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Longitudinal analysis of loneliness and inflammation at older ages: English longitudinal study of ageing

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ABSTRACT

Loneliness has been associated with adverse health outcomes, including age-related diseases with an inflammatory etiology such as cardiovascular disease. We aimed to identify potential biological pathways linking loneliness with morbidity and mortality by examining associations of loneliness with biomarkers. Participants in the English Longitudinal Study of Ageing (n = 3239) aged 50 years or older with an average age of 64 years, provided data in waves 4 (2008/2009) and 6 (2012/2013). Linear regression conditional change models had three outcomes: C reactive protein (CRP) measured in mg/L (log transformed), fibrinogen in g/L and ferritin in g/dL. In men, the onset of loneliness indicated by answering 'no' at wave 4 and 'yes' at wave 6 to question "Much of the time during the past week, you felt lonely?" was associated with a statistically significant increase in levels of CRP (β = 0.36, 95% confidence interval (0.09 to 0.62)), plasma fibrinogen (0.18 (0.04 to 0.31)) and ferritin (41.04 (6.58 to 75.50)), after full adjustment. A statistically significant increase in CRP in men was also observed for onset of loneliness assessed with the question "How often do you feel lonely?" (0.20 (0.03 to 0.38)). These associations were not mediated by depressive symptoms. Persistent loneliness (loneliness experienced at both baseline and follow-up) assessed using the University of California Los Angeles (UCLA) loneliness scale was associated with an increase in CRP (0.11 (0.004 to 0.22)) among men. Associations of the two latter loneliness measures with fibrinogen and ferritin were mainly null. Among women, the only statistically significant association was for persistent loneliness (loneliness at both waves) identified by question "Much of the time during the past week, you felt lonely?" with a reduction in levels of ferritin (-20.62 (-39.78 to -1.46)). Men may be more susceptible to loneliness-associated disease risks signaled by biological changes, including systemic inflammation. Combined social and targeted medical interventions may help to reduce health risks associated with loneliness.

1. Introduction

Loneliness, or perceived social isolation, arises from a lack of social or emotional connectedness to others and has been linked with adverse health consequences (Cacioppo et al., 2014; Miller, 2011). A meta-analysis of prospective studies reported higher all-cause mortality risk associated with loneliness, living alone, and objectively assessed social isolation such as availability and frequency of contacts (Holt-Lunstad et al., 2015). This is consistent with previous findings of 50% higher likelihood of longer survival among those with stronger social relationships (Holt-Lunstad et al., 2010). Evidence from prospective cohort studies indicates that loneliness and objectively assessed social isolation combined are both associated with 30% higher risk of developing coronary heart disease and stroke (Valtorta et al., 2016), while a recent study confirmed higher risk of CVD mortality with loneliness (Stringhini et al., 2018). An estimated 20%–30% of older adults are affected by loneliness (Ong et al., 2016), with frequently reported higher prevalence of loneliness among women (Shankar et al., 2011; Shiovitz-Ezra and Ayalon, 2011), particularly older women as they tend to outlive their partners (Vozikaki et al., 2018). A recent meta-analysis examining the effect of loneliness in men and women reported increased risk of premature all-cause mortality overall and a larger effect size in men (Rico-Uribe et al., 2018).

The biological mechanisms potentially linking loneliness with

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cardiovascular disease risk are complex and incompletely understood, but loneliness has been linked with distressed affect, lower positive affect (Steptoe et al., 2011) and an increase in some stress-related biomarkers (Shankar et al., 2011). Exposure to chronic stress leads to the activation of the hypothalamic-pituitary-adrenal (HPA) axis and autonomic nervous system with diminishing activity of the parasympathetic nervous system, leading to chronic low grade inflammation characterized by increased levels of circulating proinflammatory cytokines and acute phase reactants often in combination with reduced levels of anti-inflammatory cytokines (Kivimäki and Steptoe, 2017). There is evidence that loneliness may influence inflammation through variation in gene expression (Cole et al., 2007).

We hypothesized that loneliness leads to an increase in three biomarkers. The first is C-reactive protein (CRP), which is an established biomarker for inflammation and thus indicates future vascular risk (Ridker, 2016). The second is plasma fibrinogen. The experience of stress triggers platelet activation, increases blood viscosity and levels of fibrinogen (Kivimäki and Steptoe, 2017). Plasma fibrinogen is directly involved in pathogenesis of diseases caused by vaso-occlusion and also by processes involving increased vascular permeability and vascular wall damage, indicating several vascular risks associated with blood viscosity (Davalos and Akassoglou, 2012). The third biomarker is ferritin. Among healthy individuals ferritin can be used as a marker of total body iron stores, however, levels of ferritin markedly elevate during inflammation (Watt, 2011). Serum ferritin is an acute-phase reactant with higher levels indicating local or chronic inflammation underlying a wide range of inflammatory processes including infection, rheumatological, malignant diseases, atherosclerosis and coronary artery disease (Kell and Pretorius, 2014; Kernan and Carcillo, 2017). Ferritin release into serum in response to proinflammatory cytokines, as a leakage product from damaged cells, and in response to oxidative stress (Kernan and Carcillo, 2017) has led to the use of ferritin as a marker of inflammatory disease processes (Watt, 2011). The potentially opposing influences of iron status and inflammation suggest that associations with ferritin level should be interpreted with caution.

Most published studies examining associations between loneliness and biomarkers are cross-sectional (Mezuk et al., 2016; Nersesian et al., 2018; O'Luanaigh et al., 2012; Shankar et al., 2011); however, exposure to socioeconomic, environmental and behavioral disadvantages across the life-course may increase both risk of loneliness and systemic inflammation, so there are potential methodological challenges when assessing causality for the association of loneliness with inflammation. To resolve this, we use data from two waves of the English Longitudinal Study of Ageing (ELSA) for people aged over 50 years at baseline to examine if onset of loneliness is associated with an *increase* in CRP, fibrinogen and ferritin concentrations. Because of sex differences in the prevalence of loneliness (Shankar et al., 2011; Shiovitz-Ezra and Ayalon, 2011; Vozikaki et al., 2018) and cardiovascular disease (Benjamin et al., 2019; Roth et al., 2017), as well as differences in disease etiology, pathophysiology and progression (Regitz-Zagrosek and Kararigas, 2017), the analyses are stratified by sex.

2. Methods

2.1. Study design and population

This study uses data from English Longitudinal Study of Ageing (ELSA), which began in 2002 with over 11,000 respondents aged 50 years or older, living in private households in England (Steptoe et al., 2013). The initial sample was selected from the participants in the Health Survey for England in 1998, 1999, and 2001, using a multistage random sampling strategy. Two-yearly follow-up consisted of personal interviews and self-completion questionnaires. Every four years, in addition to the main interview, all study participants were asked if they would like to take part in data collection conducted by a registered nurse during a home visit. The nurse visit included a personal face-to-face computer assisted interview, collection of biological samples and anthropometric measurements such as height and weight. A blood sample was collected only once during the scheduled visit. Participants provided signed informed consent for the main interview, nurse visits, and blood sampling. Ethical approval was obtained from the London Multi-Centre Research Ethics Committee (MREC/01/2/91).

We used data from waves 4 (2008/09) and 6 (2012/13), comprising data for a total of 8384 members who completed interviews at both waves and were eligible for nurse visits. The response rate for nurse visits was 86% in wave 4 and 84% in wave 6 with 7834 participants visited at both time points. Of the 7834 cohort members, 575 were ineligible for blood sampling due to taking anticoagulant drugs such as warfarin, protamine, or acenocoumarol. Study nurses collected blood samples which were sent to an external laboratory for analysis (Appendix A). Other reasons for missing blood samples included inadequate samples due to various difficulties ($\approx$6%), respondent not giving consent ($\approx$9%), and samples not reaching the laboratory or taking more than five days to arrive ($\approx$4%). Overall, 4045 had data for CRP, 3868 for fibrinogen and 4045 for ferritin in both waves. There are less data available on fibrinogen due to insufficient blood volume in the samples. Depending on the outcome measure, a further 763 to 806 participants were excluded because of missing information for covariates, resulting in 3239 participants for the complete case analysis with CRP, 3105 for fibrinogen and 3239 for ferritin.

2.2. Change in loneliness

Loneliness was assessed using three exposure measures: two direct questions, and a scale developed at the University of California Los Angeles (UCLA) consisting of indirect questions related to loneliness but excluding the word 'lonely'. The changes in perceived loneliness between wave 4 and wave 6 were considered as the exposures.

The first loneliness measure is an item from The Centre for Epidemiologic Studies Depression Scale (CES-D). The question is "Much of the time during the past week, you felt lonely?" (Cheshire et al., 2009) which was asked during a computer-assisted personal interview with two possible response options 'yes' and 'no' (Appendix B). Responses to this question at baseline (wave 4) and follow-up (wave 6) were grouped to create change categories: no (wave 4)/no (wave 6); no (wave 4)/yes (wave 6); yes (wave 4)/no (wave 6); yes (wave 4)/yes (wave 6) (Appendix B). The second question, "How often do you feel lonely?" from a self-completion questionnaire, had three response options: 'hardly ever or never', 'some of the time', or 'often'. It captures the frequency of loneliness. For consistency with the other two measures, we dichotomized responses by combining 'some of the time' and 'often' into a single category labelled as 'often'. This allowed creation of a change variable with 4 categories for more straightforward interpretation than would be possible with more categories. For simplicity, the category 'hardly ever or never' is labelled 'never'. Dichotomized categories were then grouped according to answers at wave 4 and wave 6 into: never (wave 4)/never (wave 6); never (wave 4)/often (wave 6); often (wave 4)/never (wave 6); often (wave 4)/often (wave 6) (Appendix B). The direct questions may underestimate loneliness if the participant is unwilling to report loneliness or does not consciously recognize experiencing loneliness (Shiovitz-Ezra and Ayalon, 2011). A validation study of the UCLA scale provides psychometric data supporting the convergent validity based on high correlations with other measures of loneliness and construct validity supported by concordant evidence from theoretical models focusing on determinants and consequences of loneliness (Russell, 1996). ELSA included a shortened three-item version with adequate psychometric properties capturing an individual's perception of lacking relationships (Hughes et al., 2004) using the questions: "How often do you feel lack of companionship?", "How often do you feel left out?", and "How often do you feel isolated from others?". The response options were on a three-point Likert scale with the categories 1 = 'hardly ever, never'; 2 = 'some of the time'; and

3 = 'often', and the responses were summed to produce a score. The reliability of the three-item loneliness scale is indicated by a Cronbach's α of 0.72 using data from the Health and Retirement Study (Hughes et al., 2004) while higher α of 0.79 has also been reported in a study of older participants with an average age of 63 years (Mezuk et al., 2016). The distribution of the sum of scores ranged between 3 and 9 and was skewed to the right with most participants having low scores. In previous research, the scale was grouped into three levels (not lonely, score 3; moderately lonely, score 4–5; and highly lonely, score 6–9) (Mezuk et al., 2016). However, in the present study, to maintain consistent categorization with the other two measures and to keep the change variable of 4 categories instead of 9, the responses were dichotomized into the score 3 category and remaining scores (4–9). A score of 3 was labelled 'hardly ever or never', 4–9 as 'some of the time or often'. The categories were re-named as 'never' or 'often', as with the other variables. We created the change variable in a similar way as for the other variables, as described above (Appendix B).

For all three 'change in loneliness' measures, we defined *'onset of loneliness'* where participants were classified as 'no or never' at wave 4, but 'yes or often' at wave 6. The absence of loneliness at both wave 4 and 6 served as the reference category to which all other change categories were compared. The presence of loneliness at both waves is referred to as '*persistent loneliness*'.

2.3. Covariates

As potential confounding factors we considered: age (Pinquart and Sörensen, 2001), body mass index (BMI) (Pavela et al., 2018), National Statistics Socio-Economic Classification (NS-SEC) (Muscatell et al., 2018; Pinquart and Sörensen, 2001) at wave 4 and CVD (including angina, heart attack, congestive heart failure, stroke, and other heart disease) (Valtorta et al., 2016), rheumatoid arthritis and cancer diagnosed at waves 4 and 6 (Kell and Pretorius, 2014). As cardiovascular disease may increase both risk of loneliness and inflammation, included self-reported diagnosed cardiovascular diseases were angina, heart attack, congestive heart failure, stroke, and other heart disease recorded at baseline (wave 4) and follow-up (wave 6).

The association between age and CRP was nonlinear and, in the ELSA dataset, the age variable included a category of collapsed age category for 90 years or older. For this reason, as well as possible nonlinear associations with age, we used age as a categorical variable divided into 3 categories (50–60, 61–70 and ≥71 years). Registered nurses measured height and weight. Weight of over 130 kg was not measured and estimated instead. Body mass index (BMI) was calculated by dividing weight (kg) by height (m) squared and categorized according to the World Health Organization (WHO) definition of underweight (< 18.5 kg/m^2), normal weight (18.5–24.9 kg/m^2), overweight (25.0–29.9 kg/m^2), and obesity (≥30.0 kg/m^2). The NS-SEC is a social classification that has been used in the UK since 2001. It categorizes social positions based on occupational information including employment status, type of occupation, size of the employing organization as well as the number of employees. The 3 category NS-SEC was used, comprising managerial and professional occupations; intermediate; and routine and manual.

Depressive symptoms were investigated as a potential mediator on the pathway between loneliness and the biomarkers. Depression has been linked with both loneliness (Cacioppo et al., 2006) and an increase in inflammation (Stewart et al., 2009). Depressive symptoms were assessed using the 8-item Center for Epidemiologic Studies Depression Scale (CES-D), a shortened version of the original 20-item CES-D scale (Radloff, 1977). The CES-D 8-item scale was designed for use in population surveys, and has been validated in samples of older participants (Karim et al., 2015). The participants who responded positively to 4 or more questions indicating depression were categorized as having significant depressive symptoms (Au et al., 2014; Hamer and Chida, 2009; Steffick, 2000). This threshold has been commonly used and, produced comparable results with those obtained using the cut-off of 16 in the full-scale CES-D (Steffick, 2000; Zivin et al., 2010).

2.4. Statistical analysis

All biomarkers were on a continuous scale. Mean and standard deviation (SD) of fibrinogen and ferritin and median and interquartile range of CRP for categories of baseline (wave 4) characteristics were calculated. CRP was log-transformed to normalize the distribution of residuals. One-way analysis of variance was used for fibrinogen, ferritin and log-transformed CRP to determine how biomarker levels were associated with baseline characteristics. The correlation coefficients between the three loneliness measures were calculated using tetrachoric correlations for binary variables.

Multivariable linear regression using conditional change modelling (the outcome biomarker variable is conditioned on its level at baseline) was performed to estimate β coefficients and 95% confidence intervals (CI). The model estimated change in levels of CRP, fibrinogen and ferritin for each loneliness change category, and men and women were investigated separately. Interactions by sex were tested using the likelihood ratio test. The following models were used: 1) unadjusted; 2) adjusted for baseline level of biomarker; 3) additionally adjusted for age, BMI, NS-SEC, and self-reported cancer, major CVD or rheumatoid arthritis at baseline and follow-up (using both variables from waves 4 and 6).

Several sensitivity analyses were conducted. First, to investigate any effect of acute infections, we excluded participants with extreme CRP values above 50 mg/L at waves 4 and 6 (Markanday, 2015). Second, to address potential bias due to missing data, the full information maximum likelihood (FIML) method was used (Collins et al., 2001; Dong and Peng, 2013). The study sample was limited to participants visited by a nurse in waves 4 and 6 who were eligible to give a blood sample (n = 7259). The analysis was conducted using the *SEM* command in Stata. Finally, we assessed whether the association between making the transition from not lonely in wave 4 to lonely in wave 6 and increase in biomarker level was mediated through depressive symptoms measured at wave 6. The analysis was conducted using the *med4way* command which examines the proportion of mediated effect in the adjusted model (Discacciati et al., 2018; VanderWeele, 2014). The statistical analysis used Stata (version 14.1, College Station, Texas).

3. Results

At baseline (wave 4), the proportion of men classified as lonely by the questions "Much of the time during the past week, you felt lonely?", "How often do you feel lonely?" and the UCLA loneliness scale was 6%, 21% and 43%, respectively (Appendix C). Following the same order of loneliness measures, the proportion of women classified as lonely was 11%, 32% and 51%, respectively (Appendix C). The percentage of participants who made the transition from not lonely to lonely ranged between 3–11% in men (Table 1a) and 6–12% in women (Table 1b), with a similar ratio of those making the transition from lonely to not lonely among both sexes. Men who became lonely appeared to be older, more likely to be underweight or obese and less likely to belong to the highest NS-SEC category, with no clear pattern for disease diagnoses across the loneliness categories. Among women, no consistent pattern was seen for the association of baseline (wave 4) characteristics with all measures of loneliness. At baseline, the majority of men and women were 61–70 years of age, overweight, and belonged to the highest NS-SEC category (Table 2). The highest levels of CRP and fibrinogen at follow-up were among women, participants who were older or obese, and those belonging to the lowest NS-SEC category. The level of ferritin was statistically significantly higher in men, those who were overweight and those belonging to the highest NS-SEC category. The concentration of biomarkers was similar among those with and without inflammatory diseases apart from statistically significantly higher levels

Table 1a
Baseline characteristics by loneliness in men.

	"Much of the time during the past week), you felt lonely?"				"How often do you feel lonely?"				Loneliness estimated from the UCLA loneliness scale			
	No/no n (%)	No/yes n (%)	Yes/no n (%)	Yes/yes n (%)	Never/never n (%)	Never/often n (%)	Often/never n (%)	Often/often n (%)	Never/never n (%)	Never/often n (%)	Often/never n (%)	Often/often n (%)
Men, n = 1462	1328 (91)	46 (3)	54 (4)	34 (2)	1040 (71)	118 (8)	113 (8)	191 (13)	678 (46)	162 (11)	182 (12)	440 (30)
Age (years)												
50 – 60	478 (36)	16 (35)	17 (31)	14 (41)	369 (35)	35 (30)	49 (43)	72 (38)	234 (35)	49 (30)	80 (44)	162 (37)
61 – 70	545 (41)	16 (35)	22 (41)	13 (38)	434 (42)	40 (34)	48 (42)	74 (39)	292 (43)	71 (44)	65 (36)	168 (38)
71 – 99	305 (23)	14 (30)	15 (28)	7 (21)	237 (23)	43 (36)	16 (14)	45 (24)	152 (22)	42 (26)	37 (20)	110 (25)
BMI (kg/m^2)												
< 18.5	3 (0)	0	0	0	2 (0)	0	0	1 (1)	1 (0)	1 (1)	0	1 (0)
18.5 – 24.9	282 (21)	13 (28)	16 (30)	7 (21)	218 (21)	28 (24)	20 (18)	52 (27)	144 (21)	29 (18)	46 (25)	99 (23)
25.0 – 29.9	699 (53)	20 (43)	24 (44)	13 (38)	553 (53)	64 (54)	63 (56)	76 (40)	364 (54)	81 (50)	96 (53)	215 (49)
≥30	344 (26)	13 (28)	14 (26)	14 (41)	267 (26)	26 (22)	30 (27)	62 (32)	169 (25)	51 (31)	40 (22)	125 (28)
NS-SEC												
Managerial[a]	617 (46)	18 (39)	19 (35)	9 (26)	506 (49)	41 (35)	43 (38)	73 (38)	327 (48)	73 (45)	77 (42)	186 (42)
Intermediate	265 (20)	14 (30)	11 (20)	8 (24)	205 (20)	25 (21)	23 (20)	45 (24)	133 (20)	34 (21)	47 (26)	84 (19)
Routine[b]	437 (33)	14 (30)	24 (44)	17 (50)	323 (31)	52 (44)	45 (40)	72 (38)	214 (32)	55 (34)	57 (31)	166 (38)
Other	9 (1)	0	0	0	6 (1)	0	2 (2)	1 (1)	4 (1)	0	1 (1)	4 (1)
Diseases												
Cancer												
Yes	25 (2)	3 (7)	0	3 (9)	22 (2)	3 (3)	1 (1)	5 (3)	12 (2)	5 (3)	5 (3)	9 (2)
No	1303 (98)	43 (93)	54 (100)	31 (91)	1018 (98)	115 (97)	112 (99)	186 (97)	666 (98)	157 (97)	177 (97)	431 (98)
Cardiovascular disease												
Yes	81 (6)	3 (7)	4 (7)	5 (15)	68 (7)	5 (4)	4 (4)	16 (8)	38 (6)	10 (6)	7 (4)	38 (9)
No	1247 (94)	43 (93)	50 (93)	29 (85)	972 (93)	113 (96)	109 (96)	175 (92)	640 (94)	152 (94)	175 (96)	402 (91)
Rheumatoid arthritis												
Yes	64 (5)	2 (4)	3 (6)	1 (3)	41 (4)	11 (9)	9 (8)	9 (5)	23 (3)	5 (3)	10 (5)	32 (7)
No	1264 (95)	44 (96)	51 (94)	33 (97)	999 (96)	107 (91)	104 (92)	182 (95)	655 (97)	157 (97)	172 (95)	408 (93)

Note: Baseline characteristics are based on the number of participants used in the complete case analysis of CRP.
[a]Managerial and professional occupations.
[b]Routine and manual occupations.

of CRP in those with rheumatoid arthritis. The correlation coefficient for the association of UCLA loneliness scale and the "Much of the time during the past week, you felt lonely?" question is 0.68, while a higher coefficient of 0.82 was observed between the UCLA loneliness scale and "How often do you feel lonely?", and 0.82 between "How often do you feel lonely?" and "Much of the time during the past week, you felt lonely?" (Appendix E).

3.1. CRP

"Much of the time during the past week, you felt lonely?": the onset of loneliness, defined as participants who answered *no* to this question in wave 4 and *yes* in wave 6, was associated with statistically significant increase in levels of CRP in men even after full adjustment for baseline biomarker level, age, BMI, NS-SEC, diagnoses of cancer, CVD and rheumatoid arthritis at both time points (Table 3). *"How often do you feel lonely?"*: the onset (never/often) of loneliness was associated with statistically significant increase in CRP, after full adjustment. *The UCLA loneliness scale:* the onset (never/often) of loneliness was not associated with a change in CRP, but persistent (often/often) loneliness was statistically significantly associated with an increase in levels of CRP, before and after adjustment. None of the loneliness variables were associated with changes in levels of CRP among women (Table 3). Interaction by sex was statistically significant for the UCLA loneliness scale ($P_{interaction}$ = 0.05) with slightly higher P value for the question "Much of the time during the past week, you felt lonely? ($P_{interaction}$ = 0.06) with no statistically significant interaction for the "How often do you feel lonely" question (Table 3). None of the associations altered notably after excluding participants with CRP values above 50 mg/L (n = 22) (data not shown).

3.2. Fibrinogen

"Much of the time during the past week, you felt lonely?": the onset of loneliness was associated with statistically significant increase in fibrinogen in men, before and after full adjustment (Table 4). *"How often do you feel lonely?"*: the onset of loneliness was not associated with changes in biomarker level. However, men who made the transition from lonely to no longer lonely (often/never) had statistically significantly increased levels of fibrinogen. *The UCLA loneliness scale:* persistent loneliness was associated with increase in fibrinogen only in the unadjusted model among men. None of the loneliness variables were associated with changes in fibrinogen among women (Table 4). The interaction with sex was statistically significant for the "how often do you feel lonely?" question ($P_{interaction}$ = 0.01) with a higher P value for "Much of the time during the past week, you felt lonely?" ($P_{interaction}$ = 0.06) and no statistically significant interaction for sex with the UCLA loneliness scale (Table 4).

3.3. Ferritin

"Much of the time during the past week, you felt lonely?": the onset of loneliness was associated with statistically significantly increased levels of ferritin in men, including after full adjustment (Table 5). The other two loneliness variables were not associated with changes in levels of ferritin in men. *"Much of the time during the past week, you felt lonely?"*: persistent loneliness was associated with statistically significant decrease in ferritin, in minimally and fully adjusted models among women (Table 5). The interaction with sex was statistically significant for the *"Much of the time during the past week, you felt lonely?"* question ($P_{interaction}$ = 0.02) but not for the other two measures of loneliness (Table 5).

Table 1b
Baseline characteristics by loneliness in women.

	"Much of the time during the past week), you felt lonely?"				"How often do you feel lonely?"				Loneliness estimated from the UCLA loneliness scale			
	No/no	No/yes	Yes/no	Yes/yes	Never/ never	Never/ often	Often/ never	Often/ often	Never/ never	Never/ often	Often/ never	Often/ often
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Women, n = 1777	1471 (83)	115 (6)	114 (6)	77 (4)	998 (56)	204 (11)	170 (10)	405 (23)	659 (37)	220 (12)	230 (13)	668 (38)
Age (years)												
50 – 60	556 (38)	36 (31)	42 (37)	16 (21)	371 (37)	76 (37)	74 (44)	129 (32)	240 (36)	99 (45)	84 (37)	227 (34)
61 – 70	600 (41)	46 (40)	39 (34)	37 (48)	421 (42)	86 (42)	58 (34)	157 (39)	280 (42)	86 (39)	98 (43)	258 (39)
71 – 99	315 (21)	33 (29)	33 (29)	24 (31)	206 (21)	42 (21)	38 (22)	119 (29)	139 (21)	35 (16)	48 (21)	183 (27)
BMI (kg/m^2)												
< 18.5	11 (1)	0	3 (3)	1 (1)	7 (1)	3 (1)	0	5 (1)	3 (0)	2 (1)	3 (1)	7 (1)
18.5 – 24.9	478 (32)	29 (25)	35 (31)	18 (23)	323 (32)	61 (30)	53 (31)	123 (30)	208 (32)	84 (38)	75 (33)	193 (29)
25.0 – 29.9	554 (38)	46 (40)	38 (33)	28 (36)	383 (38)	78 (38)	60 (35)	145 (36)	253 (38)	72 (33)	93 (40)	248 (37)
≥30	428 (29)	40 (35)	38 (33)	30 (39)	285 (29)	62 (30)	57 (34)	132 (33)	195 (30)	62 (28)	59 (26)	220 (33)
NS-SEC												
Managerial[a]	494 (34)	36 (31)	32 (28)	18 (23)	363 (36)	65 (32)	51 (30)	101 (25)	233 (35)	76 (35)	82 (36)	189 (28)
Intermediate	452 (31)	36 (31)	30 (26)	22 (29)	295 (30)	65 (32)	51 (30)	129 (32)	195 (30)	73 (33)	66 (29)	206 (31)
Routine[b]	515 (35)	41 (36)	51 (45)	34 (44)	335 (34)	73 (36)	66 (39)	167 (41)	227 (34)	69 (31)	79 (34)	266 (40)
Other	10 (1)	2 (2)	1 (1)	3 (4)	5 (1)	1 (0)	2 (1)	8 (2)	4 (1)	2 (1)	3 (1)	7 (1)
Diseases												
Cancer												
Yes	40 (3)	3 (3)	1 (1)	1 (1)	27 (3)	6 (3)	5 (3)	7 (2)	23 (3)	3 (1)	4 (2)	15 (2)
No	1431 (97)	112 (97)	113 (99)	76 (99)	971 (97)	198 (97)	165 (97)	398 (98)	636 (97)	217 (99)	226 (98)	653 (98)
Cardiovascular disease												
Yes	67 (5)	8 (7)	14 (12)	9 (12)	43 (4)	11 (5)	13 (8)	31 (8)	31 (5)	8 (4)	8 (3)	51 (8)
No	1404 (95)	107 (93)	100 (88)	68 (88)	955 (96)	193 (95)	157 (92)	374 (92)	628 (95)	212 (96)	222 (97)	617 (92)
Rheumatoid arthritis												
Yes	93 (6)	10 (9)	8 (7)	8 (10)	59 (6)	17 (8)	14 (8)	29 (7)	37 (6)	11 (5)	15 (7)	56 (8)
No	1378 (94)	105 (91)	106 (93)	69 (90)	939 (94)	187 (92)	156 (92)	376 (93)	622 (94)	209 (95)	215 (93)	612 (92)

Note: Baseline characteristics are based on the number of participants used in the complete case analysis of CRP.
[a] Managerial and professional occupations.
[b] Routine and manual occupations.

Table 2
Baseline characteristics by biomarker levels at follow-up.

	CRP			Fibrinogen			Ferritin		
	n (%)	mg/L median (IQR)	Pvalue [a]	n (%)	g/L mean (SD)	P_{value} [a]	n (%)	g/dL mean (SD)	P_{value} [a]
All	3239	1.8 (2.8)		3105	3.33 (0.5)		3239	122.7 (116.2)	
Men	1462 (45.1)	1.6 (2.5)	< 0.001	1397 (45.0)	3.27 (0.5)	< 0.001	1462 (45.1)	156.5 (134.9)	< 0.001
Women	1777 (54.9)	1.9 (3.0)		1708 (55.0)	3.38 (0.5)		1777 (54.9)	94.9 (89.1)	
Age (years)									
50 – 60	1175 (36.3)	1.6 (2.6)	< 0.001	1130 (36.4)	3.26 (0.5)	< 0.001	1176 (36.3)	118.4 (120.5)	0.170
61 – 70	1318 (40.7)	1.7 (2.8)		1257 (40.5)	3.34 (0.5)		1317 (40.7)	127.1 (117.5)	
71 – 99	746 (23.0)	2.1 (3.0)		718 (23.1)	3.44 (0.5)		746 (23.0)	121.9 (106.6)	
BMI (kg/m^2)									
< 18.5	18 (0.6)	0.9 (2.4)	< 0.001	17 (0.5)	3.35 (0.8)	< 0.001	18 (0.6)	84.2 (59.2)	< 0.001
18.5 – 24.9	878 (27.1)	1.0 (1.6)		825 (26.6)	3.21 (0.5)		878 (27.1)	104.1 (101.9)	
25.0 – 29.9	1422 (43.9)	1.7 (2.3)		1377 (44.4)	3.31 (0.5)		1422 (43.9)	127.4 (118.0)	
≥30	921 (28.4)	2.9 (4.0)		886 (28.5)	3.49 (0.5)		921 (28.4)	134.0 (124.6)	
NS-SEC									
Managerial and professional occupations	1243 (38.4)	1.5 (2.4)	< 0.001	1198 (38.6)	3.27 (0.5)	< 0.001	1243 (38.4)	135.3 (130.8)	< 0.001
Intermediate occupations	838 (25.9)	1.8 (2.6)		793 (25.5)	3.34 (0.5)		838 (25.9)	115.9 (109.2)	
Routine and manual occupations	1133 (35.0)	2.1 (3.2)		1090 (35.1)	3.40 (0.6)		1133 (35.0)	114.7 (103.3)	
Other	25 (1.0)	3.0 (4.9)		24 (1.0)	3.39 (0.5)		25 (1.0)	89.0 (63.7)	
Diseases									
Cancer									
Yes	76 (2.4)	1.8 (2.3)	0.964	73 (2.4)	3.41 (0.6)	0.209	76 (2.4)	125.3 (113.4)	0.843
No	3163 (97.7)	1.8 (2.8)		3032 (97.6)	3.33 (0.5)		3163 (97.7)	122.7 (116.3)	
Cardiovascular disease									
Yes	45 (1.4)	2.1 (2.7)	0.408	42 (1.4)	3.48 (0.6)	0.083	46 (1.4)	122.6 (124.7)	0.993
No	3194 (98.6)	1.8 (2.8)		3063 (98.6)	3.33 (0.5)		3193 (98.6)	122.7 (116.1)	
Rheumatoid arthritis									
Yes	189 (5.8)	2.5 (4.1)	< 0.001	185 (6.0)	3.38 (0.6)	0.222	189 (5.8)	108.3 (94.5)	0.079
No	3050 (94.2)	1.7 (2.7)		2920 (94.0)	3.33 (0.5)		3050 (94.2)	123.6 (117.4)	

Note: IQR – inter-quartile range; SD – standard deviation.
[a] P value derived using one-way analysis of variance for fibrinogen, ferritin and log-transformed CRP.

Table 3
Loneliness and change in CRP in men and women.

	Men (n = 1462)				Women (n = 1777)				
Loneliness measures at wave 4/wave 6	n (%)	Model 1[a] β (95%CI)	Model 2[b] β (95%CI)	Model 3[c] β (95%CI)	n (%)	Model 1[a] β (95%CI)	Model 2[b] β (95%CI)	Model 3[c] β (95%CI)	P_{value} [d]
"(Much of the time during the past week), you felt lonely?"[e]									
No/no	1328 (91)	Reference	Reference	Reference	1471 (83)	Reference	Reference	Reference	
No/yes	46 (3)	0.29 (-0.03 to 0.61)	0.36 (0.09 to 0.63)	0.36 (0.09 to 0.62)	115 (6)	0.12 (-0.09 to 0.32)	−0.02 (-0.18 to 0.13)	−0.03 (-0.19 to 0.12)	
Yes/no	54 (4)	0.20 (-0.10 to 0.50)	0.24 (-0.01 to 0.49)	0.23 (-0.02 to 0.47)	114 (6)	0.29 (0.08 to 0.49)	0.13 (-0.02 to 0.29)	0.13 (-0.02 to 0.29)	0.061
Yes/yes	34 (2)	0.43 (0.06 to 0.80)	0.28 (-0.04 to 0.59)	0.23 (-0.08 to 0.54)	77 (4)	0.32 (0.07 to 0.56)	0.06 (-0.13 to 0.24)	0.04 (-0.15 to 0.23)	
"How often do you feel lonely?"[f]									
Never/never	1040 (71)	Reference	Reference	Reference	998 (56)	Reference	Reference	Reference	
Never/often	118 (8)	0.20 (-0.01 to 0.41)	0.22 (0.05 to 0.40)	0.20 (0.03 to 0.38)	204 (11)	0.09 (-0.07 to 0.25)	0.04 (-0.09 to 0.16)	0.03 (-0.09 to 0.15)	
Often/never	113 (8)	0.15 (-0.07 to 0.36)	0.12 (-0.06 to 0.30)	0.14 (-0.04 to 0.32)	170 (10)	0.20 (0.03 to 0.38)	0.05 (-0.08 to 0.18)	0.05 (-0.08 to 0.18)	0.210
Often/often	191 (13)	0.17 (0.001 to 0.34)	0.14 (-0.01 to 0.28)	0.11 (-0.03 to 0.26)	405 (23)	0.12 (-0.01 to 0.24)	0.01 (-0.08 to 0.10)	−0.01 (-0.10 to 0.09)	
Loneliness estimated from the UCLA loneliness scale[g]									
Never/never	678 (46)	Reference	Reference	Reference	659 (37)	Reference	Reference	Reference	
Never/often	162 (11)	−0.04 (-0.23 to 0.14)	−0.04 (-0.20 to 0.12)	−0.07 (-0.23 to 0.09)	220 (12)	0.11 (-0.06 to 0.27)	0.07 (-0.06 to 0.19)	0.07 (-0.05 to 0.19)	
Often/never	182 (12)	−0.01 (-0.19 to 0.17)	0.02 (-0.13 to 0.17)	0.04 (-0.11 to 0.19)	230 (13)	0.05 (-0.11 to 0.21)	0.03 (-0.09 to 0.15)	0.04 (-0.08 to 0.16)	0.049
Often/often	440 (30)	0.18 (0.05 to 0.32)	0.13 (0.02 to 0.24)	0.11 (0.004 to 0.22)	668 (38)	0.10 (-0.01 to 0.22)	−0.004 (-0.09 to 0.08)	−0.01 (-0.10 to 0.07)	

[a]Model 1: unadjusted model.
[b]Model 2: adjusted for baseline level of biomarker.
[c]Model 3: Model 2 with adjustment for age, BMI, NS-SEC, and diagnoses of cancer, CVD or rheumatoid arthritis at baseline and follow-up.
[d]P value for interaction by sex was obtained using likelihood ratio test.
[e]Responses to this question at baseline (wave 4) and follow-up (wave 6) were grouped to create change categories, for example, no/no indicates not feeling lonely at both baseline and follow-up, while no/yes indicates not feeling lonely at baseline and feeling lonely at follow-up.
[f]Responses to this question at baseline (wave 4) and follow-up (wave 6) were grouped to create change categories, for example, the category never/never indicates never feeling lonely at both time points, while never/often indicates never feeling at baseline and feeling often at follow-up.
[g]Loneliness was categorized based on the UCLA loneliness scale score and grouped to create change categories as above.

Table 4
Loneliness and change in fibrinogen in men and women.

Loneliness measures at wave 4/wave 6	Men (n = 1397) n (%)	Model 1[a] β (95%CI)	Model 2[b] β (95%CI)	Model 3[c] β (95%CI)	Women (n = 1708) n (%)	Model 1[a] β (95%CI)	Model 2[b] β (95%CI)	Model 3[c] β (95%CI)	P_{value} [d]
"Much of the time during the past week), you felt lonely?"[e]									
No/no	1267 (91)	Reference	Reference	Reference	1412 (83)	Reference	Reference	Reference	
No/yes	46 (3)	0.17 (0.01 to 0.32)	0.17 (0.03 to 0.30)	0.18 (0.04 to 0.31)	110 (6)	0.05 (-0.05 to 0.15)	−0.01 (-0.09 to 0.07)	−0.02 (-0.10 to 0.06)	
Yes/no	50 (4)	−0.06 (-0.22 to 0.09)	−0.06 (-0.18 to 0.07)	−0.05 (-0.18 to 0.08)	114 (7)	0.08 (-0.02 to 0.18)	0.04 (-0.04 to 0.12)	0.04 (-0.05 to 0.12)	0.058
Yes/yes	34 (2)	0.11 (-0.07 to 0.29)	0.05 (-0.11 to 0.20)	0.02 (-0.13 to 0.18)	72 (4)	0.10 (-0.03 to 0.22)	0.01 (-0.09 to 0.11)	−0.001 (-0.10 to 0.10)	
"How often do you feel lonely?"[f]									
Never/never	989 (71)	Reference	Reference	Reference	969 (57)	Reference	Reference	Reference	
Never/often	115 (8)	0.03 (-0.08 to 0.13)	0.09 (0.003 to 0.18)	0.09 (-0.001 to 0.18)	195 (11)	0.01 (-0.07 to 0.09)	0.001 (-0.06 to 0.07)	0.001 (-0.06 to 0.07)	
Often/never	106 (8)	0.10 (-0.01 to 0.21)	0.13 (0.03 to 0.22)	0.14 (0.04 to 0.23)	158 (9)	−0.04 (-0.12 to 0.05)	−0.05 (-0.12 to 0.02)	−0.05 (-0.12 to 0.02)	0.012
Often/often	187 (13)	0.03 (-0.05 to 0.12)	0.01 (-0.06 to 0.08)	0.01 (-0.07 to 0.08)	386 (23)	0.02 (-0.04 to 0.08)	−0.02 (-0.07 to 0.03)	−0.03 (-0.08 to 0.02)	
Loneliness estimated from the UCLA loneliness scale[g]									
Never/never	643 (46)	Reference	Reference	Reference	638 (37)	Reference	Reference	Reference	
Never/often	162 (12)	−0.02 (-0.12 to 0.07)	−0.03 (-0.10 to 0.05)	−0.04 (-0.12 to 0.04)	210 (12)	−0.01 (-0.09 to 0.07)	−0.01 (-0.07 to 0.06)	−0.0001 (-0.07 to 0.07)	
Often/never	173 (12)	−0.002 (-0.09 to 0.09)	0.01 (-0.06 to 0.09)	0.03 (-0.05 to 0.11)	223 (13)	−0.05 (-0.13 to 0.03)	−0.03 (-0.09 to 0.04)	−0.03 (-0.09 to 0.04)	0.126
Often/often	419 (30)	0.07 (0.005 to 0.14)	0.05 (-0.01 to 0.10)	0.05 (-0.01 to 0.10)	637 (37)	0.02 (-0.04 to 0.08)	−0.02 (-0.06 to 0.03)	−0.02 (-0.07 to 0.02)	

[a]Model 1: unadjusted model.
[b]Model 2: adjusted for baseline level of biomarker.
[c]Model 3: Model 2 with adjustment for age, BMI, NS-SEC, and diagnoses of cancer, CVD or rheumatoid arthritis at baseline and follow-up.
[d]P value for interaction by sex was obtained using likelihood ratio test.
[e]Responses to this question at baseline (wave 4) and follow-up (wave 6) were grouped to create change categories, for example, no/no indicates not feeling lonely at both baseline and follow-up, while no/yes indicates not feeling lonely at baseline and feeling lonely at follow-up.
[f]Responses to this question at baseline (wave 4) and follow-up (wave 6) were grouped to create change categories, for example, the category never/never indicates never feeling lonely at both time points, while never/often indicates never feeling at baseline and feeling often at follow-up.
[g]Loneliness was categorized based on the UCLA loneliness scale score and grouped to create change categories as above.

Table 5
Loneliness and change in ferritin in men and women.

Loneliness measures at wave 4/wave 6	Men (n = 1462)				Women (n = 1777)				P_{value} [d]
	n (%)	Model 1[a] β (95%CI)	Model 2[b] β (95%CI)	Model 3[c] β (95%CI)	n (%)	Model 1[a] β (95%CI)	Model 2[b] β (95%CI)	Model 3[c] β (95%CI)	
"Much of the time during the past week), you felt lonely?"[e]									
No/no	1327 (91)	Reference	Reference	Reference	1471 (83)	Reference	Reference	Reference	
No/yes	46 (3)	24.96 (-27.13 to 77.05)	44.80 (10.47 to 79.13)	41.04 (6.58 to 75.50)	115 (6)	−12.17 (-35.94 to 11.59)	−8.71 (-24.46 to 7.05)	−7.61 (-23.38 to 8.17)	
Yes/no	54 (4)	−19.43 (-67.65 to 28.79)	5.72 (-26.07 to 37.51)	6.88 (-24.99 to 38.75)	114 (6)	−24.52 (-48.38 to -0.66)	−7.15 (-22.98 to 8.68)	−6.07 (-21.99 to 9.85)	0.020
Yes/yes	35 (2)	9.64 (-49.84 to 69.11)	−4.61 (-43.80 to 34.58)	−10.67 (-50.22 to 28.88)	77 (4)	−18.28 (-46.97 to 10.41)	−22.77 (-41.79 to -3.75)	−20.62 (-39.78 to -1.46)	
"How often do you feel lonely?"[f]									
Never/never	1039 (71)	Reference	Reference	Reference	998 (56)	Reference	Reference	Reference	
Never/often	118 (8)	−9.38 (-43.12 to 24.36)	−0.06 (-22.34 to 22.22)	0.02 (-22.47 to 22.50)	204 (11)	7.92 (-10.95 to 26.79)	8.53 (-3.99 to 21.04)	8.73 (-3.79 to 21.26)	
Often/never	113 (8)	−11.47 (-45.87 to 22.94)	−0.26 (-22.98 to 22.46)	−0.40 (-23.28 to 22.48)	170 (10)	−7.91 (-28.28 to 12.47)	0.27 (-13.25 to 13.78)	0.39 (-13.17 to 13.94)	0.935
Often/often	192 (13)	−14.97 (-42.26 to 12.31)	−7.38 (-25.40 to 10.64)	−8.53 (-26.70 to 9.65)	405 (23)	−9.50 (-23.97 to 4.97)	−3.57 (-13.17 to 6.02)	−1.60 (-11.30 to 8.10)	
Loneliness estimated from the UCLA loneliness scale[g]									
Never/never	677 (46)	Reference	Reference	Reference	659 (37)	Reference	Reference	Reference	
Never/often	162 (11)	−6.65 (-36.99 to 23.70)	−1.92 (-21.98 to 18.14)	−3.73 (-23.82 to 16.36)	220 (12)	6.90 (-12.24 to 26.03)	1.49 (-11.20 to 14.18)	0.65 (-12.07 to 13.36)	
Often/never	182 (12)	−16.33 (-45.30 to 12.64)	−0.34 (-19.50 to 18.82)	−0.94 (-20.17 to 18.28)	230 (13)	5.26 (-13.56 to 24.08)	5.00 (-7.48 to 17.48)	5.05 (-7.42 to 17.51)	0.833
Often/often	441 (30)	−22.21 (-43.44 to -0.97)	−4.07 (-18.13 to 9.99)	−3.58 (-17.75 to 10.59)	668 (38)	−2.39 (-15.89 to 11.10)	3.05 (-5.90 to 12.00)	4.52 (-4.47 to 13.52)	

[a]Model 1: unadjusted model.
[b]Model 2: adjusted for baseline level of biomarker.
[c]Model 3: Model 2 with adjustment for age, BMI, NS-SEC, and diagnoses of cancer, CVD or rheumatoid arthritis at baseline and follow-up.
[d]P value for interaction by sex was obtained using likelihood ratio test.
[e]Responses to this question at baseline (wave 4) and follow-up (wave 6) were grouped to create change categories, for example, no/no indicates not feeling lonely at both baseline and follow-up, while no/yes indicates not feeling lonely at baseline and feeling lonely at follow-up.
[f]Responses to this question at baseline (wave 4) and follow-up (wave 6) were grouped to create change categories, for example, the category never/never indicates never feeling lonely at both time points, while never/often indicates never feeling at baseline and feeling often at follow-up.
[g]Loneliness was categorized based on the UCLA loneliness scale score and grouped to create change categories as above.

Cross-sectional associations of the loneliness measures with biomarkers are reported in appendix D.

3.4. Sensitivity analyses

In the analysis using the FIML method for handling missing data, the magnitude of associations became slightly lower, while the direction of association stayed similar to the complete-case analysis and the statistical significance remained for the majority of associations (Appendix F). In men, the association between the onset of loneliness for the *"Much of the time during the past week, you felt lonely?"* question and fibrinogen was no longer statistically significant. However, the association between the onset of loneliness indicated by *"How often do you feel lonely?"* question and fibrinogen became statistically significant after applying the FIML method.

In the analysis examining depressive symptoms as a potential mediator, there was no statistically significant mediation by depressive symptoms for the associations of the onset of loneliness using all measures of loneliness with all three biomarkers (Appendix G).

4. Discussion

Using three measures of loneliness and three markers of inflammation, we observed associations between the onset of loneliness and an increase in levels of CRP, plasma fibrinogen and ferritin, consistent with biological changes, including increased inflammation. These associations were observed among men with few statistically significant associations among women. Among men, the onset of loneliness (not lonely in the first ELSA wave, but lonely in the second) for the *"Much of the time during the past week, you felt lonely?"* question was associated with an increase in the level of all three biomarkers. Persistent loneliness (lonely at both ELSA waves) identified using the UCLA loneliness scale was associated with increased levels of CRP in men. The only statistically significant association for women was reduced levels of ferritin with persistent loneliness for the *"Much of the time during the past week, you felt lonely?"* question. The associations were independent of pre-existing or new diagnoses relevant to inflammation risk and other potential confounding factors. None of the statistically significant associations appeared to be mediated by depressive symptoms.

An important aspect of this study is its longitudinal design, as most of the previous work that we could identify was cross-sectional, limiting the ability to make causal inference (Mezuk et al., 2016; Nersesian et al., 2018; O'Luanaigh et al., 2012; Shankar et al., 2011). We assessed *change* in loneliness over time and *change* in the inflammatory markers, while taking into account potential confounding factors at baseline and follow-up. We used three measures of loneliness to include direct and indirect loneliness questions, and examined both the onset and persistent loneliness for all three measures. The assessment of loneliness varies across studies and differences were reported comparing the CES-D loneliness item compared with the three-item UCLA scale (Shiovitz-Ezra and Ayalon, 2011), indicating that these measures classify different individuals as lonely. Correlation coefficients between all three measures of loneliness in our study indicate correlation but some differences between the measures. Direct questions using the word 'loneliness' may be associated with social stigma, so the UCLA scale may identify more individuals as the word 'loneliness' is not used. Despite this, the majority of earlier studies reported no association with biomarkers and used a variety of loneliness measures. To our knowledge, only one cross-sectional study among participants aged 35–64 years, reported a positive association of a single-item loneliness question with levels of CRP and fibrinogen (Nersesian et al., 2018). No associations were reported with CRP using the 20-item UCLA loneliness scale (McDade et al., 2006), or the shorter version (Mezuk et al., 2016), or with a single item question (O'Luanaigh et al., 2012). The associations we observed may be due to methodological differences, including our use of conditional change modelling to identify change in loneliness status between two time points. One possible explanation for the lack of association with the UCLA scale in our study may be because of its dichotomization, with a large proportion (approximately half of the population) classified as lonely and therefore creating a less severe definition of loneliness.

Statistically significantly higher levels of CRP were found in men who experienced the onset of loneliness for both direct questions about loneliness. However, when using indirect loneliness variable based on the UCLA scale, it was persistent loneliness that was associated with higher CRP. In contrast with our findings, other studies reported no association between loneliness and CRP (McDade et al., 2006; Mezuk et al., 2016; O'Luanaigh et al., 2012). In our study, fibrinogen level was not clearly associated with indirect loneliness for the UCLA loneliness scale, consistent with other studies (Mezuk et al., 2016; Shankar et al., 2011). The onset of loneliness for the question *"Much of the time during the past week, you felt lonely?"* was associated with higher levels of fibrinogen among men, consistent with the results for CRP. An experimental study reported that lonely participants had higher levels of fibrinogen after induction of acute mental stress, indicating a greater acute stress response in lonely individuals (Steptoe et al., 2004). The onset of loneliness for the question *"Much of the time during the past week, you felt lonely?"* was associated with higher levels of ferritin in men, which is consistent with the results for both CRP and fibrinogen, providing a consistent pattern for an association between recent onset of loneliness and inflammation. Ferritin is used to store iron, so the decrease in ferritin among women associated with the question *"Much of the time during the past week, you felt lonely?"* may indicate a decrease in iron stores for reasons such as blood loss, changes in dietary habits; however, less likely due to chronic inflammation as an increase in levels of CRP among women was not observed in our analysis.

Previous studies of loneliness and biomarkers did not explore differences by sex (McDade et al., 2006; O'Luanaigh et al., 2012) or reported no difference by sex (Mezuk et al., 2016; Steptoe et al., 2004). Although interaction tests for our study were likely to be underpowered, we observed some indication of interactions by sex, particularly for responses to the *"Much of the time during the past week, you felt lonely?"* question and changes in all of the biomarkers. We found consistent statistically significant changes in biomarkers associations with loneliness mainly in men and estimates close to null in women. Despite this, the proportion of women who became lonely or remained lonely at both time points was statistically significantly higher than in men (chi-square tests $p < 0.001$), so statistical power is unlikely to explain this difference. As men may underreport loneliness, when they report it, it may be more severe, resulting in higher magnitude associations (Rico-Uribe et al., 2018). Moreover, men tend to be more affected by widowhood through the additional burden of adapting to performing domestic tasks (Lee et al., 2001) and are at higher risk of all-cause mortality than women after death of their spouse (Moon et al., 2011). Higher magnitude associations with a worsening biomarker profile in men are also consistent with findings from a recent meta-analysis of prospective studies, where loneliness was associated with higher risk of all-cause mortality in men and women, but with a higher magnitude association in men (Rico-Uribe et al., 2018).

We cannot determine whether loneliness has a direct immunological or neuroendocrine influence on the biomarkers, or is mediated through changes in health behavior or gene expression. There is some evidence suggesting that loneliness may result in reduced physical activity (Newall et al., 2013), higher likelihood of smoking (Dyal and Valente, 2015), and more depressive symptoms (Cacioppo et al., 2006), which in turn, may lead to changes in the levels of the biomarkers. In this study, no evidence was found for mediating effect of depressive symptoms,

and there were only 63 participants who changed their smoking status and became smokers at wave 6, so unlikely to mediate an association between loneliness and inflammatory changes. Our results are consistent with a study of leukocyte transcription dynamics using data from 14 men and women that found a high level of subjective social isolation was associated increased expression of genes related to pro-inflammatory cytokine signaling. A possible mediator for the association with loneliness is decreased activity of the anti-inflammatory glucocorticoid receptor pathway (Cole et al., 2007).

The most extensively researched mechanisms potentially linking chronic psychological stress such as loneliness with inflammation involve dysregulation of the HPA axis, sympathetic nervous system activation and depressed vagus nerve activity (Wirtz and von Kanel, 2017). These processes may result in increased levels of circulating pro-inflammatory cytokines, acute phase reactants and lower levels of anti-inflammatory cytokines leading to vascular damage including through endothelial dysfunction, coagulation activation and atherosclerosis progression (Wirtz and von Kanel, 2017). Plasma fibrinogen is a central element in hemostasis and coagulation, also with properties relevant to activation of the immune system (Davalos and Akassoglou, 2012). HPA axis dysregulation-induced inflammatory processes and the effect on blood pressure through an influence on total peripheral resistance, play a role in hypertension, atherosclerosis, and vascular disease (Hawkley et al., 2010). Serum fibrinogen increases in response to pathological conditions such as inflammation or vascular damage. Proinflammatory cytokines interleukin IL-1 and TNF-α are known to induce production of acute phase protein ferritin, particularly the H (heavy chain) ferritin, suggesting that inflammatory conditions and stress have an impact on ferritin regulation (Torti and Torti, 2002). Serum ferritin is synthesized not only in response to inflammation and oxidative damage but it also binds to proteins and enzymes involved in clotting, highlighting its role in atherosclerotic plaque formation (Watt, 2011).

The study has some potential limitations. Although we used several measures of loneliness, they do not distinguish between types of loneliness such as social, including lacking friends and acquaintances, and emotional, indicating the absence of a close relationship. Bias due to non-response, is a concern as blood samples were collected from 53% of the initial sample of participants who completed personal interviews at waves 4 and 6 and it is known that younger and healthier individuals were more likely to participate (Bridges et al., 2015). However, we conducted a sensitivity analysis using the FIML method for handling missing data, and the results of the complete-case and FIML analyses were consistent. Questions remain about causal inference: although we hypothesize that loneliness pre-dates change in biomarker levels, we cannot exclude the possibility that biomarker changes may have occurred before the experience of loneliness. One possibility is that some chronic diseases result in higher levels of fatigue and therefore social isolation, as well as influencing biomarker levels. To tackle this, we were able to show the associations are not due to the existence or onset of cancer, cardiovascular disease or rheumatoid arthritis, as these diagnoses were adjusted for at both baseline and follow-up.

5. Conclusions

The onset of loneliness was associated with systemic biological changes in men, which were not mediated by depressive symptoms. These findings may help to shed light on the mechanisms underlying the association of loneliness with disease and mortality risk. Combined social and targeted medical interventions may help to reduce health risks associated with loneliness.

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Role of the funding source

The funder of the study had no role in the study design, data analyses, interpretation or writing of the manuscript.

Ethical approval

English Longitudinal Study of Ageing received ethical approval from the London Multicentre Research Ethics Committee (MREC/01/2/91) with informed consent obtained from all study participants.

Data sharing statement

The dataset is publically available at the UK Data Service and can be downloaded upon registration for free (https://www.ukdataservice.ac.uk/).

CRediT authorship contribution statement

Snieguole Vingeliene: Conceptualization, Methodology, Formal analysis, Writing - original draft, Visualization. **Ayako Hiyoshi:** Conceptualization, Methodology, Formal analysis, Writing - review & editing, Supervision. **Marleen Lentjes:** Conceptualization, Writing - review & editing, Visualization, Supervision. **Katja Fall:** Conceptualization, Methodology, Writing - review & editing, Supervision. **Scott Montgomery:** Conceptualization, Methodology, Writing - review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

None.

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SV analyzed the data and drafted the first version of the manuscript. SM defined the broad research question and obtained funding for the study. All authors contributed substantially to the conception, design of the study and interpretation of the results; reviewed and approved the final version of the manuscript. SV takes responsibility for the integrity of the data and the accuracy of the data analysis. The authors thank Yang Cao for his advice on statistical methods.

Appendices A–G. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2019.104421.

References

Au, B., Smith, K.J., Gariepy, G., Schmitz, N., 2014. C-reactive protein, depressive symptoms, and risk of diabetes: results from the English Longitudinal Study of Ageing (ELSA). J. Psychosom. Res. 77, 180–186. https://doi.org/10.1016/j.jpsychores.2014.07.012.

Benjamin EJ et al. (2019) Heart Disease and Stroke Statistics-2019 Update: A Report From the American Heart Association Circulation 139:e56-e528 doi:10.1161/cir.0000000000000659.

Bridges, S., Hussey, D., Blake, M., 2015. The dynamics of ageing: the 2012 english longitudinal study of ageing (Wave 6): technical report. Nat Cen.

Cacioppo, J.T., Hughes, M.E., Waite, L.J., Hawkley, L.C., Thisted, R.A., 2006. Loneliness as a specific risk factor for depressive symptoms: cross-sectional and longitudinal analyses. Psychol. Aging 21, 140–151. https://doi.org/10.1037/0882-7974.21.1.140.

Cacioppo, S., Capitanio, J.P., Cacioppo, J.T., 2014. Toward a neurology of loneliness. Psychol. Bull. 140, 1464–1504. https://doi.org/10.1037/a0037618.

Cheshire, H., Hussey, D., Medina, J., Pickering, K., Wood, N., Ward, K., Taylor, K., Lessof, C., 2009. English Longitudinal Study of Ageing. Wave Four Interview Questionnaire - 2008-2009. National Centre for Social Research. https://www.elsa-project.ac.uk/study-documentation.

Cole, S.W., Hawkley, L.C., Arevalo, J.M., Sung, C.Y., Rose, R.M., Cacioppo, J.T., 2007.

Social regulation of gene expression in human leukocytes. Genome Biol. 8, R189. https://doi.org/10.1186/gb-2007-8-9-r189.
Collins, L.M., Schafer, J.L., Kam, C.M., 2001. A comparison of inclusive and restrictive strategies in modern missing data procedures. Psychol. Methods 6, 330–351.
Davalos, D., Akassoglou, K., 2012. Fibrinogen as a key regulator of inflammation in disease. Semin. Immunopathol. 34, 43–62. https://doi.org/10.1007/s00281-011-0290-8.
Discacciati, A., Bellavia, A., Lee, J.J., Mazumdar, M., Valeri, L., 2018. Med4way: a Stata command to investigate mediating and interactive mechanisms using the four-way effect decomposition. Int. J. Epidemiol. https://doi.org/10.1093/ije/dyy236.
Dong, Y., C-YJ, Peng, 2013. Principled missing data methods for researchers. Springerplus 2https://doi.org/10.1186/2193-1801-2-222. 222-222.
Dyal, S.R., Valente, T.W., 2015. A systematic review of loneliness and smoking: small effects. Big Implications Substance use & misuse 50, 1697–1716. https://doi.org/10.3109/10826084.2015.1027933.
Hamer, M., Chida, Y., 2009. Associations of very high C-reactive protein concentration with psychosocial and cardiovascular risk factors in an ageing population. Atherosclerosis 206, 599–603. https://doi.org/10.1016/j.atherosclerosis.2009.02.032.
Hawkley, L.C., Thisted, R.A., Masi, C.M., Cacioppo, J.T., 2010. Loneliness predicts increased blood pressure: 5-year cross-lagged analyses in middle-aged and older adults. Psychol. Aging 25, 132–141. https://doi.org/10.1037/a0017805.
Holt-Lunstad, J., Smith, T.B., Baker, M., Harris, T., Stephenson, D., 2015. Loneliness and social isolation as risk factors for mortality: a meta-analytic review. Perspect. Psychol. Sci. 10, 227–237. https://doi.org/10.1177/1745691614568352.
Holt-Lunstad, J., Smith, T.B., Layton, J.B., 2010. Social relationships and mortality risk: a meta-analytic review. PLoS Med. 7, e1000316. https://doi.org/10.1371/journal.pmed.1000316.
Hughes, M.E., Waite, L.J., Hawkley, L.C., Cacioppo, J.T., 2004. A short scale for measuring loneliness in large surveys: results from two population-based studies. Res. Aging 26, 655–672. https://doi.org/10.1177/0164027504268574.
Karim, J., Weisz, R., Bibi, Z., ur Rehman, S., 2015. Validation of the Eight-item Center for Epidemiologic Studies Depression Scale (CES-D) Among Older Adults Vol 34. Springer, Germany. https://doi.org/10.1007/s12144-014-9281-y.
Kell, D.B., Pretorius, E., 2014. Serum ferritin is an important inflammatory disease marker, as it is mainly a leakage product from damaged cells. Metallomics 6, 748–773. https://doi.org/10.1039/c3mt00347g.
Kernan, K.F., Carcillo, J.A., 2017. Hyperferritinemia and inflammation. Int. Immunol. 29, 401–409. https://doi.org/10.1093/intimm/dxx031.
Kivimäki, M., Steptoe, A., 2017. Effects of stress on the development and progression of cardiovascular disease. Nat. Rev. Cardiol. 15, 215–229. https://doi.org/10.1038/nrcardio.2017.189.
Lee, G.R., DeMaris, A., Bavin, S., Sullivan, R., 2001. Gender differences in the depressive effect of widowhood in later life. J. Gerontol. B Psychol. Sci. Soc. Sci. 56, S56–61. https://doi.org/10.1093/geronb/56.1.s56.
Markanday, A., 2015. Acute phase reactants in infections. Evidence-Based Review and a Guide for Clinicians Open forum infectious diseases 2, ofv098. https://doi.org/10.1093/ofid/ofv098.
McDade, T.W., Hawkley, L.C., Cacioppo, J.T., 2006. Psychosocial and behavioral predictors of inflammation in middle-aged and older adults: the Chicago health, aging, and social relations study. Psychosom. Med. 68, 376–381. https://doi.org/10.1097/01.psy.0000221371.43607.64.
Mezuk, B., Choi, M., DeSantis, A.S., Rapp, S.R., Diez Roux, A.V., Seeman, T., 2016. Loneliness, depression, and inflammation: evidence from the multi-ethnic study of atherosclerosis. PLoS One 11, e0158056. https://doi.org/10.1371/journal.pone.0158056.
Miller, G., 2011. Social neuroscience. Why loneliness is hazardous to your health. Science 331, 138–140. https://doi.org/10.1126/science.331.6014.138.
Moon, J.R., Kondo, N., Glymour, M.M., Subramanian, S.V., 2011. Widowhood and mortality: a meta-analysis. PLoS One 6, e23465. https://doi.org/10.1371/journal.pone.0023465.
Muscatell, K.A., Brosso, S.N., Humphreys, K.L., 2018. Socioeconomic status and inflammation: a meta-analysis. Mol. Psychiatry. https://doi.org/10.1038/s41380-018-0259-2.
Nersesian, P.V., Han, H.R., Yenokyan, G., Blumenthal, R.S., Nolan, M.T., Hladek, M.D., Szanton, S.L., 2018. Loneliness in middle age and biomarkers of systemic inflammation: findings from Midlife in the United States. Soc. Sci. Med. 209, 174–181. https://doi.org/10.1016/j.socscimed.2018.04.007.
Newall, N.E., Chipperfield, J.G., Bailis, D.S., Stewart, T.L., 2013. Consequences of loneliness on physical activity and mortality in older adults and the power of positive emotions. Health Psychol. 32, 921–924. https://doi.org/10.1037/a0029413.
O'Luanaigh, C., et al., 2012. Loneliness and vascular biomarkers: the Dublin healthy ageing study. Int. J. Geriatr. Psychiatry 27, 83–88. https://doi.org/10.1002/gps.2695.
Ong, A.D., Uchino, B.N., Wethington, E., 2016. Loneliness and health in older adults: a mini-review and synthesis. Gerontology 62, 443–449. https://doi.org/10.1159/000441651.
Pavela, G., Kim, Y.I., Salvy, S.J., 2018. Additive effects of obesity and loneliness on C-reactive protein. PLoS One 13, e0206092. https://doi.org/10.1371/journal.pone.0206092.
Pinquart, M., Sörensen, S., 2001. Influences on loneliness in older adults: a meta-analysis. Basic Appl. Soc. Psych. 23, 245–266. https://doi.org/10.1207/153248301753225702.
Radloff, L.S., 1977. The CES-D scale: a self-report depression scale for research in the general population. Appl. Psychol. Meas. 1, 385–401. https://doi.org/10.1177/014662167700100306.
Regitz-Zagrosek, V., Kararigas, G., 2017. Mechanistic pathways of sex differences in cardiovascular disease. Physiol. Rev. 97, 1–37. https://doi.org/10.1152/physrev.00021.2015.
Rico-Uribe, L.A., Caballero, F.F., Martin-Maria, N., Cabello, M., Ayuso-Mateos, J.L., Miret, M., 2018. Association of loneliness with all-cause mortality: a meta-analysis. PLoS One 13, e0190033. https://doi.org/10.1371/journal.pone.0190033.
Ridker, P.M., 2016. From C-Reactive protein to Interleukin-6 to Interleukin-1: moving upstream to identify novel targets for atheroprotection. Circ. Res. 118, 145–156. https://doi.org/10.1161/CIRCRESAHA.115.306656.
Roth, G.A., et al., 2017. Global, regional, and national burden of cardiovascular diseases for 10 causes, 1990 to 2015. J. Am. Coll. Cardiol. 70, 1–25. https://doi.org/10.1016/j.jacc.2017.04.052.
Russell, D.W., 1996. UCLA Loneliness Scale (Version 3): reliability, validity, and factor structure. J. Pers. Assess. 66, 20–40. https://doi.org/10.1207/s15327752jpa6601_2.
Shankar, A., McMunn, A., Banks, J., Steptoe, A., 2011. Loneliness, social isolation, and behavioral and biological health indicators in older adults. Health Psychol. 30, 377–385. https://doi.org/10.1037/a0022826.
Shiovitz-Ezra, S., Ayalon, L., 2011. Use of direct versus indirect approaches to measure loneliness in later life. Res. Aging 34, 572–591. https://doi.org/10.1177/0164027511423258.
Steffick, D.E., 2000. Documentation of Affective Functioning Measures in the Health and Retirement Study. Institute for Social Research, University of Michigan, Ann Arbor, Michigan.
Steptoe, A., Breeze, E., Banks, J., Nazroo, J., 2013. Cohort profile: the English longitudinal study of ageing. Int. J. Epidemiol. 42, 1640–1648. https://doi.org/10.1093/ije/dys168.
Steptoe, A., Leigh, E.S., Kumari, M., 2011. Positive affect and distressed affect over the day in older people Psychology and Aging 26, 956–965. https://doi.org/10.1037/a0023303.
Steptoe, A., Owen, N., Kunz-Ebrecht, S.R., Brydon, L., 2004. Loneliness and neuroendocrine, cardiovascular, and inflammatory stress responses in middle-aged men and women. Psychoneuroendocrinology 29, 593–611. https://doi.org/10.1016/s0306-4530(03)00086-6.
Stewart, J.C., Rand, K.L., Muldoon, M.F., Kamarck, T.W., 2009. A prospective evaluation of the directionality of the depression-inflammation relationship. Brain Behav. Immun. 23, 936–944. https://doi.org/10.1016/j.bbi.2009.04.011.
Stringhini, S., Zaninotto, P., Kumari, M., Kivimaki, M., Lassale, C., Batty, G.D., 2018. Socio-economic trajectories and cardiovascular disease mortality in older people. Engl. Longit. Stud. Ageing Int. J. Epidemiol. 47, 36–46. https://doi.org/10.1093/ije/dyx106.
Torti, F.M., Torti, S.V., 2002. Regulation of ferritin genes and protein. Blood 99, 3505–3516. https://doi.org/10.1182/blood.v99.10.3505.
Valtorta, N.K., Kanaan, M., Gilbody, S., Ronzi, S., Hanratty, B., 2016. Loneliness and social isolation as risk factors for coronary heart disease and stroke: systematic review and meta-analysis of longitudinal observational studies. Heart 102, 1009–1016. https://doi.org/10.1136/heartjnl-2015-308790.
VanderWeele, T.J., 2014. A unification of mediation and interaction: a 4-way decomposition. Epidemiology 25, 749–761. https://doi.org/10.1097/EDE.0000000000000121.
Watt, R.K., 2011. The many faces of the octahedral ferritin protein. Biometals 24, 489–500. https://doi.org/10.1007/s10534-011-9415-8.
Wirtz, P.H., von Kanel, R., 2017. Psychological stress, inflammation, and coronary. Heart Disease Current cardiology reports 19, 111. https://doi.org/10.1007/s11886-017-0919-x.
Vozikaki, M., Papadaki, A., Linardakis, M., Philalithis, A., 2018. Loneliness among older European adults: results from the survey of health, aging and retirement in Europe. J. Public Health (Bangkok) 26, 613–624. https://doi.org/10.1007/s10389-018-0916-6.
Zivin, K., Llewellyn, D.J., Lang, I.A., Vijan, S., Kabeto, M.U., Miller, E.M., Langa, K.M., 2010. Depression among older adults in the United States and England. Am. J. Geriatr. Psychiatry 18, 1036–1044. https://doi.org/10.1097/JGP.0b013e3181dba6d2.