 2010; Shankar et al., 2011), we found that middle-aged and older adults living alone experienced a flattened diurnal cortisol slope and higher levels of CRP and IL-6. A potential explanation for these findings is that people living with others might engage more in behaviors beneficial to health compared to people living alone, a hypothesis that has found empirical support since the 1980s (e.g., Umberson, 1987, for a recent study, see Schrempft et al., 2019). In our study, however, the relationships between living status and diurnal cortisol slope and CRP were robust to the inclusion of health behaviors, suggesting that other pathways contributed to these associations. In this regard, additional

explanations include the idea that people living with others have more direct access to different types of social support (e.g., emotional, instrumental, informational) (Gariepy et al., 2016), experience less negative affect (Lam and García-Román, 2020) and more satisfaction with interpersonal relationships (Mellor et al., 2008), and are less likely to be exposed to social (e.g., job loss) and physical (e.g., disease; Jain et al., 2017) threats. All these factors have been previously associated with healthier daily cortisol profiles (for a review, see Miller et al., 2007) and reduced systemic inflammation (for review, see Uchino, 2006). However, not all of these alternative pathways might contribute equally to explain our findings. For example, in our analyses, living alone continued to be associated with daily cortisol and CRP levels after controlling for depressive symptoms and loneliness, indicating that heightened negative affect did not mediate the effects of social isolation on health-related biology. Further supporting this claim, we found no statistically significant indirect effect linking living status to either daily cortisol slope or CRP through self-reported loneliness.

In several of the studies cited above, marital status, rather than living status, was used as a proxy of social isolation. In our study, living status was chosen as a broader category reflecting the objective condition of being alone. Although we did not report marital status directly, it is very likely that the majority of the people we categorized as "not living alone" were indeed married. However, a question emerges as to whether the effects observed here could be attributed to some of the benefits and interpersonal processes specific to being married (e.g., intimacy, perceived responsiveness). Although possible, this explanation appears unlikely because variability in these processes exists (e.g., not all marriages share the same level of intimacy and marital satisfaction), and this variability has been shown to map onto variability in various health outcomes, including daily cortisol secretion (Saxbe et al., 2008; Slatcher et al., 2015) and inflammation (Whisman and Sbarra, 2012). Future studies focusing on long-distance romantic relationships and romantic couples living apart are required to disentangle the unique contribution of interpersonal romantic processes and living status on health-related endocrine and immune processes.

Turning to the loneliness results, we found that participants with higher levels of loneliness had higher IL-6 and CRP levels. In models adjusted for social isolation, however, the relationship between loneliness and CRP disappeared. Our results are congruent with recent meta-analytic evidence in favor of a positive relationship between loneliness and IL-6, but not CRP (Smith et al., 2020). Smith and colleagues suggested that this differential effect of loneliness on inflammatory markers might be related to the fact that experiences of loneliness are often transient (Qualter et al., 2015); that is, when experiencing states of loneliness, people are more likely to engage in affiliative behaviors aimed at reducing the emotional discomfort associated with loneliness. Notably, social motivation is highlighted as a crucial adaptation to loneliness by the "social homeostatic model" (Matthews and Tye, 2019, see also the "social allostatic load model" by Quadt et al., 2020), a recently developed theoretical framework on the behavioral and neural adaptations to deficits in social connections. Further, acute and chronic stressors impact the innate immune system differently, with the former ones more likely to influence the first line of defense of the innate immune system (i.e., the release of cytokines), and the latter ones more likely to modulate downstream inflammatory markers, such as CRP (Hawkley et al., 2007; Marsland et al., 2017). Accordingly, it can be hypothesized that loneliness, when experienced as a transient psychological state, is more likely to act as an acute stressor and thus be related to cytokines rather than acute-phase proteins. In a recent study, Vingeliene and colleagues (2019) provided partial support for this hypothesis by showing that persistent loneliness (i.e., loneliness experienced at both baseline and follow-up) was associated with higher CRP levels. However, in the same study, the authors also found that CRP levels were higher in those participants who reported loneliness at follow-up but not at baseline, somewhat weakening the idea of an exclusive effect of persistent loneliness on CRP levels. This hypothesis remains a theoretically interesting one that awaits further empirical testing.

Loneliness and depression are highly correlated; however, they are deemed as distinct constructs. Specifically, loneliness is thought to be an antecedent of depression (Cacioppo et al., 2010). In our study, we found that the association between loneliness and CRP was no longer significant after accounting for depressive symptoms (for a similar approach, see Shankar et al., 2011; Steptoe et al., 2013). These results might support the hypothesis that depression acts as an intermediary for the association between loneliness and health outcomes, including systemic inflammation (Vingeliene et al., 2019). However, the few studies that have formally tested this mediating model using longitudinal designs failed to support this hypothesis (e.g., Luo et al., 2012; Vingeliene et al., 2019). In our study, loneliness was not associated with daily cortisol slope or any other daily cortisol parameters. This is somewhat surprising given that several studies found higher loneliness to be associated with higher levels of evening cortisol (Montoliu et al., 2019; Pressman et al., 2005) and a flatter cortisol decline throughout the day (Johar et al., 2020). That said, inconsistent findings in this narrow literature are not uncommon. For example, in one of the early studies on this topic, Kiecolt-Glaser et al. (1984) found loneliness to be positively correlated with urinary cortisol levels in a sample of hospitalized psychiatric patients. This finding was not replicated about 20 years later in a larger community-based sample of middle-aged and older adults (Hawkley et al., 2006). As described above, to the extent to which experiences of loneliness are transient, they might not be associated with chronic alterations in the activity of the HPA axis, as indexed by individual differences in cortisol slopes averaged across several days (Wang et al., 2014; but see, Zilioli et al., 2017).

Another unexpected finding that emerged from our study was that none of the associations between our predictors (i.e., social isolation and loneliness) and outcomes (i.e., cortisol and inflammation) was moderated by age. Loneliness seems to be stable throughout the lifespan (Mund et al., 2020). Some researchers have speculated that loneliness and social isolation might have more pronounced effects on older adults' than younger adults' physical health (Hawkley and Capitanio, 2015). This hypothesis is in line with the idea that feelings of social disconnectedness, particularly from familiar and emotionally close others, might be more harmful among older adults who place a greater premium on emotionally meaningful experiences than younger adults (Carstensen et al., 1999). The few existing empirical findings in this area, however, seem to suggest otherwise. For instance, in a recent meta-analysis, Holt-Lunstad and colleagues (2015) found that social isolation and loneliness were more strongly associated with mortality risk among adults aged 65 and younger. Our results supported neither hypothesis. However, one limitation of our study was the underrepresentation of older adults (only about 19% of our participants aged 60 and older, and about 10% of them aged 65 years and older). Studies using more age-balanced samples are required to test these competing hypotheses properly. Another limitation of our study concerns its cross-sectional nature, limiting our interpretations of the temporal relationships between social isolation and loneliness and diurnal cortisol and inflammation. This limitation also applies to our mediation analyses. Specifically, our cross-sectional mediation likely yielded biased estimates of the parameters of the corresponding longitudinal mediation model (Maxwell et al., 2011). Longitudinal studies are warranted to understand how these associations unfold over time. In addition to longitudinal studies, these associations could be tested for more robust causal inference through intervention studies (e.g., Creswell et al., 2012) and quasi-experiments, in which conditions of social isolation and loneliness would naturally emerge (e.g., moving abroad for work). Although this study included a national sample of middle-aged and older adults in the US, our sample was predominantly White. More studies on the endocrine and immune correlates of social isolation and loneliness are needed among racial minorities (e.g., Zilioli et al., 2019). Lastly, two important caveats should be considered when assessing the results of

this study. First, the validity and reliability of our findings rely on the validity and reliability of the scales used. One of the reasons for conducting our study using the MIDUS Refresher sample instead of the MIDUS original cohort was based on the fact that loneliness in the refresher sample was assessed using the UCLA Loneliness Scale. By contrast, one loneliness item was used in the MIDUS original cohort (Nersesian et al., 2018). Regardless, future work should continue assess loneliness using multiple-item scales. Second, in our sensitivity analyses, some of the associations reported above fell short of statistical significance after the inclusion of specific covariates. Future studies with larger sample sizes are required to corroborate our findings.

In sum, the current study aimed at advancing the psychoneuroendocrinology and psychoneuroimmunology of social isolation and loneliness by 1. considering the simultaneous impact of these two constructs on both daily cortisol secretion and systemic inflammation; 2. determining whether loneliness mediated any of the effects of social isolation on daily cortisol and systemic inflammation; and, 3. testing any moderating role of age. Above and beyond loneliness, we found that living alone was associated with a flattened diurnal cortisol slope and higher CRP levels. On the other hand, loneliness was associated with higher IL-6 levels, above and beyond our measures of social isolation. Loneliness did not mediate any of the effects of social isolation on either cortisol or CRP, and age did not moderate any of the relationships reported above. Our findings support the idea that loneliness and social isolation have unique and independent endocrine and immune effects and are relevant to mental and physical health in light of the well-established impact of endocrine and immune dysregulations on several mental health disorders and aging-related diseases. We hope these findings can advance the science of the biobehavioral pathways through which social well-being exerts its effects on health across the lifespan.

CRediT authorship contribution statement

Samuele Zilioli: Study conceptualization and writing. **Yanping Jiang**: Data analyses, Study conceptualization and writing.

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Declaration of Competing Interest

The authors confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. The authors further confirm that any aspect of the work covered in this manuscript that has involved human subjects has been conducted with the ethical approval of all relevant bodies.

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