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Loneliness and stress-related inflammatory and neuroendocrine responses in older men and women

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Summary Loneliness is a predictor of mortality and increased cardiovascular morbidity. Inflammation is a potential pathway through which loneliness might impact health. The aim of the study was to investigate the relationship between loneliness and inflammatory interleukin-6 (IL-6), interleukin-1 receptor antagonist (IL-1Ra) and monocyte chemotactic protein-1 (MCP-1) responses to standardized mental stress. A secondary purpose was to evaluate whether individual variations in cortisol responses influenced the hypothesised relationship between loneliness and inflammation. Saliva samples and blood were taken from 524 healthy middle-aged men and women from the Whitehall II cohort at baseline, immediately after the stress tasks and 45 min later. Loneliness was measured using the revised UCLA loneliness scale. Greater loneliness was associated with larger IL-6 ($p = 0.044$) and IL-1Ra ($p = 0.006$) responses to psychological stress and higher MCP-1 ($p < 0.001$) levels in women, independently of age, grade of employment, body mass index and smoking status. No associations were observed in men. Cortisol responsivity was inversely related to loneliness in women, with the odds of being a cortisol responder decreasing with increased loneliness independently of covariates ($p = 0.008$). The impact of loneliness on health in women may be mediated in part through dysregulation of inflammatory and neuroendocrine systems.

1. Introduction

The impact of loneliness on health has become increasingly well recognised. Loneliness is independently predictive of mortality (Penninx et al., 1997; Patterson and Veenstra, 2010; Shiovitz-Ezra and Ayalon, 2010; Tilvis et al., 2011) and increases the risk of having a heart condition (Sorkin et al., 2002). There is some evidence that the effects of loneliness may be differentially experienced by men and women, although the literature on gender differences in loneliness levels is mixed (Pinquart and Sörensen, 2001; Theeke, 2009). For example, Thurston and Kubzansky (2009) showed that loneliness was prospectively linked with incident coronary heart disease (CHD) over a 19-year follow-up period, after controlling for standard risk factors. But effects were only found in women, and not in men.

Identification of the biological mechanisms through which loneliness might impact health would clarify the processes

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underlying these epidemiological observations. The biological concomitants of loneliness can be investigated using a number of different research strategies, including clinical assessments of variables such as blood pressure, naturalistic monitoring of biological function in everyday life, and mental stress testing (Steptoe and Poole, 2010). Mental stress testing involves the measurement of biological responses to acute challenges, and has the advantage that detailed dynamic responses can be studied under controlled conditions, reducing the impact of other factors that may confound associations. Loneliness has been linked with changes in cardiovascular processes such as elevated systolic blood pressure (Hawkley et al., 2006, 2010), greater diastolic blood pressure reactions to acute stress in women (Steptoe et al., 2004), elevated total peripheral resistance, lower heart rate variability and decreased cardiac output (Cacioppo et al., 2002; Hawkley et al., 2003). Ong et al. (2012) recently reported that loneliness was associated with greater blood pressure responses to social evaluative threat and with delayed post-stress recovery, and that this effect was accentuated by greater age. Elevated cortisol levels have been observed in both lonely students (Cacioppo et al., 2000; Pressman et al., 2005; Doane and Adam, 2010) and lonely adults (Steptoe et al., 2004; Adam et al., 2006). Cortisol is involved in regulating inflammation through activation of the glucocorticoid (GC) receptor mechanisms, leading to inhibition of pro-inflammatory signalling pathways (Raison and Miller, 2003; Rhen and Cidlowski, 2005). Previously we have shown that cortisol responders have significantly smaller cytokine responses compared with cortisol non-responders (Kunz-Ebrecht et al., 2003). Therefore, as loneliness has been linked with elevated cortisol levels, the heightened risk of diseases related to inflammation in the lonely may appear paradoxical.

One explanation is that loneliness may compromise the regulation by GCs of pro-inflammatory transcription pathways. Cole et al. (2007) analysed genome-wide transcriptional activity, and identified 209 transcriptions representing 144 genes that were differentially expressed in lonely compared to non-lonely individuals. Loneliness was associated with an over-expression of genes with elements for the pro-inflammatory NF-κB/Rel transcription pathway and impaired expression of anti-inflammatory GC response genes. Verifying the increased inflammatory signalling observed in the genomic analyses, lonely individuals had significantly greater circulating C-reactive protein (CRP) concentrations than non-lonely individuals.

Additionally, several studies have found a link between altered immunoregulation and stress responsivity in the lonely. For example, loneliness has been associated with reduced natural kill cell activity and less lymphocyte transformation by the Epstein Barr virus in response to examination stress (Kiecolt-Glaser et al., 1984a,b) and increased human herpes virus antibody titers following an environmental stressor (Dixon et al., 2001).

Overall, these data point towards an association between loneliness, stress responsivity and inflammation. Previously we observed an independent association between loneliness and heightened fibrinogen concentrations in response to psychological stress (Steptoe et al., 2004). By contrast, some studies involving samples taken at rest have not found a link between loneliness and inflammatory markers (O'Luanaigh et al., 2012; Shankar et al., 2011).

In the present study, we investigated the relationship of loneliness with inflammatory responses to standardized mental stress, assessing interleukin-6 (IL-6), interleukin-1 receptor antagonist (IL-1Ra), and the chemokine monocyte chemotactic protein-1 (MCP-1). Elevated IL-6 levels have been prospectively associated with coronary disease (Danesh et al., 2008) and heightened circulating IL-1Ra and certain polymorphisms of the IL-1Ra gene have been linked with coronary events (van Minkelen et al., 2009; Fragoso et al., 2010). MCP-1 is not as widely studied as IL-6 and IL-1Ra, but it is thought to play an important role in pathogenesis of vascular diseases by promoting recruitment of leukocytes to sites of inflammation in the vessel wall (Niu and Kolattukudy, 2009; Hansson and Hermansson, 2011). Elevated MCP-1 concentrations have been positively associated with subclinical arthrosclerosis and incident CHD (Herder et al., 2006; Tang et al., 2007). Gender differences in the relationship between loneliness and health have been reported (Thurston and Kubzansky, 2009) and gender may be a moderator both of inflammatory cytokine (Steptoe et al., 2002) and cortisol responses (Kudielka and Kirschbaum, 2005), with evidence of greater cortisol responses to acute stress in older women (Kudielka et al., 2004; Seeman et al., 1995). We tested for gender differences in association between loneliness and biological stress responses. We reasoned that that if inflammation mediates the influence of loneliness on disease risk, then loneliness would be positively associated with stress responses in IL-6, IL-1Ra and MCP-1. We were also interested in investigating whether individual variations in cortisol responses influenced the hypothesised relationship between loneliness and inflammation.

2. Method

2.1. Participants

Participants were recruited from the Whitehall II epidemiological cohort of British civil servants (Marmot et al., 1991) for psychophysiological stress testing between 2006 and 2008. The primary purpose of the study was to investigate biological responses to acute stress in relation to socioeconomic factors and subclinical coronary atherosclerosis and cardiovascular risk (e.g. Hamer et al., 2010; Steptoe et al., 2010; Hamer and Steptoe, 2012). Participants had no history or objective sign of CHD and no diagnosis or treatment for hypertension, diabetes, inflammatory diseases, allergies, anxiety or major depression. Civil service employment grade was used as an indicator of socioeconomic status, and recruitment was stratified to include a range of employment grades. 543 men and women age 53—76 years took part in stress testing, of whom 524 provided data on loneliness and at least one of the key biological measures (IL-6, IL-1Ra or MCP-1). People included and excluded from the statistical analyses did not differ in age, gender distribution, grade of employment, body mass index (BMI), smoking status or loneliness. In the seven days prior to testing, participants were prohibited from taking anti-inflammatory or anti-histamine medication and were rescheduled if they reported colds or other infections on the day of testing. Participants were also instructed

to refrain from drinking caffeinated beverages or smoking for at least 2 h before testing and to avoid vigorous exercise and alcohol from the previous evening. All participants gave full informed consent to take part in the study and ethical approval was granted by the UCLH Committee on the Ethics of Human Research.

2.2. Psychological measures

Loneliness was assessed with the revised UCLA loneliness scale as part of a questionnaire battery completed before the start of the session (Russell et al., 1980). This self-report questionnaire consists of 20 items which are rated on a four-point Likert scale from 1 = never to 4 = often. Total loneliness scores were calculated by summing the responses for all items. Total scores ranged from 20 to 80, with higher scores indicating greater loneliness. The internal consistency (Cronbach's alpha) of the scale was 0.94 in this sample. Depression was also assessed before the session using the Center for Epidemiologic Studies Depression scale (CESD), a standard measure of depressive symptomatology (Radloff, 1977).

During the laboratory session, subjective stress ratings were periodically obtained on a 7-point scale, with higher values indicating greater stress.

2.3. Mental stress tasks

Mental stress was induced in the laboratory using two 5-min behavioural tasks. The first was a computerized version of the Stroop colour-word interference task which involved successive presentation of target colour words (e.g. red, blue) printed in another colour (Muldoon et al., 1992). In the task, there were four names of colours printed in incongruous colours at the bottom of the computer screen. Participants were requested to press the computer key that corresponded to the position at the bottom of the screen of the name of the colour in which the target word was printed. To ensure sustained demands, the rate of presentation of stimuli was adjusted to the performance of the participant. The second task was mirror tracing, which involved tracing with a metal stylus a star that could only be seen in mirror image (Owens et al., 1993). Each time the stylus came off the star a mistake was registered and a loud beep was emitted by the apparatus (Lafayette Instruments Corp., Lafayette, IN, USA). Participants were told that the average person could complete five circuits of the star in the available time. These tasks were selected because they have been shown to stimulate similar appraisals of involvement and engagement from participants across the social gradient (Steptoe et al., 2002), and have been used in a number of previous studies in our laboratory (Steptoe et al., 1999).

2.4. Biological measures

Blood samples were collected in EDTA tubes and centrifuged immediately at 2500 rpm for 10 min at room temperature. Plasma was removed from the tube and aliquoted into 0.5 ml portions and stored at $-80\ ^{\circ}C$ until analysis. Plasma IL-6 was assayed using a Quantikine® high sensitivity two-site enzyme-linked immunosorbent assay (ELISA) from R&D Systems (Oxford, UK). The sensitivity of the assay ranged from 0.016 to 0.110 pg/ml and the intra and inter assay coefficient of variations (CVs) of 7.3% and 7.7% respectively. IL-1Ra and MCP-1 were assayed in duplicate using fluorescent-labelled capture antibody beads from Millipore (Milliplex Human Cytokine/Chemokine kit, Millipore Corporation, US), and concentrations were determined with Luminex flow cytometer technology from Bio Rad (Bio-Plex, Hercules, CA, US). The limit of detection for IL-1Ra was 2.3 pg/ml and the mean intra and inter assay CVs were 4.6% and 6% respectively. For MCP-1 the limit of detection was 1.2 pg/ml and the mean intra and inter assay CVs were 6.1% and 12% respectively. Saliva samples were obtained for the assessment of cortisol. The samples were collected using Salivettes (Sarstedt, Leicester, UK), which were stored at $-20\ ^{\circ}C$ until analysis. Cortisol levels were assessed using a time resolved immunoassay with fluorescence detection, at the University of Dresden. The intra- and inter-assay coefficients of variation were less than 8%. As noted in previous analyses of this dataset (Hamer et al., 2010), the cortisol responses to this protocol were small. We therefore used the procedure described by Hamer et al. (2010) to classify participants as cortisol responders or non-responders. Individuals were defined as cortisol responders if salivary cortisol increased $\geq$1 nmol/l above baseline either immediately after tasks or 20 min later. This corresponds to a 20% increase relative to baseline. Responses above and below this level are differentially associated with subclinical coronary artery disease as defined by coronary artery calcification (Hamer et al., 2010), and also predict the development of future hypertension (Hamer and Steptoe, 2012).

2.5. Procedure

Participants were tested individually in a light and temperature-controlled laboratory. Scheduling demands required that sessions were held both in the morning and the afternoon. At the start of the session height, weight, waist and hip circumference measurements were taken using standardized techniques. Measurement devices for the assessment of cardiovascular activity (not described here) were attached, and a venous cannula was inserted for the collection of blood samples. After a rest period of 30 min, the baseline blood sample was drawn, saliva was collected for the analysis of cortisol, and a subjective rating of stress was obtained. Then the two behavioural tasks were administered in random order. A subjective stress rating, second blood and saliva samples were taken immediately after tasks. A further blood sample was drawn 45 min after tasks and saliva was collected at 20 and 45 min post tasks.

2.6. Statistical analysis

Plasma IL-6 values were normally distributed, but both IL-1Ra and MCP-1 were skewed so were log transformed before analysis. Men and women were compared using χ^2 tests for categorical and *t*-tests for continuous variables. Preliminary analyses of subjective stress, IL-6, IL-1Ra and MCP-1 responses to tasks were carried out using repeated measures analysis of variance with gender as the between-person factor and trial (baseline, task, 45 min post-task) as the within-person factor. The Greenhouse–Geisser correction

of p values was applied to correct for sphericity where appropriate, but raw degrees of freedom are presented in Section 3. Significant effects were further explored using Tukey's least significant differences (LSD) test. In the light of the gender differences in responses described in Section 3, associations between loneliness and biological responses were analysed separately for men and women. Multivariable linear regressions on baseline values of IL-6, IL-1Ra and MCP-1, and regressions on responses following stress were carried out. The independent variables in these analyses were loneliness, age, grade of employment, BMI, and smoking status. The variance in biological responses accounted for by loneliness was quantified by η^2 in the analyses of variance and by computing the change in variance (Δr^2) accounted for by loneliness once other factors had been included in the regression models. To ensure that differences in biological markers were attributable to loneliness, preliminary analyses were conducted. These analyses indicated that time of day of stress testing did not impact on the associations with loneliness, so time of day was not included in the final models. Depressed mood was also measured in this study. However, preliminary analyses indicated that it was unrelated to the patterns of IL-6, IL-1Ra and MCP-1 response described in the Results, so it was not included in the final statistical models.

Results are presented as unstandardized regression coefficients (B) with 95% confidence intervals (C.I.). In the case of cortisol, logistic regression was carried out on the likelihood of being a cortisol responder; adjusted odds ratios and 95% C.I. are presented. Comparisons were also made following grouping individuals into loneliness tertiles, and comparing the highest (mean loneliness 47.0 ± 7.8) and lowest (mean loneliness 24.0 ± 2.6) tertiles. However, the pattern of results was the same as that observed using regression across the whole range of loneliness scores, so the latter analyses are presented in Section 3. Significant effects from the regression analyses were illustrated by comparing lower, intermediate and higher tertiles of loneliness using analysis of covariance.

3. Results

3.1. Characteristics of participants

The characteristics of participants in the study are summarized in Table 1. Participants were aged 63.0 ± 5.7 years on average, with mean age being greater in women than men ($t = 3.52$, $p < 0.001$). There was no significant difference in the distribution of grade of employment, but men had higher BMIs ($t = 2.97$, $p = 0.003$). Mean loneliness ratings did not differ between men and women.

3.2. Responses to stress

Subjective stress ratings were higher in women than men (F (1, 516) = 7.06, $p < 0.001$), and the trial (F (2, 1032) = 1291.6, $p < 0.001$) and gender by trial interaction (F (2, 1032) = 3.33, $p = 0.036$, $\eta^2 = 0.006$) were also significant. Mean ratings at each time point are shown in Table 2. Subjective stress increased following tasks in both men and women, returning to baseline levels at 45 min post-task, but the increase with tasks was greater in women than men.

The main effect for trial was significant in the analysis of IL-6 (F (2, 1004) = 139.4, $p < 0.001$), together with the gender by trial interaction (F (2, 1004) = 14.48, $p < 0.001$, $\eta^2 = 0.028$). As can be seen in Table 2, IL-6 increased in both men and women following stress, with the largest responses 45 min post-task. The increase was larger in women than men and also emerged sooner, so that differences from baseline were evident in the blood sample taken immediately after the task period. There was no significant change across trials in IL1-Ra (F (2, 668) = 0.05, $p = 0.95$). However, men had higher mean levels of IL1-Ra throughout (F (1, 334) = 8.81, $p = 0.003$, $\eta^2 = 0.026$). As in the case of IL-6, there was also a significant gender by trial interaction in the analysis of MCP-1 (F (2, 910) = 4.90, $p = 0.008$, $\eta^2 = 0.011$). The concentration of MCP-1 rose following stress in both men and women (see Table 2). Values returned to baseline during the recovery period in men, but remained significantly elevated in women. Across the whole study period, MCP-1 concentration was higher in men than women (F (1, 455) = 5.44, $p = 0.020$, $\eta^2 = 0.020$). Cortisol rose from a mean of 6.48 ± 4.1 nmol/l at baseline to 7.75 ± 5.1 nmol/ following tasks; 206 individuals (39.9%) were defined as cortisol responders, including 114 (40.7%) men and 92 (39.0%) women; these proportions did not differ.

3.3. Loneliness and subjective and biological responses

Loneliness was positively associated with stress ratings at baseline in both men and women ($B = 0.009$, C.I. = 0.01—0.017, $p = 0.034$, and 0.012, C.I. = 0.001—0.022, $p = 0.033$ respectively). Stress ratings following tasks and the changes in stress ratings in response to tasks were not related to loneliness, but ratings during recovery were again positively

Table 1 Characteristics of participants in the study Means ± standard deviations and N (%).

	Men (n = 283)	Women (n = 241)	p difference
Age (years)	61.18 ± 5.8	63.93 ± 5.4	0.001
Grade of employment			
Higher	111 (39.2%)	90 (37.3%)	0.064
Intermediate	122 (43.1%)	83 (34.4%)	
Lower	50 (17.7%)	68 (28.2%)	
Body mass index (kg/m^2)	26.33 ± 3.6	25.32 ± 4.2	0.003
Current smoker	15 (5.3%)	14 (5.8%)	0.85
Loneliness	35.00 ± 11.4	34.2 ± 9.7	0.39

Table 2 Subjective and biological responses to stress Means ± SD.

	Baseline	Task	45 min post-task
Subjective stress			
Men	1.42 ± 0.78^{a}	3.98 ± 1.33^{b}	1.41 ± 0.82^{a}
Women	1.44 ± 0.86^{a}	4.32 ± 1.49^{b}	1.53 ± 0.92^{a}
IL-6 (pg/ml)			
Men	1.38 ± 0.85^{a}	1.39 ± 0.86^{a}	1.57 ± 0.93^{b}
Women	1.30 ± 0.82^{a}	1.35 ± 0.90^{b}	1.69 ± 1.09^{c}
IL-1Ra (log) (pg/ml)			
Men	2.66 ± 0.81	2.62 ± 0.85	2.66 ± 0.83
Women	2.39 ± 0.86	2.42 ± 0.82	2.37 ± 0.85
MCP-1 (log) (pg/ml)			
Men	4.96 ± 0.35^{a}	5.03 ± 0.37^{b}	4.97 ± 0.35^{a}
Women	4.89 ± 0.31^{a}	4.94 ± 0.33^{b}	4.93 ± 0.34^{b}
Cortisol responders *n* (%)		114 (40.7%)	92 (39.0%)
Men			
Women			

[a,b,c]Values in rows with different superscripts are significantly different from one another ($p < 0.05$).

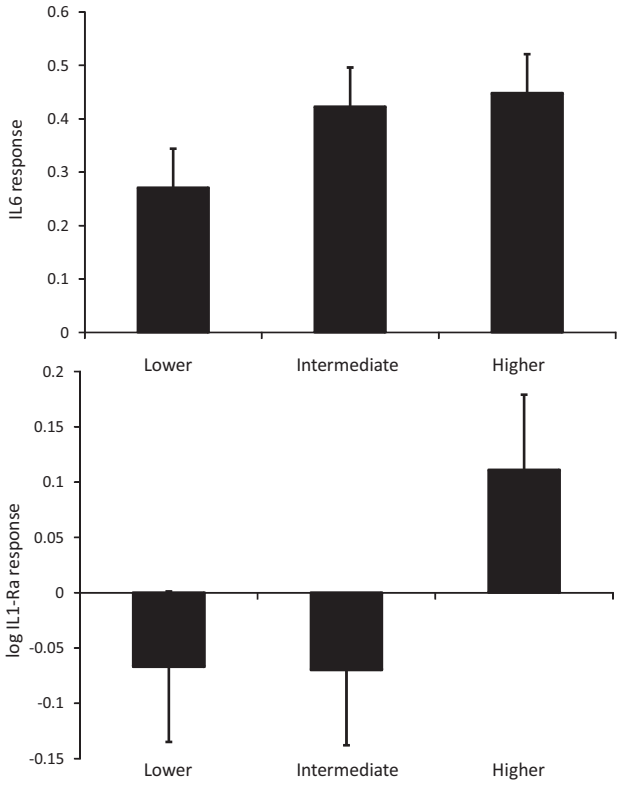

Figure 1 Mean changes in plasma IL-6 (pg/ml) between baseline and 45 min post tasks (upper panel) and IL-1Ra (pg/ml) between baseline and 45 min post tasks (lower panel) in women divided into lower ($n = 69$), intermediate ($n = 82$) and higher tertiles ($n = 72$) of loneliness. Mean loneliness scores were 24.1 ± 2.4, 32.6 ± 2.7 and 46.1 ± 7.2 for the lower, intermediate and higher tertiles respectively. Values are adjusted for age, grade of employment, BMI and smoking status. Error bars are standard error of the mean.

associated with loneliness ($B = 0.010$, C.I. = 0.001—0.018, $p = 0.026$, $\Delta r^2 = 0.019$, and 0.014, C.I. = 0.002—0.026, $p = 0.020$, $\Delta r^2 = 0.028$, for men and women respectively). Thus loneliness was related to subjective stress ratings under resting conditions, but not in the task phase of the study.

There were no significant associations between loneliness and any biological responses to stress in men, but effects were observed in women. In the case of plasma IL-6, there was no association with loneliness at baseline ($B = 0.006$, C.I. = −0.004 to 0.016, $p = 0.21$), but regression on the change in IL-6 between baseline and 45 min post-task showed a significant association with loneliness ($B = 0.009$, C.I. = 0.001—0.018, $p = 0.044$, $\Delta r^2 = 0.006$). This effect was independent of age, grade of employment, BMI and smoking status, and is illustrated in Fig. 1 (upper panel), where the women in the study have been divided into tertiles of loneliness. Greater loneliness was associated with larger plasma IL-6 increases following stress.

In the analyses of IL1-Ra, there was again no association with loneliness at baseline ($B = 0.007$, C.I. −0.006 to 0.020, $p = 0.28$). However, increases in IL1-Ra both immediately after tasks ($B = 0.011$, C.I. 0.003—0.019, $p = 0.006$, $\Delta r^2 = 0.016$,) and 45 min later ($B = 0.011$, C.I. 0.003—0.019, $p = 0.006$, $\Delta r^2 = 0.014$) were positively associated with loneliness after adjustment for covariates. The changes between baseline and 45 min post-task responses are shown in Fig. 1 (lower panel), where it is apparent that IL-1Ra fell slightly following tasks in less lonely participants, and only increased in those experiencing higher loneliness.

The pattern of results was somewhat different for MCP-1, there was no association between loneliness and responses to stress ($p = 0.28$) or rate of recovery ($p = 0.38$). Instead, loneliness was positively associated with MCP-1 levels throughout all trials including baseline ($B = 0.008$, C.I. 0.004—0.012, $p < 0.001$, $\Delta r^2 = 0.056$), following tasks ($B = 0.008$, C.I. 0.004—0.013, $p < 0.001$, $\Delta r^2 = 0.053$), and 45 min later ($B = 0.005$, C.I. 0.001—0.010, $p = 0.027$, $\Delta r^2 = 0.021$), adjusting for covariates. The relationship between loneliness and MCP-1 aggregated across all trials

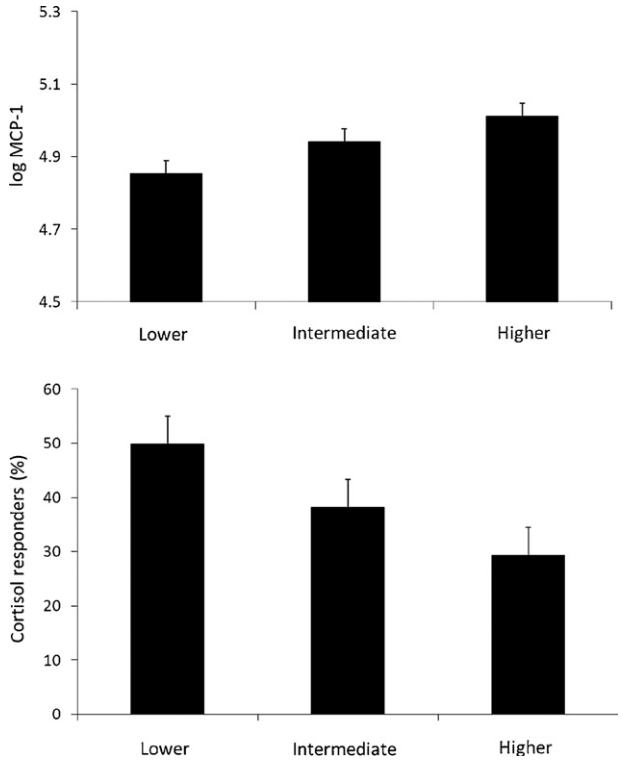

Figure 2 Upper panel: mean levels of plasma MCP-1 (pg/ml) during the study in women divided into lower ($n = 70$), intermediate ($n = 95$) and higher ($n = 74$) tertiles of loneliness. Lower panel: proportion of participants who were cortisol responders to stress in women divided into lower ($n = 73$), intermediate ($n = 96$) and higher ($n = 72$) tertiles of loneliness. Values are adjusted for age, grade of employment, BMI and smoking status. Error bars are standard error of the mean.

is shown in Fig. 2 (upper panel). None of the associations between loneliness and inflammation were altered when the subjective stress response was included as an additional covariate.

In contrast with the cytokine and chemokine results, cortisol responses to stress showed an inverse association with loneliness in women. In logistic regression, the odds of being a cortisol responder were reduced by 4.2% for every unit increase in loneliness rating after adjustment for age, grade of employment, BMI and smoking status (odds ratio = 0.958, C.I. = 0.928—0.989, $p = 0.008$). This pattern can be seen in Fig. 2, (lower panel) where it can be seen that 49.8% of participants in the lowest loneliness tertile were cortisol responders, compared with 29.3% in the highest tertile. By contrast, there was no association between baseline cortisol and loneliness in women ($B = 0.001$, C.I. = 0.004—0.007, $p = 0.64$).

In the light of the different relationships between loneliness and inflammatory markers and cortisol, we tested whether cortisol responsivity contributed to the positive associations observed between loneliness and inflammation in women. Contrary to this hypothesis, the inclusion of cortisol responsivity did not alter the associations previously described between loneliness, IL-6 stress responses, IL-1Ra stress responses, and MCP-1 levels.

4. Discussion

We reasoned that if inflammation contributes to the impact of loneliness on disease risk, then loneliness would be positively associated with inflammatory responses to laboratory stress. The results of the study were partly consistent with this notion, since greater loneliness was associated with larger IL-6 and IL-1Ra responses to psychological stress and greater MCP-1 levels in women, independently of age, grade of employment, BMI and smoking status. But no associations were observed in men. We were also interested in investigating whether individual variations in cortisol responsivity influenced the relationship between loneliness and inflammation. The results showed cortisol responsivity was inversely related to loneliness, with the odds of being a cortisol responder decreasing with increased loneliness independently of covariates. However, cortisol responsivity did not alter the positive associations observed between loneliness and inflammation in women.

Loneliness was assessed with the revised UCLA loneliness scale in this study. The mean loneliness ratings were comparable with those of other studies of older men and women. The average of 34.6 ± 10.6 was similar to the mean of 36.3 ± 9.5 reported in our earlier study of a different subsample of Whitehall II study participants (Steptoe et al., 2004), and compares with 32.5 ± 7.4 in a recent study of men and women aged 65.80 (Ong et al., 2012), 36.0 ± 9.9 in adults aged 50—67 years in the Chicago Health, Aging, and Social Relations Study (Cacioppo et al., 2006; Hawkley et al., 2010), and a median of 35 in a study of older adults in sheltered living facilities (White et al., 2002). The mean loneliness rating in the high loneliness tertile was 47.0 ± 7.8. This compares with an average of 44.0 in the 'high loneliness' subgroup an earlier study of biological factors (Cacioppo et al., 2002), and with 46.0 ± 5.6 in the high loneliness/isolated group of the study of loneliness and gene expression reported by Cole et al. (2007).

To our knowledge, no other studies have investigated the relationship between loneliness and IL-6 and IL-1Ra responses to acute mental stress. Previous research has linked heightened IL-6 concentrations with feelings of social disconnection (Eisenberger et al., 2010) and poor social relations (Friedman et al., 2005). In the present study, greater loneliness was associated with elevated IL-6 and IL-1Ra responses in women. Elevated IL-6 and IL-1Ra levels are associated with future coronary disease (Danesh et al., 2008; van Minkelen et al., 2009), and IL-6 also predicts cognitive and physical decline at older ages (Weaver et al., 2002; Cohen et al., 2003). It is unclear why associations were observed in women but not in men. However, we found in an earlier analysis that IL-6 and IL-1Ra responses to psychological stress were greater in women than men (Steptoe et al., 2002), a finding replicated here for IL-6. It may be that this greater responsiveness provides greater scope for the effects of psychosocial factors such as loneliness to be expressed.

MCP-1 is a pro-inflammatory chemokine that is known to play a major role in the development of cardiovascular disease (Hansson and Hermansson, 2011). We found that MCP-1 showed a small but significant increase in both men and women. This cannot be unequivocally attributed to stress in the absence of a no task control group. Nevertheless, the fact that values returned towards baseline by 45 min argues

for a transient perturbation rather than an increase due to passage of time. Interestingly, increases in MCP-1 were more sustained in women than men, consistent with the heightened cytokine responses. Loneliness was positively associated with elevated MCP-1 concentrations in women throughout the session, which were maintained from baseline to recovery. Few studies have examined the effects of potentially adverse psychological characteristics on MCP-1. However, raised MCP-1 levels have been found in major depression patients (Rajagopalan et al., 2001), as well as in men reporting mild depressive symptoms (Suarez et al., 2003).

The observed relationship between loneliness and heightened inflammation in women extends the findings of a previous analysis in which diastolic blood pressure reactivity to stress was positively associated with loneliness in women but not in men (Steptoe et al., 2004). It may be that the inflammatory processes which are implicated in coronary atherogenesis (Tedgui and Mallat, 2006) are particularly sensitive to the effects of loneliness in women. This idea is consistent with results from a recent large epidemiological study which found chronic loneliness to be prospectively associated with incident CHD in women but not in men (Thurston and Kubzansky, 2009).

The greater sensitivity of women to the impact of loneliness on biological responses may result from gender differences in the importance of social relationships to the maintenance of well-being. Taylor et al. (2000) have argued that females employ a ''tend-and-befriend'' response to stress, prioritizing the creation and maintenance of social relationships in managing adversity. This pattern may make women more sensitive to the effects of loneliness, potentially accounting for the heightened inflammatory stress responses observed in lonely women, despite there being no difference in loneliness scores between men and women.

However, not all studies have reported a stronger association between loneliness and coronary disease or cardiovascular mortality in women than men (Herlitz et al., 1998; Sorkin et al., 2002). Indeed Olsen et al. (1991) found that frequent loneliness was associated with increased cardiovascular mortality among older men but not women. Furthermore, loneliness has been associated greater concentrations of inflammatory markers such as CRP (Cole et al., 2007) and elevated fibrinogen responses to psychological stress (Steptoe et al., 2004) in both sexes. Men and women differed in their subjective response to stress, with women reporting greater subjective stress ratings both at baseline and 45 min post-task, which may have influenced the sex difference observed in the study. However, there was no association between loneliness and the magnitude of subjective stress responses in women. This makes it unlikely that lonely women showed larger inflammatory responses because of variations in the experience of acute subjective stress, rather than because of more enduring differences in biological regulation.

In this study loneliness was linked with reduced cortisol responsivity to stress independently of covariates in women. The odds of being a cortisol responder were reduced by 4.2% for every unit increase in loneliness ratings. In our previous study we found no association between loneliness and cortisol responsivity to acute stress (Steptoe et al., 2004). Cole et al. (2007) also found no differences in circulating cortisol between lonely and non-lonely individuals, while Doane and Adam (2010) reported elevated cortisol levels in the lonely, though neither study tested people under acutely stressful conditions. The larger sample size in the present compared with our previous study (524 versus 202) may account for our greater power to detect differences. These findings suggest that lonely people show insufficient GC signalling to inhibit inflammatory responses under stress, not only because of attenuated GC receptor sensitivity but decreased hormone release. Thus decreased cortisol responses to stress, reduced GC receptor sensitivity, and increased activity of pro-inflammatory transcription pathways may all contribute to heightened inflammatory responses in lonely individuals. However, our test of whether cortisol responsivity contributed to the positive associations observed between loneliness and inflammation was inconclusive. Contrary to hypothesis, the inclusion of cortisol responsivity status in the regression did not attenuate the positive associations observed between loneliness and IL-6 stress responses, IL-1Ra stress responses and MCP-1 levels in women. The absolute magnitude of cortisol responses was small in this study compared with experiments in which social evaluative challenges are administered (Dickerson and Kemeny, 2004), and this may have reduced our ability to test this mediating pathway.

The study was cross-sectional in nature so it is not possible to infer causality. Longitudinal research is needed to elucidate the degree to which chronic loneliness and changes in loneliness over time are associated with increased systemic inflammation and negative health outcomes. The study has a number of other limitations. It involved healthy middle-aged men and women of white European ethnicity, thus the patterns of results may not generalise to other groups. Loneliness was only measured at one time point in this study, and it may be that loneliness measured on one occasion does not have the same biological correlates as chronic loneliness (Cacioppo et al., 2003). It is possible that a more detailed cytokine profile would have generated additional results. No control group of participants not exposed to stress was included, since we have previously observed that the concentration of biomarkers such as IL-6 does not change over time in the absence of behavioural challenge (Steptoe et al., 2001; Brydon et al., 2005).

Despite these considerations, this study builds upon the literature examining the effects of loneliness on biological processes relevant to health. Greater loneliness was associated with larger IL-6, IL-1Ra responses to psychological stress and greater MCP-1 levels in women, independent of covariates. This suggests that the impact of loneliness on health in women could be mediated in part through dysregulation of inflammatory and neuroendocrine systems.

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Conflicts of interest statement

None declared.

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