**Systemic low-grade inflammation and subsequent depressive symptoms: Is there a mediating role of physical activity?**

Philipp Franka (philipp.frank.16@ucl.ac.uk), Aradhna Kaushala (aradhna.kaushal.14@ucl.ac.uk), Lydia Poolea (lydia.poole@ucl.ac.uk), Samantha Lawesa(samantha.lawes.13@ucl.ac.uk), Trudie Chalderb (trudie.chalder@kcl.ac.uk) & Dorina Cadara (d.cadar@ucl.ac.uk)

aDepartment of Behavioural Science and Health, University College London, 1-19 Torrington Place,

WC1E 7HB, London, UK

bDepartment of Psychological Medicine, Institute of Psychiatry, Psychology and Neuroscience, King’s College London, 16 De Crespigny Park, SE5 8AB London, UK

***Corresponding author***: Philipp Frank

 University College London

 1-19 Torrington Place, WC1E 7HB, London, UK

 Phone: +44(0) 7826 388750

 philipp.frank.16@ucl.ac.uk

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**HIGHLIGHTS**

* Systemic inflammation is associated with lower levels of physical activity
* Regular physical activity reduces the risk of developing depressive symptoms
* Low physical activity mediates the inflammation-depressive symptoms relationship

***Keywords:*** Depressive symptoms, systemic low-grade inflammation, physical activity, mediation analysis

**ABSTRACT**

**Objective***:* Systemic low-grade inflammation has been associated with the onset of depression, but the exact mechanisms underlying this relationship remain elusive. This study examined whether physical activity (PA) explained the association between elevated plasma levels of inflammatory markers and subsequent depressive symptoms.

**Design***:* Prospective cohort design.

**Method:** The sample consisted of 3,809 non-depressed men and women (aged 50+) recruited from the English Longitudinal Study of Ageing (ELSA). Serum levels of inflammatory markers (C-reactive protein (CRP), fibrinogen) and covariates (age, sex, education, wealth, body mass index, smoking, cholesterol, triglycerides) were measured at baseline (wave 4, 2008/09). Self-reported weekly moderate/vigorous (high) PA versus no weekly moderate/vigorous (low) PA was examined at a four-year follow-up (wave 6, 2012/13), using a single-item question. Depressive symptoms were assessed at baseline, four years (wave 6, 2012/13) and six years post baseline (wave 7, 2014/15), using the 8-item version of the Centre for Epidemiological Studies Depression Scale (CES-D).

**Results***:* Participants with higher baseline concentrations of inflammatory markers were significantly more likely to report low PA levels four years later (CRP: OR: 1.25; 95% CI, 1.05-1.48; fibrinogen: OR: 1.18; 95% CI, 1.05-1.39). Moreover, low PA was associated with higher odds of elevated depressive symptoms at follow-up (OR: 1.59; 95% CI, 1.15-2.19). Mediation analyses revealed that low PA explained a total of 36.71% of the relationship between high CRP and elevated depressive symptoms, and 33.26% between higher levels of fibrinogen and elevated depressive symptoms six years later. No direct association was found between systemic low-grade inflammation and subsequently elevated depressive symptoms.

**Conclusion***:* These results suggest that low PA is a significant partial mediator of the relationship between systemic low-grade inflammation and subsequent elevated depressive symptoms in a nationally representative cohort of older adults.

**1. INTRODUCTION**

Depression is a growing public health concern and a leading cause of disability worldwide, particularly among older adults ([Mathers & Loncar, 2006](#_ENREF_48)). Previous evidence suggests that approximately 18% of adults aged 65 years and older suffer from depression in England ([Zivin et al., 2010](#_ENREF_7)). Late-life depression has been linked to a wide range of adverse health outcomes, including an increased risk of morbidity ([Frasure-Smith & Lespérance, 2003](#_ENREF_19); [Lee, Choi, & Lee, 2001](#_ENREF_39); [Meyers, 1996](#_ENREF_50)), cognitive decline ([Jorm, 2000](#_ENREF_1)) and mortality ([Alexopoulos, 2005](#_ENREF_2); [Blazer, 2003](#_ENREF_10)). However, the exact biological mechanisms underlying the pathogenesis of depression remain elusive ([Franceschi & Campisi, 2014](#_ENREF_18)).

One hypothesis that has been previously suggested for the aetiology of late-life depression is the inflammation hypothesis of depression ([Alexopoulos & Morimoto, 2011](#_ENREF_3); [Franceschi & Campisi, 2014](#_ENREF_18)). This hypothesis posits that ageing shifts the body into a chronic proinflammatory state, based on an increased immune response in the peripheral nervous system (PNS), impaired immune communication between the PNS and the central nervous system (CNS) and a discordant and increased CNS response to inflammatory processes in the periphery ([Franceschi & Campisi, 2014](#_ENREF_18)). Furthermore, it suggests that these age-related biological changes can, in susceptible individuals, initiate the development of ‘sickness behaviour’ or depressive-like symptoms, such as fatigue, irritability, anhedonia and social withdrawal ([Dantzer, O’Connor, Freund, Johnson, & Kelley, 2008](#_ENREF_15)). Plausible biological pathways exist for the effects of systemic low-grade inflammation on mood, including the activation of the tryptophan-degrading enzyme indoleamine 2,3-dioxygenase, the inhibition of serotonin synthesis, and the impairment of antioxidant defences ([Valkanova et al., 2013](#_ENREF_64)).

Systemic low-grade inflammation describes the persistent production of proinflammatory factors, as opposed to an acute inflammatory state, in response to immune challenge (Pietzner et al., 2017). Low-grade inflammation is typically measured via serum concentrations of circulating proinflammatory markers (e.g. the cytokines interleukin-8 (IL-8), interleukin-6 (IL-6), tumour necrosis factor α (TNF-α), the acute phase protein C-reactive protein (CRP) and the coagulation protein fibrinogen). To date, prospective evidence supporting the depressogenic effect of systemic low-grade inflammation has yielded mixed results. For example, a previous meta-analysis evaluated the cumulative evidence of eleven prospective cohort studies on the relationship between systemic inflammation and subsequent depressive symptoms ([Valkanova et al., 2013](#_ENREF_64)). Overall, the results revealed a significant association between high baseline levels of inflammatory markers (CRP and IL-6) and subsequent depressive symptoms (N=18,527). However, only two of these studies assessed the inflammation-depression pathway in ageing cohorts ([Stewart et al., 2009](#_ENREF_62); [van den Biggelaar et al., 2007](#_ENREF_65)), hampering the generalisability to the older population.

Martínez-Cengotitabengoa et al. (2016) reviewed the longitudinal evidence of six studies focusing uniquely on older adults (≥ 60 years). Five of these studies reported significant associations between IL-6, IL-8 or TNF-α and future depressive symptoms ([Baune et al., 2012](#_ENREF_9); [Bremmer et al., 2008](#_ENREF_11); [Forti et al., 2010](#_ENREF_17); [Milaneschi et al., 2009](#_ENREF_51); [van den Biggelaar et al., 2007](#_ENREF_65)). However, Stewart et al. (2009) found that neither baseline CRP nor IL-6 predicted depressive symptomatology six years later in a sample of 263 American older adults (≥ 50 years). These results concur with the findings of another prospective investigation, which reported no significant associations between elevated levels of CRP and subsequent depressive symptoms in 3,397 community-dwelling older adults (≥ 50 years) ([Au et al., 2014](#_ENREF_5)). Possible explanations for inconsistencies in findings include differences in the study design, study population and variability in measurements used to assess both low-grade inflammation and depressive symptoms ([Martínez-Cengotitabengoa et al., 2016](#_ENREF_47)).

Physical activity (PA) has been linked to both the regulation of the innate immune system and the treatment of depressive disorders. For example, regular PA has been found to significantly reduce systemic inflammation in older adults ([Hamer et al., 2009](#_ENREF_24)). Moreover, people engaging in regular PA are less likely to develop future depressive symptoms ([Hamer et al., 2009](#_ENREF_24); [Joshi et al., 2016](#_ENREF_33); [Ku et al., 2017](#_ENREF_37); [Mammen & Faulkner, 2013](#_ENREF_45); [Pasco et al., 2011](#_ENREF_54); [Roh et al., 2015](#_ENREF_59); [Yoshida et al., 2015](#_ENREF_67)). Hence, regular PA may play a central role in the maintenance of both mental and physical health. However, until now, there have been no prospective studies examining whether PA acts as an intermediate mechanism in the relationship between systemic low-grade systemic inflammation and subsequent depressive symptoms. Therefore, the present study aimed to assess whether low PA mediated the relationship between elevated serum levels of proinflammatory biomarkers (CRP and fibrinogen) and subsequent depressive symptoms in English community-dwelling older adults, using prospective data from the English Longitudinal Study of Ageing (ELSA).

**1.1. Hypotheses**

It was hypothesised that:

1. Higher baseline levels of inflammatory markers (CRP and fibrinogen) would be associated with elevated depressive symptoms at follow-up (path a, Figure 1);
2. Low PA would mediate the association between higher levels of inflammatory markers and subsequent elevated depressive symptoms (path b\*c, Figure 1).

*< Figure 1 here >*

**2. METHOD**

**2.1. Study Design**

ELSA is an ongoing, nationally representative, prospective cohort study of men and women aged ≥ 50 years living in England. ELSA was established in 2002, using objective and subjective measures to assess the causes and consequences of health-related outcomes (Steptoe et al., 2012). The original sample consisted of people born on/before 29th February 1952, who were recruited from English households in the context of the Health Survey for England ([Sproston & Mindell, 2006](#_ENREF_2)). Participant selection was based on multistage stratified probability sampling, selecting postcode sectors at the first stage and household addresses at the second stage. Since 2002, data collection has been conducted bi-annually. Wave 4 (2008/09) was considered as the baseline in the present study. This wave includes a rich variety of psychosocial data collected via face-to-face computer-assisted personal interviews (CAPI) and biological data collected during nurse visits at participants’ homes (e.g. blood samples, body mass index, etc.). The follow-up period reported here is wave 7 (2014/15), the latest wave of data collection available at the time of these analyses. All participants provided written consent prior to their participation in the study. Ethical approval was granted by the London Multi-Centre Research Ethics Committee (<https://www.elsa-project.ac.uk>).

**2.2. Analytical Sample**

A total of 9,886 participants attended the core assessment at wave 4. Participants with missing blood samples at baseline were omitted from the analytical sample (n=3,985). Missing biological data were mainly due to participants’ unwillingness to consent or ineligibility to provide blood samples (e.g. due to clotting or bleeding disorders and/or use of anticoagulant medication). Participants with baseline levels of CRP equal to or higher than 10mg/L were also excluded (n=411) as these concentrations indicate acute infection rather than systemic low-grade inflammation ([Koenig et al., 1999](#_ENREF_35)). Additionally, participants with elevated depressive symptoms at baseline and wave 6 were omitted from the present analysis (n=976). Following further exclusion of participants with missing or invalid data (covariates, mediator and outcome; n=705), the final analytical sample consisted of 3,809 adults (see Figure 2 for the analytical sample flow-chart).

*< Figure 2 here >*

**2.3. Measures**

***2.3.1. Depressive symptoms at wave 7 (2014/15)***

Depressive symptoms were assessed using the 8-item version of the Centre for Epidemiological Studies Depression Scale (CES-D) ([Radloff, 1977](#_ENREF_56)), which has been well validated in previous studies (Irwin et al., 1999; Lyness et al., 1997). Participants responded on a binary response scale (yes = 1; no = 0), indicating whether they had experienced a) low mood, b) low energy levels/effort doing things, c) enjoyment in life, d) sadness, e) loneliness and/or f) restless sleep during the past week. CES-D scores ranged from 0 (no depressive symptoms) to 8. The total score was dichotomised, using a well-recognised cut-off value of ≥ 4 to indicate ‘elevated depressive symptoms’ (Hamer et al., 2009; [Steffick, 2000](#_ENREF_61)). Cronbach’s alpha was 0.68.

***2.3.2. C-reactive protein and fibrinogen at wave 4 (2008/09)***

Serum concentrations of CRP (mg/L) were analysed at the Royal Victoria Infirmary laboratory in Newcastle (UK), using the N Latex CRP mono Immunoassay on the Behring Nephelometer II Analyser ([Graig et al., 2004](#_ENREF_23)). CRP values were dichotomised, using a validated threshold of (*≥* 3mg/L) to denote high levels of peripheral inflammation ([Pearson et al., 2003](#_ENREF_55)).

Fibrinogen (g/L) was analysed via the Clauss Thrombin clotting method on the Organon Teknika MDA 180 analyser (Graig et al., 2004). Fibrinogen was treated as a continuous variable, with higher values indicating greater levels of systemic inflammation.

***2.3.3. Mediator at wave 6 (2012/13)***

*Physical Activity*

Participants were asked to report how often they engaged in weekly mild, moderate and/or vigorous PA. Response options included ‘*hardly ever or never’, ‘one to three times a month’, ‘more than once a week’* and *‘once a week’*. Before answering these questions, participants were shown examples of activities on a card in order to help them to interpret PA types and intensities. Examples of mild activities included home repairs and laundry; the moderate PA category included walking at a moderate pace, gardening, dancing, cleaning the car and floor or stretching exercises; vigorous intensity activities included swimming, tennis, digging with a spade, running/jogging, aerobics/gym workout and cycling. The present analysis used the item *‘moderate/vigorous PA at least once a week’* as a measure of PA. Responses were categorised into *‘high PA’* (moderate/vigorous PA at least once a week) versus *‘low PA’* (no weekly moderate/vigorous PA) based on the NHS guidelines for PA in adults (NHS, 2013). PA scores were reversed, with a score of “0” indicating “high PA” and a score of “1” indicating “low PA”.

***2.3.4. Baseline covariates***

*Sociodemographic variables*

Sociodemographic variables included age, sex, education and socio-economic position (SEP). Education was measured by asking participants to report their highest attained educational qualification. SEP was indexed by total household wealth, the most detailed and reliable indicator of SEP in ELSA ([Marmot et al., 2002](#_ENREF_46)). The imputation of total household wealth was based on over 20 components of wealth and dept, which were either observed or computed during the CAPI. These included participants’ financial wealth (including investments and savings), the worth of any property, business assets, physical wealth (e.g. jewellery) and net of debt (including mortgage). For a more detailed description of how household wealth was calculated, see Marmot et al. (2002). In this study, total household wealth was divided into quintiles.

*Health-related variables*

Body Mass Index (BMI) was assessed during nurse visits at participants’ homes. BMI was calculated by deriving participants’ height and weight and subsequently using the standard formula (kg/m2).

Data on smoking were obtained by asking participants whether they had ever smoked. Participants who responded positively were further queried to indicate whether they still smoke. Responses were categorised into *‘no’* (never and past smoker) and *‘yes'* (current smoker).

*Metabolic markers*

High-density lipoprotein (HDL) cholesterol and triglycerides were measured via blood samples collected during nurse visits at participants’ homes. HDL cholesterol and triglycerides were measured with an Olympus 640 analyser using a direct method (no precipitation) and an enzymatic assay respectively (Graig et al., 2004). The unit of measurement for both metabolic markers was mmol/L.

**2.4. Statistical Analyses**

Baseline characteristics of the study participants are reported as means and standard deviations (SD) for continuous variables and proportions for categorical variables. Significant differences in means and proportions were tested via two-sided t-tests and chi-square tests of independence respectively.

Mediation assumes that the relationship between the independent variable (systemic low-grade inflammation) and the dependent variable (depressive symptoms) is mediated by a third variable (low PA). The total effect refers to the effect of systemic low-grade inflammation on subsequent depressive symptoms. The indirect effect represents the amount of mediation exerted through low PA on the relationship between elevated levels of inflammatory markers and subsequent depressive symptoms. The direct effect is defined as the effect of systemic low-grade inflammation on subsequent depressive symptoms while controlling for the mediator variable ([Rucker et al., 2011](#_ENREF_3)).

Mediation was assessed initially by fitting a series of multivariate regression models (see Figure 1). First, multivariate logistic regression models were employed to test the associations between inflammatory markers (fibrinogen and CRP) and subsequent depressive symptoms respectively (step 1, Figure 1). Second, the relationship between baseline levels of inflammatory markers and weekly moderate/vigorous PA was analysed (step 2, Figure 1). All analyses (step 1 and step 2) were adjusted for age, sex, education, total household wealth, BMI, smoking, cholesterol and triglyceride levels. Covariates were entered in a stepwise fashion (*Model 1:* adjusted for age and sex; *Model 2:* Model 1 & further adjusted for sociodemographic variables; *Model 3:* Model 2 & further adjusted for BMI and smoking; *Model 4:* Model 3 & further adjusted for metabolic factors). Third, further multivariate logistic regression analyses were performed to test whether low PA at wave 6 was associated with elevated depressive symptoms at follow-up (step 3, Figure 1). Analyses were independent of age, sex, baseline inflammatory markers, education, wealth, BMI, smoking, cholesterol and triglyceride levels. The results of all multivariate regression analyses are presented as adjusted odds ratios (OR) with 95% confidence intervals (CI).

Previously, it was originally assumed that a significant total effect (i.e. step 1) was a necessary condition for establishing mediation (Baron & Kenny, 1986). However, it has since been widely agreed that mediation can occur in the absence of total or direct effect (Emsley et al., 2010; Goldsmith et al., 2018; Shrout & Bolger, 2002). For example, mediation may be present if the mediated and direct effect have opposing signs, i.e. the effects under investigation are in opposite directions. Conceptually, our analysis anticipates that elevated baseline levels of proinflammatory markers will be associated with low PA at wave 6 but a higher incidence of depressive symptoms at wave 7. Therefore, the conditions for mediation are satisfied.

The significance of the indirect pathway, i.e. whether low PA explained the relationship between elevated levels of inflammatory markers and subsequent depressive symptoms was assessed using the *binary mediation* package for Stata. All mediation analyses were adjusted for age, sex, education, wealth, BMI, cholesterol and triglycerides. Bootstrapping (1000 replications) was employed as a validated method to estimate the bias-corrected confidence intervals of the indirect effect (step 4) (Mackinnon, 2008). All statistical analyses were conducted using STATA version 14.1.

***2.4.1 Sensitivity Analyses***

Three sensitivity analyses were conducted. First, further mediation analyses were performed to investigate the indirect effect of low PA on the association between systemic inflammation and elevated depressive symptoms at follow-up, when additionally adjusting for sleep problems and diet at baseline (wave 4) (Supplementary Table S1). Sleep problems and unhealthy dietary habits have both been implicated in the regulation of the innate immune system and the pathogenesis of depression (Baglioni et al., 2011; Irwin, Olmstead and Carroll, 2016; Lassale et al., 2018). Second, to further assess the temporal precedence in the relationship between systemic low-grade inflammation, PA and depressive symptoms, mediation models were re-estimated with adjustment for baseline levels of PA (Supplementary Table S2). In addition, we conducted supplementary analyses to assess whether additionally accounting for baseline levels of PA may have resulted in a potential over-adjustment. Hence, we analysed the cross-sectional association between baseline levels of systemic inflammation and PA (Supplementary Table S3), as well as the prospective association between baseline PA and PA at wave 6 (Supplementary Table S4). Subsequently, we ran a McNemar’s test to assess whether there was a significant difference between participants’ PA levels at baseline and wave 6. Stability of PA levels over time (i.e. baseline to wave 6) may indicate that accounting for baseline PA resulted in a potential over-adjustment of the model. Third, given the possibility of collider bias (also known as *endogenous selection bias*) (Elwert & Winship, 2014), further mediation models were conducted, including participants with elevated depressive symptoms at baseline and wave 6 (Supplementary Table S5).

**3. RESULTS**

**3.1. Sample Characteristics**

Baseline characteristics of the sample (N = 3,809) are presented in Table 1. The average age of participants was 64.59 ± 8.28 years. Slightly more women (51.59%) than men (48.41%) participated in this study. High baseline concentrations of CRP (*≥* 3mg/L) were found in approximately 27% of participants. The mean concentration of baseline fibrinogen was 3.28 ± 0.49 g/L. Roughly 30% of the overall sample were categorised into the low PA group at wave 6.

Excluded participants tended to be of older age (66.90 vs. 64.59 years; p<0.001), had a higher incidence of elevated baseline levels of CRP (30.26% vs. 26.26%; p=0.018), fibrinogen (3.28 vs. 3.33 mg/L; p=0.004) and cholesterol (5.53 vs. 5.65 mmol/L; p=0.002), and were less likely to have completed a university degree (16.67% vs. 21.92%; p<0.001). They were also less likely to engage in weekly moderate/vigorous PA at wave 6 (41.82% vs. 29.09%; p<0.001), and more likely to be in the lowest quintile of household wealth (17.60% vs. 11.08%; p<0.001) and current smokers (12.86% vs. 10.48%; p=0.027).

*< Table 1 here >*

**3.2. Step 1: Inflammatory Markers and Depressive Symptoms**

The results of the hierarchical multivariate logistic regression models testing the association between higher baseline levels of inflammatory markers and depressive symptoms at a six years follow-up are presented in Table 2.

*< Table 2 here >*

*C-reactive protein.* Compared to participants with normal CRP levels at baseline, those with high CRP were not significantly more likely to subsequently develop elevated depressive symptoms at follow-up (Model 1: OR: 1.17; 95% CI, 0.85-1.61).

*Fibrinogen.* Similarly, higher fibrinogen concentrations at baseline were unrelated to elevated depressive symptoms at follow-up, relative to lower baseline levels of fibrinogen (Model 1: OR: 1.14; 95% CI, 0.84-1.54).

**3.3. Step 2: Inflammatory Markers and Physical Activity**

The fully adjusted multivariate logistic regression models for the associations between inflammatory markers at baseline and low PA at wave 6 are presented in Table 3.

*< Table 3 here >*

*C-Reactive Protein.* Participants with elevated levels of CRP at baseline had significantly higher odds of low PA at wave 6 (OR: 1.25; 95% CI, 1.05-1.48), relative to those with normal CRP levels.

*Fibrinogen.*Participants with higher levels of fibrinogen also had significantly greater odds of low PA (OR: 1.18; 95% CI, 1.05-1.39), compared to those with lower levels of fibrinogen at baseline.

**3.4. Step 3: Physical Activity and Depressive Symptoms**

Table 4 displays the fully adjusted logistic regression models for the associations between low PA at wave 6 and depressive symptoms at wave 7.

*< Table 4 here >*

*Low PA.* Participants who did not engage in weekly moderate/vigorous PA were significantly more likely to report elevated depressive symptoms at follow-up, compared to physically active participants (model additionally adjusted for baseline CRP: OR: 1.59; 95% CI, 1.15-2.19; model additionally adjusted for baseline fibrinogen: OR: 1.59; 95% CI, 1.15-2.19).

**3.5. Step 4: Mediation Analyses**

Binary mediation analysis was performed to test whether low PA mediated the relationship between systemic low-grade inflammation and subsequently elevated depressive symptoms (see Table 5).

*C-reactive protein.* Low PA significantly mediated the relationship between high CRP and subsequently elevated depressive symptoms (indirect effect: β = 0.010; 95% CI, 0.001-0.022), explaining a total of 36.71% of this association. Neither a significant total (β = 0.019, 95% CI, -0.096-0.112) nor direct effect (β = 0.009, 95% CI, -0.101-0.103) was found in this model.

*Fibrinogen*. Low PA also acted as a partial mediator of the relationship between higher levels of fibrinogen and elevated depressive symptoms at follow-up, explaining a total of 33.27% (indirect effect: β = 0.005; 95% CI, 0.0003-0.0140). No significant total (β = 0.016, 95% CI, -0.069-0.109) or direct effect (β = 0.011, 95% CI, -0.076-0.102) was found in this model.

*< Table 5 here >*

Figure 3 provides a summary of the mediation analyses conducted, using graphical illustrations.

*< Figure 3 here >*

**3.6. Sensitivity Analyses**

Our first sensitivity analysis revealed similar results to those in our main analyses; low PA at wave 6 remained a significant mediator after additionally adjusting for sleep problems and dietary habits at baseline (wave 4) (Supplementary Table S1).

Our second sensitivity analysis indicated that additionally adjusting for baseline levels of PA resulted in a non-significant indirect effect of low PA (wave 6) on the relationship between baseline levels of systemic low-grade inflammation and depressive symptoms at wave 7 (Supplementary Table S2). Interestingly, our supplementary analyses suggest that this attenuation to statistical non-significance may be due to over-adjustment. Higher levels of systemic inflammation were cross-sectionally associated with low PA levels at baseline (Supplementary Table S3). Moreover, PA levels at baseline were significantly associated with PA levels four years later (wave 6) (Supplementary Tables S4). Furthermore, the results of the McNemar’s test showed that there was no significant difference between participants’ PA levels at baseline and wave 6 (p=0.64). Therefore, additionally controlling for baseline levels of PA may have resulted in over-adjustment.

Lastly, our third sensitivity analyses revealed that low PA at wave 6 continued to be a significant mediator after including participants with elevated depressive symptoms at baseline and wave 6 (Supplementary Table S5).

**4. DISCUSSION**

The present study investigated whether low PA mediated the relationship between higher serum levels of proinflammatory markers (CRP and fibrinogen) and subsequently elevated depressive symptoms in non-depressed English community-dwelling older adults. The present findings suggest that neither elevated baseline levels of CRP or fibrinogen are significantly associated with elevated depressive symptoms six years later. This is in line with the results of two previous longitudinal studies ([Au et al., 2014](#_ENREF_5); [Stewart et al., 2009](#_ENREF_62)). For example, Au and colleagues (2014) assessed the prospective relationship between CRP and subsequent depressive symptoms (CES-D ≥ 4) in 3,397 ELSA participants. The results indicated no evidence for a significant association between high baseline levels of CRP and elevated depressive symptoms at a six-year follow-up, after adjusting for metabolic and health-related variables.

Interestingly, earlier studies repeatedly showed significant effects of TNF-α, IL-6, or IL-8 on subsequent depressive symptoms in adult populations (≥ 50 years) ([Forti et al., 2010](#_ENREF_17); [Milaneschi et al., 2009](#_ENREF_51)), but not for CRP or fibrinogen (Baune et al., 2012; Bremmer et al., 2008). For instance, Baune et al. (2012) found that both IL-6 and IL-8, but not CRP, predicted the onset of depressive symptomatology two years later in a sample of 1,037 Australian older adults. Similarly, Bremmer et al. (2008) reported that elevated baseline levels of IL-6, but not CRP or fibrinogen, were significantly associated with the onset of major depression 12 months later in 1,285 Dutch adults, using the 20-item version of the CES-D and clinical interviews to assess depressive symptomatology. Inconsistencies in findings may also be due to the lack of regard for persistency of repeated exposure to systemic low-grade inflammation (Bell et al., 2017; Kivimäki et al., 2014). A recent epidemiological investigation examined whether repeated exposure to elevated levels of CRP (*≥* 3mg/L) increased the risk of developing future depressive symptoms, using prospective data from a population-based sample of adults aged 50 and older (Bell et al., 2017). The results indicated that participants with elevated levels of CRP on only one occasion were not significantly more likely to develop depressive symptoms at follow-up compared to normal levels of CRP. However, participants with increased plasma CRP on multiple occasions had significantly higher odds of developing depressive symptoms at follow-up, relative to those without elevated levels of systemic inflammation.

To the best of our knowledge, this is the first study to demonstrate that weekly moderate/vigorous PA represents a significant partial mediator of the relationship between systemic low-grade inflammation and subsequently elevated depressive symptoms. This coincides with previous literature suggesting that PA has a central role in both mental health and the regulation of peripheral inflammatory processes. Regular PA has consistently been associated with a lower risk of developing depressive symptoms in people aged 50 years and older ([Chang et al., 2017](#_ENREF_13); [Hamer et al., 2009](#_ENREF_24); [Joshi et al., 2016](#_ENREF_33); [Ku et al., 2017](#_ENREF_37); [Lindwall et al., 2011](#_ENREF_40); [Pasco et al., 2011](#_ENREF_54); [Roh et al., 2015](#_ENREF_59); [Yoshida et al., 2015](#_ENREF_67)). Moreover, previous studies suggested reciprocal links between systemic inflammation and PA ([Abramson & Vaccarino, 2002](#_ENREF_1); [Geffken et al., 2001](#_ENREF_21); [Hamer & Steptoe, 2007](#_ENREF_25); [Kasapis & Thompson, 2005](#_ENREF_34)). In the present study, low PA was significantly associated with elevated depressive symptomatology two years later. Moreover, elevated baseline levels of CRP and fibrinogen were significantly associated with low PA four years later. Hence, our results suggest that people with elevated levels of systemic inflammation may be less likely to engage in weekly moderate or vigorous PA, which may increase the risk of developing elevated levels of depressive symptoms. Importantly, PA explained approximately one-third of the total effect of systemic inflammation on subsequently elevated depressive symptoms. Future research is needed to identify other intermediate mechanisms explaining this relationship.

Interestingly, our second sensitivity analysis revealed that additionally accounting for baseline levels of PA changed the indirect effect of low PA (wave 6) on the association between baseline levels of systemic inflammation and elevated depressive symptoms at wave 7 to statistical non-significance. However, our supplementary analyses indicated that there was no significant difference between participants’ PA levels at baseline and wave 6. This suggests that PA levels were likely to be stable over time in the present sample. Hence, additionally adjusting for baseline levels of PA may have resulted in over-adjustment, potentially explaining the statistically non-significant indirect effect of low PA. In sum, our findings contribute to the notion that regular PA may not only be a valuable tool in preventing subsequent depressive symptoms but may also have a potential anti-inflammatory role.

**4.1. Biological Mechanisms**

The exact biological mechanisms linking inflammatory processes to depressive symptoms through the effects of PA are not entirely known. However, it has been suggested that systemic low-grade inflammation and PA act on the same effector systems of the body: the hypothalamic pituitary adrenal axis (HPA-axis) and the sympathetic nervous system (SNS) (Cotman, Berchtold & Christie, 2007; [Irwin, Olmstead, & Carroll, 2016](#_ENREF_26)). For example, elevated levels of proinflammatory cytokines have repeatedly been related to the hyperactivation of the HPA-axis, hypersecretion of stress hormones (e.g. cortisol), impaired serotonin and dopamine production, as well as the initiation of oxidative stress (Irwin et al., 2016). Moreover, regular PA has been found to increase the body’s serotonin synthesis, improve the noradrenergic neurotransmission, trigger the release of endorphins, and reduce chronically increased SNS activity ([Cotman et al., 2007](#_ENREF_14); [Meeusen & De Meirleir, 1995](#_ENREF_49)). PA has also been linked to increases in muscle-derived IL-6, which, in this context, can act as an anti-inflammatory myokine by inhibiting TNF-α (Pedersen, 2017). More importantly, these shared biological mechanisms have been found to play a major role in the pathophysiology of depression neurotransmitters ([Franceschi & Campisi, 2014](#_ENREF_18)).

**4.2. Strengths and Limitations**

This study has a number of strengths, including the large sample size, the prospective study design, as well as the statistical methods employed to examine potential mediating mechanisms of low PA in the temporal relationships between inflammatory markers and subsequent depressive symptoms. Moreover, as far as we are aware, this is the first study to date which has explored this mediating mechanism in a large representative sample of English older adults.

However, findings need to be interpreted in light of several limitations. First, the measure of PA was based on self-report rather than objective methods, bearing the risk of self-report bias. Second, our PA measure was based on a single-item question focusing on the frequency rather than the duration of weekly moderate or vigorous PA. Third, PA ratings may have been influenced by psychosocial factors, such as mood or socioeconomic circumstances. To address this issue, participants with elevated depressive symptoms at baseline and four years post-baseline were excluded from the analytical sample. Moreover, all analyses were adjusted for education and wealth. Lastly, the generalisability of the present findings is limited to older Caucasian adults only, since the ELSA population is largely comprised of white participants.

**4.3. Implications**

Taken together, the present findings suggest that low PA is a significant partial mediator of the relationship between systemic low-grade inflammation and subsequent depressive symptomatology – lending support to the second hypothesis of this study. Hence, systemic low-grade inflammation is likely to represent an indirect risk factor for the development of new depressive symptoms through the mechanism of low PA. This finding may have implications for clinicians and healthcare providers since PA represents a modifiable health behaviour. As such, targeting systemic low-grade inflammation via tailored interventions (e.g. exercise) may be an effective way to treat adults with elevated depressive symptoms.

Evidence suggests that even low levels of physical exercise (e.g. walking less than 150 minutes per week) significantly reduce the risk of developing future depressive symptoms by 8% to 63% ([Ball et al., 2009](#_ENREF_7); [Brown, Ford et al., 2005](#_ENREF_12); [Jonsdottir et al., 2010](#_ENREF_32)). Accordingly, regular PA (three sessions per week; 45-60 minutes over a period of 10 to 14 weeks) has been included in the clinical guidelines of the National Institute for Health and Care Excellence (NICE) for the treatment of mild to moderate depression (NICE, 2009). Targeting insufficient PA may alleviate not only depressive symptomatology but also reduce chronically elevated levels of peripheral inflammation – thus diminishing the risk of developing other inflammatory diseases (e.g. cardiovascular diseases).

**4.4. Conclusion**

In conclusion, this study revealed that low PA significantly mediated the relationship between systemic low-grade inflammation and subsequently elevated depressive symptoms in community-dwelling older adults.

**Competing Interests**

All the authors declare no financial relationships with any organisations that might have an interest in the submitted work; no other relationships or activities that could appear to have influenced the proposed work.

**Author Contributions**

Conception, design and planning of the study by PF & DC; analysis and interpretation of data by PF & DC, writing, revision and contribution to the manuscript by all authors.

**Ethical Approval**

The National Research Ethics Service (London Multicentre Research Ethics Committee (MREC/01/2/91) (http://www.nres.npsa.nhs.uk) granted ethical approval. All participants provided informed consent prior to the study.

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**Data Sharing**

The data are linked with the UK Data Archive and freely available through the UK data services and can be accessed here: https://discover.ukdataservice.ac.uk. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes on Aging or other funding bodies mentioned above. The developers and funders of ELSA do not bear any responsibility for the analyses or interpretations presented here.

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**FIGURE TITLES**

Figure 1. Hypothesised mediation model

Figure 2. Participant flow-chart ELSA cohort

Figure 3. Mediation models of the relationship between elevated baseline levels

of inflammatory biomarkers and subsequent depressive symptoms mediated by low physical activity

**Figure 1**: Hypothesised mediation model

Inflammatory biomarkers

a (step 1)

Depressive symptoms

d

Low physical activity

c (step 3)

b (step 2)

Hypothesised mediation models: a = direct effect; b\*c = indirect effect; d = total effect; b\*c + d = sum of indirect and direct effect

Step 1 = Inflammatory markers in relation to depressive symptoms

Step 2 = Inflammatory markers in relation to mediator variable (PA)

Step 3 = Mediator variables in relation to depressive symptoms

**Figure 2**: Analytical Sample Flow-Chart

Missing blood samples (wave 4, 2008/09)

(n=3,985)

Core participants at baseline (wave 4, 2008/09)

**N = 9,886**

**Main analytical sample**

**N = 3,809**

Exclusion of participants with missing outcome data (n=648)

Core participants with no missing exposure

**N = 5,901**

Exclusion of individuals with depressive symptoms at wave 4 (2008/09) and wave 6 (2012/13)

Wave 4 (n=674)

Wave 6 (n=302)

Exclusion of participants with CRP ≥ 10mg/L (wave 4, 2008/09)

(n=411)

Missing covariates:

Age (n=0)

Sex (n=0)

Education (n=1)

Wealth Status (n=16)

BMI (n=27)

Smoking (n=3)

Cholesterol (n=0)

Triglycerides (n=0)

Exclusion of participants with missing mediator data (n=10)

**Figure 3**: Mediation models of the relationship between elevated baseline levels of inflammatory biomarkers and subsequent depressive symptoms mediated by low physical activity

**Model 1:** Effect of elevated levels of CRP

High CRP

b = 1.25\*

c = 1.59\*\*

a = 1.07

Depressive symptoms

Low PA

on subsequent depressive symptoms

through low PA (b\*c = 0.006; 95% CI,

0.001-0.014)

**Model 2:** Effect of elevated levels of

Fibrinogen

b = 1. 18\*

c = 1.59\*\*

a = 1.05

Depressive symptoms

Low PA

fibrinogen on subsequent depressive

symptoms through physical inactivity

(b\*c = 0. 005; 95% CI, 0. 0003-0.0140)

**Note.** \* = p < 0.05; \*\* = p < 0.005; numbers = Odds ratio (OR)

**TABLE TITLES**

Table 1. Descriptive characteristics of the sample at baseline (2008/09), follow-

up (2014/15) and wave 6 (2012/13) (N = 3,809)

Table 2. Association between high CRP/fibrinogen (2008/09) and depressive

symptoms at follow-up (2014/15) (N = 3,809)

Table 3. Associations between baseline inflammatory markers (2008/09) and

low physical activity at wave 6 (2012/13) (N = 3,809)

Table 4. Associations between low PA at wave 6 (2012/13) and depressive

symptoms at follow-up (2014/15) (N = 3,809)

Table 5. Mediation of the association between baseline inflammatory markers

(wave 4, 2008/09) and depressive symptoms at follow-up (wave 7, 2014/15) through low physical activity at wave 6 (2012/13) (N = 3,809)

|  |  |  |
| --- | --- | --- |
| Age (mean ± SD) |  | 64.59 ± 8.28 |
| Sex *Men* *Women* |  | 1,844 (48.41%)1,965 (51.59%) |
| Elevated depressive symptoms at wave 7 (CES-D *≥ 4*) |  | 186 (4.88%) |
| C-reactive protein (mg/L) *Elevated* *Normal* |  | 1,014 (26.62%)2,795 (73.38%) |
| Fibrinogen (g/L) |  | 3.28 ± 0.49 |
| Moderate or vigorous physical activity (wave 6)  *Yes* *No* |  | 2,701 (70.91%)1,108 (29.09%) |
| Education  *University degree/equivalent* *Less than university* *No qualification* |  | 785 (21.60%)2,189 (56.48)835 (21.92%) |

**Table 1**:Descriptive characteristics of the sample at baseline (2008/09), follow-up (2014/15) and wave 6 (2012/13) (N = 3,809)

**Note**: Number (percentage) and mean (standard deviation)

**Table 1 (*continued):*** Descriptive characteristics of the sample at baseline (2008/09), follow-up (2014/15) and wave 6 (2012/13) (N = 3,809)

|  |  |  |
| --- | --- | --- |
| Wealth (quintiles) *Lowest quintile*  *2nd quintile*  *3rd quintile*  *4th quintile*  *Highest* quintile BMI *Underweight* *Normal* *Overweight* *Obese*Current smoker  *Yes*  *No*Cholesterol (mmol/L)Triglyceride (mmol/L) |  | 422 (11.08%)641 (16.83%)779 (20.45%)879 (23.08%)1,088 (28.56%)21 (0.55%)1,038 (27.25)1,706 (44.79%)1,044 (27.41%)399 (10.48%)3,410 (89.52%)5.65 ± 1.171.70 ± 1.00 |

**Note**: Number (percentage) and mean (standard deviation

**Table 2:** Association between high CRP/fibrinogen (2008/09) and depressive symptoms at follow-up (2014/15) N = 3,809

|  |  |  |
| --- | --- | --- |
| Model  |  | Elevated depressive symptoms (CES-D ≥ 4)  |
|  |  | Adjusted odds ratio (95% CI) | *P-value* |
| Model 1 *CRP* *Fibrinogen* |  | 1.17 (0.85, 1.61)1.14 (0.84, 1.54) | *0.345**0.407* |
| Model 2  *CRP* *Fibrinogen* |  | 1.10 (0.79, 1.52)1.07 (0.79, 1.46) | *0.573**0.658* |
| Model 3 *CRP* *Fibrinogen* |  | 1.08 (0.77, 1.52)1.04 (0.76, 1.42) | *0.639**0.818* |
| Model 4 *CRP*  *Fibrinogen* |  | 1.07 (0.76, 1.51)1.05 (0.76, 1.44) | *0.687**0.776* |

All analyses were adjusted for age, sex, education, wealth status, BMI, smoking, cholesterol and triglycerides.

**Table 3:** Associations between baseline inflammatory markers (2008/09) and low physical activity at wave 6 (2012/13)

|  |  |
| --- | --- |
| Inflammatory markers | Low Physical ActivityN = 3,809 |
|  | Odds Ratio(95% CI) | *P-value* |
| CRP (*≥* 3 mg/L) | 1.25 (1.05, 1.48) | *0.010* |
| Fibrinogen (g/L) | 1.18 (1.01, 1.39) | *0.035* |

All analyses were adjusted for age, sex, education, wealth status, BMI, smoking, cholesterol and triglycerides.

**Table 4:** Associations between low PA at wave 6 (2012/13) and depressive symptoms at follow-up (2014/15)

|  |  |  |
| --- | --- | --- |
| Mediators |  | Elevated depressive symptoms (CES-D ≥ 4)N = 3,809 |
|  |  | Odds ratio(95% CI) | *P-value* |
| Low physical activity Model 1 Model 2 |  | 1.59 (1.15, 2.19)1.59 (1.15, 2.19) | *0.005**0.005* |

Model 1: adjusted for age, sex, education, wealth status, BMI, smoking, cholesterol, triglycerides & baseline CRP; Model 2: adjusted for age, sex, education, wealth status, BMI, smoking, cholesterol, triglycerides & baseline fibrinogen.

**Table 5:** Mediation of the association between baseline inflammatory markers (wave 4, 2008/09) and depressive symptoms at follow-up (wave 7, 2014/15) through low physical activity at wave 6 (2012/13) (N = 3,809)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Independent variable (wave 4) | Mediator(wave 6) | Outcome(wave 7) | Total indirect effect  | Total effect  | Total direct effect  | Total effect mediated |
|  |  |  |  *Coefficient*(Bc CI\*) | *Coefficient*(Bc CI) | *Coefficient* (Bc CI) | *%* |
| CRP | Low PA | Depressive symptoms | 0.010 (0.001, 0.022) | 0.019 (-0.096-0.112) | 0.009(-0.101, 0.103) | 36.71% |
| Fibrinogen | Low PA | Depressive symptoms | 0.005(0.0003, 0.0140) | 0.016(-0.069, 0.109) | 0.011(-0.076, 0.102) | 33.27% |

All analyses adjusted for age, sex, sociodemographic variables (education, wealth status), BMI, smoking, metabolic factors (cholesterol and triglycerides).

\* Bc CI = Bias corrected 95% confidence intervals

**SUPPLEMENTARY ANALYSES**

Table S1. Mediation of the association between baseline inflammatory markers (wave 4, 2008/09) and depressive symptoms at follow-up (wave 7, 2014/15) through low physical activity at wave 6 (2012/13), additionally adjusted for sleep problems and dietary habits (N = 3,160)

Table S2.Mediation of the association between baseline inflammatory markers

(wave 4, 2008/09) and depressive symptoms at follow-up (wave 7,

2014/15) through low physical activity at wave 6 (2012/13) (N = 3,809),

additionally adjusted for baseline PA levels

Table S3. Cross-sectional association between levels of inflammatory markers and low physical activity at baseline (wave 4, 2008/2009)

Table S4. Prospective association between baseline low physical activity (wave 4, 2008/09) and low physical activity at wave 6 (2012/13)

Table S5. Mediation of the association between baseline inflammatory markers

(wave 4, 2008/09) and depressive symptoms at follow-up (wave 7,

2014/15) through low physical activity at wave 6 (2012/13), including

people with depressive symptoms at wave 4 and wave 6 (N = 4,435)

**Sensitivity Analysis (I)**

For the purpose of our first sensitivity analysis, the CES-D item on “restless sleep” was omitted to avoid shared variance when additionally adjusting for sleep problems. A validated threshold of ≥ 3 was used, representing “elevated depressive symptoms” (Steffick, 2000; White et al., 2016).

 Moreover, we included *sleep problems* and *dietary habits* as additional covariates. These health-related covariates were measured as follows:

*Sleep problems*

Sleep problems were measured via three items of the Jenkins Sleep Problems Scale, referring to the most common sleep complaints (difficulty falling asleep, difficulty staying asleep, and morning tiredness) (Jenkins et al., 1988). Items were rated on a four-point Likert scale ranging from 1 to 4 (*1 = not during the last month; 2 = less than once a week; 3 = once or twice a week; 4 = three or more times a week*). An average summary score was created and subsequently divided into quartiles. Scores in the fourth quartile were classified as disturbed sleep (Kumrai et al., 2008). For the present study, the variable sleep disturbance was dichotomised. Responses were categorised into ‘no sleep problems’ for scores in the lower three quartiles and ‘sleep problems’ for scores in the highest quartile.

*Dietary habits*

Dietary habits were measured by assessing participants’ daily fruit and vegetable intake. Participants were asked to indicate whether they consumed at least 5 portions of fruit/vegetables per day. Responses were categorised into *‘yes’* (≥ 5 portions of fruit/vegetables per day) and *‘no’* (< 5 portions of fruit/vegetables per day

**Table S1:** Mediation of the association between baseline inflammatory markers (wave 4, 2008/09) and depressive symptoms at follow-up (wave 7, 2014/15) through low physical activity at wave 6 (2012/13), additionally adjusted for sleep problems and dietary habits (N = 3,160)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Independent variable (wave 4) | Mediator(wave 6) | Outcome(wave 7) | Total indirect effect  | Total effect  | Total direct effect  | Total effect mediated |
|  |  |  |  *Coefficient*(Bc CI\*) | *Coefficient*(Bc CI) | *Coefficient* (Bc CI) | *%* |
| CRP | Low PA | Depressive symptoms(CES-D 7-item version) a | 0.007(0.001, 0.016) | 0.011(-0.083, 0.092) | 0.004(-0.092, 0.086) | 65.83% |
| Fibrinogen | Low PA | Depressive symptoms(CES-D 7-item version) a | 0.007(0.001, 0.016) | 0.020(-0.066, 0.112) | 0.013(-0.073, 0.103) | 34.03% |

All analyses adjusted for age, sex, sociodemographic variables (education, wealth status), BMI, dietary habits, sleep problems, smoking, metabolic factors (cholesterol and triglycerides).

\* Bc CI = Bias corrected 95% confidence intervals

a Elevated depressive symptoms = CES-D ≥ 3; CES-D item *on ‘restless sleep’* excluded to avoid shared variance

**Sensitivity Analysis (II)**

**Table S2:** Mediation of the association between baseline inflammatory markers (wave 4, 2008/09) and depressive symptoms at follow-up (wave 7, 2014/15) through low physical activity at wave 6 (2012/13) (N = 3,809), additionally adjusted for baseline PA levels

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Independent variable (wave 4) | Mediator(wave 6) | Outcome(wave 7) | Total indirect effect  | Total effect  | Total direct effect  | Total effect mediated |
|  |  |  |  *Coefficient*(Bc CI\*) | *Coefficient*(Bc CI) | *Coefficient* (Bc CI) | *%* |
| CRP | Low PA | Depressive symptoms | 0.004 (-0.0004, 0.011) | 0.006 (-0.081-0.091) | 0.010(-0.078, 0.096) | - |
| Fibrinogen | Low PA | Depressive symptoms | 0.003(-0.0006, 0.010) | 0.008(-0.076, 0.096) | 0.011(-0.072, 0.099) | - |

All analyses adjusted for age, sex, sociodemographic variables (education, wealth status), BMI, smoking, metabolic factors (cholesterol and triglycerides), and baseline PA levels.

\* Bc CI = Bias corrected 95% confidence intervals

**Table S3:** Cross-sectional association between inflammatory markers and low physical activity at baseline (wave 4, 2008/2009)

|  |  |
| --- | --- |
| Inflammatory markers | Low Physical ActivityN = 3,809 |
|  | Odds Ratio(95% CI) | *P-value* |
| CRP (*≥* 3 mg/L) | 1.37 (1.16, 1.62) | *< 0.001* |
| Fibrinogen (g/L) | *1.32 (1.12, 1.54)* | *0.001* |

All analyses were adjusted for age, sex, baseline PA, education, wealth status, BMI, smoking, cholesterol and triglycerides.

**Table S4:** Prospective association between baseline low physical activity (wave 4, 2008/09) and low physical activity at wave 6 (2012/13)

|  |  |
| --- | --- |
|  | Low Physical Activity (wave 6)N = 3,809 |
|  | Odds Ratio(95% CI) | *P-value* |
| Low Physical Activity (baseline) | 3.01 (2.56, 3.54) | *< 0.001* |

All analyses were adjusted for age, sex, education, wealth status, BMI, smoking, cholesterol and triglycerides.

**Sensitivity Analysis (III)**

**Table S5:** Mediation of the association between baseline inflammatory markers (wave 4, 2008/09) and depressive symptoms at follow-up (wave 7, 2014/15) through low physical activity at wave 6 (2012/13), including people with depressive symptoms at wave 4 and wave 6 (N = 4,435)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Independent variable (wave 4) | Mediator(wave 6) | Outcome(wave 7) | Total indirect effect  | Total effect  | Total direct effect  | Total effect mediated |
|  |  |  |  *Coefficient*(Bc CI\*) | *Coefficient*(Bc CI) | *Coefficient* (Bc CI) | *%* |
| CRP | Low PA | Depressive symptoms | 0.009(0.002, 0.017) | 0.037(-0.021, 0.089) | 0.028(-0.027, 0.079) | 24.16% |
| Fibrinogen | Low PA | Depressive symptoms | 0.010(0.003, 0.017) | 0.018(-0.036, 0.075) | 0.008(-0.045, 0.067) | 54.85% |

All analyses adjusted for age, sex, sociodemographic variables (education, wealth status), BMI, smoking, metabolic factors (cholesterol and triglycerides).

\* Bc CI= Bias corrected 95% confidence intervals

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