DOI:
10.2337/dc19-0813

Document Version
Peer reviewed version

Title:

The prospective association between inflammation and depression in type 2 diabetes stratified by sex

Running head:

Inflammation and depression in type 2 diabetes

Authors:

Calum D. Moulton MRCPsych[1], John C. Pickup D. Phil[2], Anna S. Rokakis MSc[1], Stephanie A. Amiel MD[2], Khalida Ismail PhD[1*]*, Daniel Stahl PhD[3]*

Affiliations:

1. Department of Psychological Medicine, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, SE5 9RJ, UK
2. Division of Diabetes and Nutritional Sciences, Faculty of Life Sciences and Medicine, King's College London, London SE1 1UL and SE5 9RJ, UK
3. Department of Biostatistics and Health Informatics, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, SE5 8AB, UK

Corresponding author:

Dr Calum Moulton, Department of Psychological Medicine, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, SE5 9RJ, UK
Email: calum.moulton@kcl.ac.uk; tel.: 02078485657

Word count: 3360

Number of figures: 1

Number of tables: 3

*Daniel Stahl and Khalida Ismail are co-senior authors.

Abstract

Background

We tested whether inflammation is associated with worsening depressive symptoms in type 2 diabetes and examined whether sex moderated this association.

Research Design and Methods

In a prospective cohort study of people with newly diagnosed type 2 diabetes, we measured depressive symptoms over 2-year follow-up using the Patient Health Questionnaire-9 (PHQ-9). The independent variable was a composite inflammation burden score at diagnosis of diabetes, derived from high-sensitivity C-reactive protein, white cell count, interleukin (IL)-1β, IL-1 receptor antagonist, monocyte chemotactic protein-1 and vascular endothelial growth factor concentrations. General linear models assessed i) the association between overall inflammation burden and estimated marginal mean PHQ-9 score (natural log (ln)-transformed) at 2 years; and ii) the interaction between elevated (above-median) inflammation burden and sex on ln-PHQ-9 score. Models were adjusted for age, ethnicity, body mass index, blood pressure, cholesterol, HbA_{1c}, antidepressants, anti-inflammatory medications and baseline ln-PHQ-9 score.

Results

Of 1174 people with complete inflammation data, mean age was 56.7 (11.0) years, 46.1% were of non-white ethnicity and 44.1% female. After full adjustment, inflammation burden was not associated with worsening ln-PHQ-9 score (p=0.65). However, there was a significant interaction between sex and inflammation on 2-year ln-PHQ-9 score (β=0.32, p=0.005), showing that the difference by inflammation burden in females was 0.32 larger than in males. In post-hoc comparisons, ln-PHQ-9 score was higher in females than males with elevated inflammation (p=0.003) but not with low inflammation burden (p=0.34).

Conclusion

In type 2 diabetes, female sex confers specific vulnerability to the effects of inflammation on depressive symptoms.

Introduction

Depressive symptoms are reported by 10-30% of people with type 2 diabetes – twice as often as in the general population – and are associated with 1.5-3-fold increased frequency of diabetes complications and premature mortality.[1–3] In a psychological model of the association, depressive symptoms could be formulated as negative cognitions and emotions to the burden of a chronic disease, in turn leading to poor diabetes outcomes through reduced diabetes self-care.[4] This model, however, provides limited opportunities to understand disease mechanisms and improve outcomes. For example, in cohort studies, depressive symptoms are not always associated with worsening in glycaemic control over time,[5] suggesting that depression does not consistently lead to worsening diabetes self-care. In neuroimaging research, the brain changes typical of depression – such as hippocampal atrophy – mirror those seen in type 2 diabetes.[6,7] Moreover, the link is bidirectional: whereas type 2 diabetes is associated with increased risk of incident depressive symptoms, the converse association is even stronger.[8]

A unifying explanation for these observations is that depressive symptoms and type 2 diabetes are not distinct conditions, but rather may be linked by shared disease mechanisms, such as elevated inflammation.[9] In support, pro-inflammatory cytokines and elevated acute phase proteins are associated with onset of depression and type 2 diabetes, respectively,[10,11] whilst some anti-inflammatory therapies have demonstrated benefit in the two conditions separately.[12,13] To date, research testing inflammation as a link between depression and type 2 diabetes has been scarce. In cross-sectional studies in people with type 2 diabetes, those with comorbid depressive symptoms had higher concentrations of acute phase proteins and cytokines, even after adjustment for confounders such as body mass index (BMI), smoking, and age.[14–16] However, no prospective cohort study has tested the association between inflammation and the course of depressive symptoms over time in people with type 2 diabetes, such that potential causality cannot yet be inferred.

Like depressive symptoms, female sex is associated with increased risk of cardiovascular complications in people with type 2 diabetes, even accounting for sex differences in other major cardiovascular risk factors.[17] Depressive symptoms are also nearly twice as prevalent in females than males with established type 2 diabetes.[18] In depression research, female sex appears to increase vulnerability to the effects of inflammation on the brain,[19] and anti-

inflammatory diabetes treatments may improve depressive symptoms more significantly in females.[20] Collectively, this suggests that inflammation could lead predominantly to depressive symptoms in females with type 2 diabetes and further could provide an explanation for the poor overall prognosis of female diabetes.

Using an incident type 2 diabetes cohort, we therefore aimed to test the hypotheses that elevated inflammation is associated with worsening depressive symptoms in people with type 2 diabetes and secondly that this association is stronger in females than in males.

Research Design and Methods

Setting and study design: The South London Diabetes (SOUL-D) study is a population-based prospective cohort of people with newly diagnosed type 2 diabetes recruited within six months of diagnosis and followed over 2 years.[21] The study was set in the inner-city boroughs of Lambeth, Southwark, and Lewisham in South London, which collectively have approximately 0.75 million residents from diverse ethnic- and socioeconomic backgrounds. All General Practice (GP) surgeries in these boroughs were invited to participate. Local protocols for diagnosis of type 2 diabetes followed World Health Organisation criteria.[22] Ethical approval was granted by the King's College Hospital Research Ethics Committee (reference 08/H0808/1) and by Lambeth, Southwark, and Lewisham Primary Care Trusts (reference RDLSLB 410). All participants gave written informed consent, including for access to their medical records. Details of inclusion criteria and recruitment timetable have been published.[21]

Outcomes: The outcome at 2-year follow-up was depressive symptoms, as measured by total Patient Health Questionnaire-9 (PHQ-9) score. Due to positive skew, we natural log-transformed PHQ-9 scores. As discussed previously, the PHQ-9 is a 9-item questionnaire based on the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) diagnostic criteria for clinical depression, which has been validated for use in people with type 2 diabetes.[23] Missing PHQ-9 data (where <20% of responses were missing) were imputed using case mean substitution, discussed in detail previously.[5]

Independent variable: The independent variable was a composite measure of inflammation burden based on six markers of inflammation: high-sensitivity C-reactive protein (hs-CRP),

interleukin-1β (IL-1β), IL-1 receptor antagonist (IL-1RA), monocyte chemotactic protein-1 (MCP-1), vascular endothelial growth factor (VEGF), and white blood cell count (WBC). As well as measuring a range of inflammatory pathways – including acute phase-, cytokine- and chemokine responses – these markers were selected because of their positive individual associations with depressive symptoms in the baseline SOUL-D cohort.[14] As in previous research,[24] a composite inflammation burden score was calculated by transforming each measure into a z-distribution, whose mean is 0 and standard deviation is 1, before averaging the individual biomarker z-scores. This approach minimises multiple testing and reduces the influence of the biological variability of each measure. Serum hs-CRP was measured using an Advia 2400 analyzer (Siemens Diagnostics, Frimley, UK) with detection limit 0.1 mg/L; WBC was measured using an Advia 2120 analyzer (Siemens Diagnostics); and IL-1β, IL-1RA, VEGF and MCP-1 were measured from serum samples centrifuged from venous blood samples taken after an overnight fast, stored between −40 and −80°C using cytokine-array biochip kits (Randox, Belfast, UK) and analyzed using the Randox Evidence Investigator; the inter- and intra-assay coefficients of variation for all analytes measured using these kits are <15 and <10%, respectively.

Confounders: We considered the following potential confounders *a priori*: age, ethnicity (white versus non-white), BMI, blood pressure, smoking status, HbA_{1c} (measured by affinity chromatography [Primus Ultra2, Kansas City, USA]), serum total cholesterol (measured using Siemens Advia 2400 Analyzer, detection limit 0.01 mmol/L), prescription of any anti-inflammatory medications (systemic steroids, nonsteroidal anti-inflammatory drugs [NSAIDs]) and antidepressant medications.

Statistical analyses

Baseline analysis: Data were analysed using IBM SPSS version 25.0. We compared the following baseline characteristics of the cohort stratified by low- or elevated inflammation burden at baseline: sociodemographic variables (age, self-report ethnicity [African/Caribbean, white, South Asian/other]); baseline biomedical variables (smoking status, total cholesterol, BMI, HbA_{1c}, inflammatory markers, anti-inflammatory medications, antidepressant medications, blood pressure, inflammatory marker concentrations); and baseline PHQ-9 score. For continuous variables, we used Student's t-test for normally distributed data and the Mann-Whitney U test for skewed data, and for categorical variables

we used chi-square tests. Results were presented as mean (SD) or median (interquartile range) for skewed data, unless otherwise stated. Compared to those included in the analysis, we assessed the characteristics of people missing inflammation data at baseline.

Prospective association between inflammation burden and depressive symptoms: the prospective analysis comprised unadjusted- and adjusted general linear models in which the outcome was 2-year PHQ-9 score (natural log-transformed). We firstly tested whether elevated inflammation burden (total inflammation z-score) was associated with elevated PHQ-9 score at follow-up in the whole cohort after adjustment for baseline PHQ-9 score. We next adjusted the model for the range of potential confounders. Associations were reported using the standardised beta coefficient and its associated p-value.

Interaction between inflammation and sex on course of depressive symptoms: We next used general linear models to test the interaction between sex and inflammation burden on 2-year PHQ-9 score. We firstly measured the beta-coefficient of the interaction, which presents the difference in PHQ-9 score between low- and elevated inflammation status in females compared to males. We next interrogated the directionality of any interaction using the estimated marginal mean (EMM) difference in PHQ-9 score at follow-up by comparing the differences in PHQ-9 score between females and males within the low- and elevated inflammation groups. Estimated marginal means are presented at mean values for each covariate (rather than resetting them to 0, as in linear regression models), which provides a clinically relevant measure of the association between inflammation and depression course in a typical patient (in this case a patient with average baseline depression score and average score or average proportion for other covariates and categorical variables respectively). The unadjusted interaction model comprised the outcome 2-year PHQ-9 score (natural log-transformed) and the independent variables of sex, inflammation status, sex*inflammation status, and baseline PHQ-9 score. We next adjusted the model for the full range of confounders. Interactions were also displayed graphically.

Sensitivity analyses: To account for missing data, we performed multiple imputation analyses with 20 multiply imputed datasets. Incomplete variables were imputed under fully conditional specification, using iterative Markov chain Monte Carlo (MCMC) estimation method. All variables were included in the imputation process, including interactions between PHQ-9 score (ln-transformed) and sex at both time points. The parameters of

interest were estimated in each imputed dataset separately and combined using Rubin's rules.[25] Finally, to account for participants who may have had elevated inflammation due to an acute infection, we conducted a further sensitivity analysis excluding those with hs-CRP concentration >10mg/L at baseline.[26]

Results

Ninety-six out of 136 (70.6%) GP surgeries agreed to participate. From their diabetes registers, a target population of 3008 newly-diagnosed type 2 diabetes patients were identified, of whom 2406 people were potentially eligible and invited to participate, and of whom 1735 people consented. Of these, 1174 (67.7%) provided complete data on all 6 inflammatory markers and were included in the current analysis. The mean age of this subset was 56.7 (11.0) years, 44.1% were female and 46.1% of non-white ethnicity.

Compared with those included in the analysis, those with missing inflammation data were younger (55.0 [11.0] versus 56.7 [11.0] years, p=0.003), had slightly higher BMI (32.5 [6.7] versus 31.8 [6.4] kg/m^2, p=0.032), slightly lower total cholesterol (4.50 [1.1] versus 4.62 [1.1] mmol/L, p=0.044), higher baseline depressive symptoms (3 [1-7] versus 2 [0-6], p=0.009), were more often of non-white ethnicity (65.0% versus 48.7%, p<0.001), smokers at baseline (24.5% versus 19.0%, p=0.011), and less likely to be prescribed NSAIDs/steroids (23.2% versus 30.0%, p=0.003), or antidepressants at baseline (1.2% versus 7.3%, p<0.001). However, there were no differences in proportion of female sex (p=0.14), baseline HbA1c (p=0.21), BMI (p=0.99), systolic blood pressure (p=0.47) or diastolic blood pressure (p=0.89) (test statistics not shown).

Baseline comparisons: Compared to those with low inflammation burden, people with elevated inflammation burden were older, had higher BMI, higher total cholesterol, higher hs-CRP, higher baseline PHQ-9 score, were more often of African/Caribbean ethnicity than white, smokers at baseline, and prescribed antidepressant medication. There were no differences by inflammation burden in sex, South Asian ethnicity, blood pressure, glycaemic control and prescription of anti-inflammatory medication (Table 1).

[Insert Table 1 here]

Follow-up: A total of 803 (68.4%) of people provided both inflammation and 2-year PHQ-9 data. Compared to those followed up, people with missing data were younger (55.1 [11.6] years versus 57.4 [10.7] years, p=0.001), more likely to be female (49.1% versus 41.8%, p=0.021), of non-white ethnicity (52.8% versus 43.0%, p=0.002), to have low inflammation burden (56.1% versus 46.6%, p=0.002), had higher baseline HbA1c (7.14 [1.5] versus 6.90 [1.4], p=0.006), and higher baseline diastolic blood pressure (84.0 [11.2] versus 82.6 [10.4], p=0.04). However, there were no differences by attrition in proportion of smokers (p=0.64), prescription of NSAIDs/steroids at baseline (p=0.34), prescription of antidepressants at baseline (p=0.73), baseline BMI (p=0.13), baseline systolic blood pressure (p=0.55), baseline total cholesterol (p=0.98) and baseline total PHQ-9 score (p=0.84) (test statistics not shown).

Prospective association between inflammation burden and course of depressive symptoms: Before or after full adjustment, there was no association between inflammation burden at baseline and higher PHQ-9 score at 2 years in the whole cohort (Table 2).

[Insert Table 2 here]

Unadjusted interaction between inflammation burden and sex on course of depressive symptoms: In the unadjusted general linear model, there was a significant interaction between female sex and elevated inflammation burden on change in PHQ-9 score (β =0.36, p=0.001), meaning that the difference between low- and elevated inflammation status was 0.36 larger in females compared to males (Table 2). A post hoc analysis of the interactions revealed no differences in depressive symptoms between males and females with low inflammation burden (EMM difference = 0.07, p=0.37), whilst depressive symptoms were significantly higher in females than males in the subgroup with elevated inflammation burden (EMM difference = 0.29, p<0.001) (Table 3, Figure 1a).

[Insert Table 3 and Figure 1 here]

Adjusted interaction between inflammation burden and sex on depressive symptoms: After adjustment for the full range of confounders, including baseline depressive symptoms, the interaction remained significant (β=0.32, p=0.005): the difference between low- and elevated inflammation burden in females was 0.32 larger than in males (Table 2). Post hoc pairwise comparisons again showed that there was no significant difference between males and

females in the low inflammation group (EMM difference = 0.08, p=0.34), whereas females had higher depressive symptoms than males in the elevated inflammation group (EMM difference = 0.23, p=0.003), conditioned on average values of covariates (Table 3, Figure 1b).

Sensitivity analyses: Using multiple imputation for missing data did not alter the conclusions: the unadjusted interaction (β = 0.36, p<0.001) and fully adjusted interaction (β = 0.31, p<0.001) between female sex and elevated inflammation remained strongly significant. Supplemental Table 1 shows the full breakdown of unadjusted- and adjusted interactions after multiple imputation. Exclusion of the 147 people with baseline hs-CRP >10mg/L – potentially indicating acute infection – likewise did not alter the conclusions: the fully adjusted interaction remained significant (β = 0.31, p=0.009) and females had higher depressive symptoms than males in subgroup with elevated inflammation (EMM difference = 0.24 p=0.007).

[Insert Supplemental Table 1 here]

Discussion

In a prospective cohort study of people with newly diagnosed type 2 diabetes recruited from primary care, elevated inflammation at diagnosis of type 2 diabetes – as estimated using a composite measure of acute phase, cytokine and chemokine markers – was not associated with worsening depressive symptoms over 2 years. However, elevated inflammation burden was associated with greater worsening depressive symptoms in females compared to males. These findings remained robust to adjustment for a range of pro-inflammatory and anti-inflammatory confounders, including BMI and smoking status.

Comparison with previous literature: Depressive symptoms are commonly reported in people with type 2 diabetes and are associated with poor biomedical outcomes.[1,2] In addition to psychological and behavioural factors, there is increasing evidence that biological mechanisms may provide a link between depressive symptoms and type 2 diabetes. In particular, previous studies have demonstrated a cross-sectional association between elevated inflammation and depressive symptoms in people with type 2 diabetes,[14,15] although no cohort study has tested this prospectively. Our study therefore marks a clear advance in the

literature by demonstrating that inflammation is associated with preferential worsening in depressive symptoms in females compared to males with type 2 diabetes.

Although depressive symptoms are nearly twice as prevalent in females with type 2 diabetes compared to males,[18] the reasons for this predominance are poorly understood. A possible explanation is that females are more likely to report depressive symptoms than males, for example due to different social processes influencing presentation of psychological distress for the different sexes. However, this is not supported by the similar reporting of depressive symptoms between males and females with low burden of inflammation in our study. Likewise, adjustment for increased BMI – another possible reason for female vulnerability to depression[27] – did not attenuate our findings.

Echoing prospective findings from the general population,[28,29] our results suggest that worse depressive symptoms in females with type 2 diabetes could result from elevated inflammation. This is likely to occur through dialogue with dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis. Specifically, activation of the HPA axis by stress should normally lead to an increase in glucocorticoid sensitivity, enabling cortisol to inhibit- and thus regulate inflammatory responses. Whereas males demonstrate this response consistently, stress appears to exert the opposite effect in females, leading to decreased glucocorticoid sensitivity and exaggerated inflammatory responses.[30] Notably, females in our sample were typically of postmenopausal age, by which time HPA axis dysfunction has become most pronounced, probably due to loss of modulation by gonadal steroids.[31] The presence of a dysfunctional HPA axis thereby exposes females to exaggerated effects on inflammation, leading to increased risk of cardiovascular disease in the periphery and potentially an increased risk of depression centrally.[32,33] Central effects may occur through pro-inflammatory cytokines activating the enzyme indoleamine 2,3-dioxygenease, which diverts tryptophan metabolism from serotonin towards neurotoxic metabolites in the brain.[33]

Interpretation: Our findings suggest that depressive symptoms and female sex – both poor prognostic factors in type 2 diabetes respectively – are biologically linked by inflammation. The result is an 'inflammatory depression' that predominantly affects females, runs a persistent course and is poorly explained by lifestyle factors such as smoking and obesity. Both female sex and inflammation are strongly associated with cardiovascular risk in type 2

diabetes,[17,32] and notably the typical hs-CRP concentration for the elevated inflammation group in our study was in the range for high cardiovascular risk.[26] As such, the inflammatory depression of female type 2 diabetes could be an important and potentially modifiable biomarker of future cardiovascular disease and mortality, which requires testing over longer-term follow-up.

Therapeutically, the strong association between female sex and inflammation suggests a need to 'gender' therapy towards reducing the burden of inflammation in females. For example, in managing depression, elevated innate inflammation is associated with poor response to the usual first-line antidepressants: selective serotonin reuptake inhibitors (SSRIs).[34] In females with depressive symptoms, clinicians may therefore consider proactive switching to antidepressants that have greater anti-inflammatory properties, such as tricyclic antidepressants.[34] Likewise, within the repertoire of current diabetes treatments, females with type 2 diabetes could be treated earlier with therapies known to have greater anti-inflammatory properties. For example, incretin-based therapies and thiazolidenediones have potent effects on inflammation,[35,36] and could even be a novel treatment for depression by modifying inflammation.[20]

As well as repositioning of anti-inflammatory diabetes treatments, our results support trials of anti-inflammatory agents for depressive symptoms in type 2 diabetes, which have not yet been performed. In the general population, anti-inflammatory- and HPA-axis modifying therapies have demonstrated inconsistency in improving depressive symptoms,[12,37] and our findings suggest that benefit would be maximised by specifically recruiting females to such clinical trials. Future experimental medicine studies are needed to profile the dynamic immune responses conferring vulnerability to female depressive symptoms in type 2 diabetes, thereby providing clearer targets for interventional studies. A better understanding of the bidirectional interplay between the HPA axis and inflammation is required, including predisposing factors to HPA axis dysfunction, such as changes in gonadal hormones. Finally, lifecourse epidemiological research is needed to delineate the temporal relationship between inflammation, depression and type 2 diabetes, as well as possible upstream aetiologies such as stressful life events.

Strengths and limitations: our study is strengthened by its unique multi-ethnic and socioeconomically diverse cohort, which is representative of the global type 2 diabetes population. By recruiting all patients within 6 months of diagnosis of type 2 diabetes, confounding effects of diabetes complications on inflammation and depression were minimised. A composite measure of inflammation that included acute phase, cytokine and chemokine responses provided a broad yet rigorous test of inflammation. Our data were limited by 32% missing values for inflammation burden at baseline, although there were no sex differences between those missing data and those included. Although a similar attrition rate over 2 years is a further limitation of our study, maximising follow-up is particularly challenging in an urban population with high rates of social deprivation, multimorbidity, geographical mobility, and a primary care setting in which several GP surgeries closed during the study. Furthermore, multiple imputation of missing data resulted in no significant changes to the study findings. We did not assess for periodontitis, which could be an important cause of inflammation, depression and type 2 diabetes.[38,39] We measured depressive symptoms continuously using a validated self-report questionnaire, which will likely over-identify depressive symptoms compared to a diagnostic interview. However, there is strong evidence that subthreshold depressive symptoms are prognostically important in people with diabetes.[40] Finally, longer follow-up is needed to confirm the persistence of inflammatory depression in females, as well as associated biomedical sequelae.

Summary

In the early stages of type 2 diabetes, inflammation shows no overall association with worsening in depressive symptoms over 2-year follow-up. However, inflammation demonstrates a preferential association with worsening depressive symptoms in females compared to males, which is not explained by potential confounders such as obesity. Future studies should test whether inflammation is a modifiable target for reducing the gender gap in psychological- and biomedical outcomes in people with type 2 diabetes.

Figure captions

Figure 1: General linear model testing the interaction between sex and inflammation burden on the 2-year course of depressive symptoms in the SOUL-D cohort: a) adjusted only for baseline depressive symptoms; b) adjusted for baseline depressive symptoms and the full range of confounders.

Author contributions

S.A.A and K.I conceived the South London Diabetes Cohort. C.D.M, D.S, J.C.P and K.I designed the current manuscript and C.D.M wrote the first draft. C.D.M, D.S and A.R performed the statistical analyses. All authors revised the manuscript for important intellectual content. C.D.M is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Declarations of interest

J.C.P reports consultancy and/or speaker fees from Insulet, Medtronic, Roche and Theras. K.I reports speaker fees from Eli Lilly, Janssen, Novo Nordisk and Sanofi. C.D.M, A.R, S.A.A and D.S report no potential dualities of interest.

Funding

This report presents independent research funded by the National Institute for Health Research (NIHR) under its Programme Grants for Applied Research scheme (RP-PG-0606-1142) and part-funded by the NIHR Biomedical Research Centre at South London and South London and Maudsley NHS Foundation Trust. Additional funding was received from the EFSD/Lilly Mental Health in Diabetes Programme (2010). C.D.M is supported by the JMAS Sim fellowship funded by the Royal College of Physicians of Edinburgh. K.I and D.S are part-funded by the National Institute for Health Research (NIHR) Biomedical Research Centre at South London and Maudsley NHS Foundation Trust and King's College London. The views expressed in this publication are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health.

Acknowledgements

The authors would like to thank the people who volunteered to take part in this study. The authors also thank the SOUL-D research team, King's College London, London, UK (J. Schonbeck, J. Valka, N. Iles, B. Jackson, E. Britneff, L. East, J. Hunt, S. Mann, G. Knight, L. Marwood, R. Stopford, J. P. Laake, K. Twist, M. Hussain and A. Bayley), the staff at participating general practices, the Primary Care Research Network (PCRN-GL) for their assistance in recruiting the general practices, the Diabetes Research Network, and the South London Comprehensive Local Research network.

References

1 Anderson RJ, Freedland KE, Clouse RE, Lustman PJ. The prevalence of comorbid depression in adults with diabetes: a meta-analysis. *Diabetes Care* 2001; **24**: 1069–78.

2 de Groot M, Anderson R, Freedland KE, Clouse RE, Lustman PJ. Association of depression and diabetes complications: a meta-analysis. *Psychosom Med* 2001; **63**: 619–30.

3 Winkley K, Sallis H, Kariyawasam D, *et al.* Five-year follow-up of a cohort of people with their first diabetic foot ulcer: the persistent effect of depression on mortality. *Diabetologia* 2012; **55**: 303–10.

4 Katon WJ. Epidemiology and treatment of depression in patients with chronic medical illness. *Dialogues Clin Neurosci* 2011; **13**: 7–23.

5 Ismail K, Moulton CD, Winkley K, *et al.* The association of depressive symptoms and diabetes distress with glycaemic control and diabetes complications over 2 years in newly diagnosed type 2 diabetes: a prospective cohort study. *Diabetologia* 2017. DOI:10.1007/s00125-017-4367-3.

6 Moulton CD, Costafreda SG, Horton P, Ismail K, Fu CHY. Meta-analyses of structural regional cerebral effects in type 1 and type 2 diabetes. *Brain Imaging Behav* 2015; **9**. DOI:10.1007/s11682-014-9348-2.

7 Cole J, Costafreda SG, McGuffin P, Fu CHY. Hippocampal atrophy in first episode depression: a meta-analysis of magnetic resonance imaging studies. *J Affect Disord* 2011; **134**: 483–7.

8 Mezuk B, Eaton WW, Albrecht S, Golden SH. Depression and type 2 diabetes over the lifespan: a meta-analysis. *Diabetes Care* 2008; **31**: 2383–90.

9 Moulton CD, Pickup JC, Ismail K. The link between depression and diabetes: the search for shared mechanisms. *Lancet Diabetes Endocrinol* 2015; **3**: 461–71.

10 Wang X, Bao W, Liu J, *et al.* Inflammatory markers and risk of type 2 diabetes: a systematic review and meta-analysis. *Diabetes Care* 2013; **36**: 166–75.

11 Valkanova V, Ebmeier KP, Allan CL. CRP, IL-6 and depression: a systematic review and meta-analysis of longitudinal studies. *J Affect Disord* 2013; **150**: 736–44.

12 Husain MI, Strawbridge R, Stokes PR, Young AH. Anti-inflammatory treatments for mood disorders: Systematic review and meta-analysis. *J Psychopharmacol* 2017; **31**: 1137–48.

13 Larsen CM, Faulenbach M, Vaag A, *et al.* Interleukin-1-receptor antagonist in type 2 diabetes mellitus. *N Engl J Med* 2007; **356**: 1517–26.

14 Laake JP, Stahl D, Amiel SA, *et al.* The association between depressive symptoms and systemic inflammation in people with type 2 diabetes: findings from the South London Diabetes Study. *Diabetes Care* 2014; **37**: 2186–92.

15 Hayashino Y, Mashitani T, Tsujii S, Ishii H. Elevated levels of hs-CRP are associated with high prevalence of depression in japanese patients with type 2 diabetes: the Diabetes Distress and Care Registry at Tenri (DDCRT 6). *Diabetes Care* 2014; **37**: 2459–65.

16 Herder C, Schmitt A, Budden F, *et al.* Association between pro- and anti-inflammatory cytokines and depressive symptoms in patients with diabetes-potential differences by diabetes type and depression scores. *Transl Psychiatry* 2018; **7**: 1.

17 Peters SAE, Huxley RR, Woodward M. Diabetes as a risk factor for stroke in women compared with men: a systematic review and meta-analysis of 64 cohorts, including 775,385 individuals and 12,539 strokes. *Lancet (London, England)* 2014; **383**: 1973–80.

18 Ali S, Stone MA, Peters JL, Davies MJ, Khunti K. The prevalence of co-morbid depression in adults with Type 2 diabetes: a systematic review and meta-analysis. *Diabet Med* 2006; **23**: 1165–73.

19 Derry HM, Padin AC, Kuo JL, Hughes S, Kiecolt-Glaser JK. Sex Differences in Depression: Does Inflammation Play a Role? *Curr Psychiatry Rep* 2015; **17**: 78.

20 Moulton CD, Hopkins CWP, Ismail K, Stahl D. Repositioning of diabetes treatments for

depressive symptoms: A systematic review and meta-analysis of clinical trials. *Psychoneuroendocrinology* 2018; **94**: 91–103.
21 Winkley K, Thomas SM, Sivaprasad S, *et al.* The clinical characteristics at diagnosis of type 2 diabetes in a multi-ethnic population: the South London Diabetes cohort (SOUL-D). *Diabetologia* 2013; **56**: 1272–81.
22 Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med* 1998; **15**: 539–53.
23 Twist K, Stahl D, Amiel SA, Thomas S, Winkley K, Ismail K. Comparison of depressive symptoms in type 2 diabetes using a two-stage survey design. *Psychosom Med* 2013; **75**: 791–7.
24 von Scholten BJ, Reinhard H, Hansen TW, *et al.* Markers of inflammation and endothelial dysfunction are associated with incident cardiovascular disease, all-cause mortality, and progression of coronary calcification in type 2 diabetic patients with microalbuminuria. *J Diabetes Complications* 2016; **30**: 248–55.
25 Rubin D. Multiple imputation for nonresponse in surveys. New York: Wiley, 1987.
26 Torres JL, Ridker PM. Clinical use of high sensitivity C-reactive protein for the prediction of adverse cardiovascular events. *Curr Opin Cardiol* 2003; **18**: 471–8.
27 Mulugeta A, Zhou A, Power C, Hypponen E. Obesity and depressive symptoms in mid-life: a population-based cohort study. *BMC Psychiatry* 2018; **18**: 297.
28 Niles AN, Smirnova M, Lin J, O'Donovan A. Gender differences in longitudinal relationships between depression and anxiety symptoms and inflammation in the health and retirement study. *Psychoneuroendocrinology* 2018; **95**: 149–57.
29 Beydoun MA, Beydoun HA, Dore GA, *et al.* White blood cell inflammatory markers are associated with depressive symptoms in a longitudinal study of urban adults. *Transl Psychiatry* 2016; **6**: e895.
30 Rohleder N, Schommer NC, Hellhammer DH, Engel R, Kirschbaum C. Sex differences in glucocorticoid sensitivity of proinflammatory cytokine production after psychosocial stress. *Psychosom Med* 2001; **63**: 966–72.
31 Young E, Korszun A. Sex, trauma, stress hormones and depression. *Mol Psychiatry* 2010; **15**: 23–8.
32 Pickup JC, Mattock MB. Activation of the innate immune system as a predictor of cardiovascular mortality in Type 2 diabetes mellitus. *Diabet Med* 2003; **20**: 723–6.
33 Arnone D, Saraykar S, Salem H, Teixeira AL, Dantzer R, Selvaraj S. Role of Kynurenine pathway and its metabolites in mood disorders: A systematic review and meta-analysis of clinical studies. *Neurosci Biobehav Rev* 2018; **92**: 477–85.
34 Uher R, Tansey KE, Dew T, *et al.* An inflammatory biomarker as a differential predictor of outcome of depression treatment with escitalopram and nortriptyline. *Am J Psychiatry* 2014; **171**: 1278–86.
35 Negrotto L, Farez MF, Correale J. Immunologic Effects of Metformin and Pioglitazone Treatment on Metabolic Syndrome and Multiple Sclerosis. *JAMA Neurol* 2016; **73**: 520–8.
36 Satoh-Asahara N, Sasaki Y, Wada H, *et al.* A dipeptidyl peptidase-4 inhibitor, sitagliptin, exerts anti-inflammatory effects in type 2 diabetic patients. *Metabolism* 2013; **62**: 347–51.
37 McAllister-Williams RH, Anderson IM, Finkelmeyer A, *et al.* Antidepressant augmentation with metyrapone for treatment-resistant depression (the ADD study): a double-blind, randomised, placebo-controlled trial. *The lancet Psychiatry* 2016; **3**: 117–27.
38 Hashioka S, Inoue K, Hayashida M, Wake R, Oh-Nishi A, Miyaoka T. Implications of Systemic Inflammation and Periodontitis for Major Depression. *Front Neurosci* 2018; **12**: 483.
39 Pussinen PJ, Salomaa V. Oral health: a neglected aspect of diabetes care. *lancet Diabetes Endocrinol* 2018; **6**: 917–9.
40 Schmitz N, Gariepy G, Smith KJ, *et al.* Recurrent subthreshold depression in type 2 diabetes: an important risk factor for poor health outcomes. *Diabetes Care* 2014; **37**: 970–8.

Table 1: Baseline characteristics of the South London Diabetes cohort stratified by inflammation burden at baseline

Variable	Category	Total cohort[a]	Elevated inflammation burden[a] (n=592)	Low inflammation burden[a] (n=582)	p-value[b]
Sociodemographic variables					
Age, years (SD)		56.7 (11.0)	57.6 (10.9)	55.7 (11.0)	0.003
Sex (%)	Male	656 (55.9)	323 (54.6)	333 (57.2)	-
	Female	518 (44.1)	269 (45.4)	249 (42.8)	0.36
Ethnicity (%)	White	633 (54.0)	394 (66.6)	239 (41.1)	-
	African/Caribbean	417 (35.5)	124 (20.9)	293 (50.3)	<0.001[c]
	South Asian/other	124 (10.6)	74 (12.5)	50 (8.6)	0.59[c]
Baseline vascular risk factors					
Smoker status (%)	Smoker	216 (18.9)	152 (26.3)	64 (11.4)	-
	Non-smoker	924 (81.1)	427 (73.7)	497 (88.6)	<0.001
Mean BMI, kg/m^2 (SD)		31.8 (6.4)	32.6 (6.8)	30.9 (5.8)	<0.001
Mean systolic BP, mmHg (SD)		136.1 (17.4)	136.3 (17.5)	136.0 (17.3)	0.78
Mean diastolic BP, mmHg (SD)		83.0 (10.5)	83.1 (10.6)	82.8 (10.4)	0.61
Mean HbA1c, % (SD)		6.98 (1.4)	7.02 (1.4)	6.91 (1.4)	0.35
Mean HbA1c, mmol/mol (SD)		52.8 (10.6)	53.2 (10.6)	52.0 (10.5)	0.35
Mean total cholesterol, mmol/L (SD)		4.62 (1.1)	4.70 (1.1)	4.53 (1.0)	0.007
Baseline inflammatory variables					
Prescribed NSAIDs/opioids (%)	Yes	348 (30.0)	187 (31.9)	161 (28.0)	-
	No	814 (70.0)	400 (68.1)	414 (72.0)	0.15
Median hs-CRP [IQR]		2.7 [1.1-6.3]	4.6 [1.9-9.0]	1.6 [0.8-3.9]	<0.001
Median IL-1B [IQR]		1.02 [0.73-1.87]	1.16 [0.79-2.64]	0.91 [0.69-1.54]	<0.001
Median IL-1RA [IQR]		437.0 [290.0-695.8]	620.2 [399.4-916.0]	323.8 [223.1-473.4]	<0.001
Median MCP-1 [IQR]		102.3 [59.6-152.2]	138.7 [92.3-189.2]	72.3 [49.9-109.9]	<0.001
Median VEGF [IQR]		76.0 [45.6-118.1]	114.4 [82.1-163.7]	48.9 [33.1-71.7]	<0.001
Median WBC [IQR]		7.6 [6.4-9.0]	8.6 [7.4-10.1]	6.7 [5.7-7.7]	<0.001
Baseline psychological variables					
Median PHQ-9 score [IQR]		2 [0-6]	3 [1-7]	2 [0-6]	<0.001
Prescribed antidepressants (%)	Yes	84 (7.2)	53 (8.9)	31 (5.3)	-
	No	1089 (92.8)	538 (91.1)	551 (94.7)	0.016

a) Inflammation burden comprises baseline concentrations of white cell count, interleukin-β, interleukin-1 receptor antagonist, high-sensitivity C-reactive protein, monocyte chemotactic protein-1 and vascular endothelial growth factor. Each concentration is z-transformed and all 6 measures are averaged to calculate an overall score. This is split by the median value to define elevated- and low inflammation burden. b) Parametric continuous data are presented as mean (SD) and compared using Student's t-test; non-parametric continuous data are presented as median [interquartile range] and compared using Mann-Whitney *U* test; categorical variables presented as frequency (%) and compared using chi-square tests. c) Compared to white ethnicity. Key: BMI, body mass index; BP, blood pressure; CRP, high-sensitivity C-reactive protein; NSAIDs, non-steroidal anti-inflammatory drugs; PHQ-9, patient health questionnaire-9; SD; standard deviation.

Table 2: Multivariate analysis testing the associations between inflammation burden and sex on 2-year depressive symptoms in the SOUL-D cohort			
Independent variables			
Model 1: inflammation as independent variable		**Adjusted for baseline PHQ-9 score only**	**Fully adjusted[a]**
Overall inflammation burden (continuous)	β (95% CI), p-value	0.05 (-0.03 to 0.14), p=0.23	0.02 (-0.07 to 0.11), p=0.65
Inflammation burden (binary) (1=elevated, 0=low)	β (95% CI), p-value	0.09 (-0.02 to 0.19),p=0.11	0.06 (-0.05 to 0.18), p=0.30
Model 2: inflammation, sex and their interaction as independent variables			
Inflammation burden (1=elevated, 0=low)	β (95% CI), p-value	-0.06 (-0.20 to 0.08), p=0.37	-0.07 (-0.22 to 0.08), p=0.35
Sex (1=female, 0=male)	β (95% CI), p-value	-0.07 (-0.23 to 0.09) p=0.37	-0.08 (-0.25 to 0.09), p=0.34
Interaction (female sex*elevated inflammation burden)	β (95% CI), p-value	0.36 (0.15 to 0.57), p=0.001	0.32 (0.10 to 0.53), p=0.005
	Total number in model	N=797	N=706
Multivariable general linear models with outcome 2-year Patient Health Questionnaire-9 (PHQ-9) score (natural log-transformed). Reference groups are low inflammation burden, male sex, and low inflammation*male sex. a) Adjusted for baseline PHQ-9 score (log-transformed), age, non-white ethnicity, body mass index, baseline systolic blood pressure, baseline smoking status, baseline serum cholesterol, baseline $HbA_{1c,}$ prescription of anti-inflammatory medication and prescription of antidepressant medication. Key: CI, confidence interval			

Table 3: General linear models testing the interactions between sex and inflammation burden on 2-year depressive symptoms in the SOUL-D cohort

i) Pairwise comparisons between males and females within low- and elevated inflammation groups: adjusted for baseline depressive symptoms only[a]

Inflammation burden	(I) Sex	(J) Sex	Mean Difference (I-J)	Std. Error	Sig.	95% CI Lower Bound	95% CI Upper Bound
Low	Male	Female	-0.07	0.08	0.37	-0.23	0.09
Elevated	Male	Female	-0.29	0.07	<0.001	-0.43	-0.14

ii) Pairwise comparisons between males and females within low- and elevated inflammation groups: fully adjusted[b]

Inflammation burden	(I) Sex	(J) Sex	Mean Difference (I-J)	Std. Error	Sig.	95% CI Lower Bound	95% CI Upper Bound
Low	Male	Female	0.08	0.09	0.34	-0.09	0.25
Elevated	Male	Female	-0.23	0.08	0.003	-0.39	-0.08

Outcome is estimated marginal mean Patient Health Questionnaire-9 (PHQ-9) score (natural log-transformed) at 2 years. a) Adjusted only for baseline PHQ-9 score (natural log-transformed). b) Adjusted for baseline PHQ-9 score (natural log-transformed), age, non-white ethnicity, body mass index, baseline systolic blood pressure, baseline smoking status, baseline serum cholesterol, baseline $HbA_{1c,}$ prescription of anti-inflammatory medication and prescription of antidepressant medication. Key: CI, confidence interval.