Supplementary Material

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ONLINE SUPPLEMENTARY MATERIAL

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METHODS

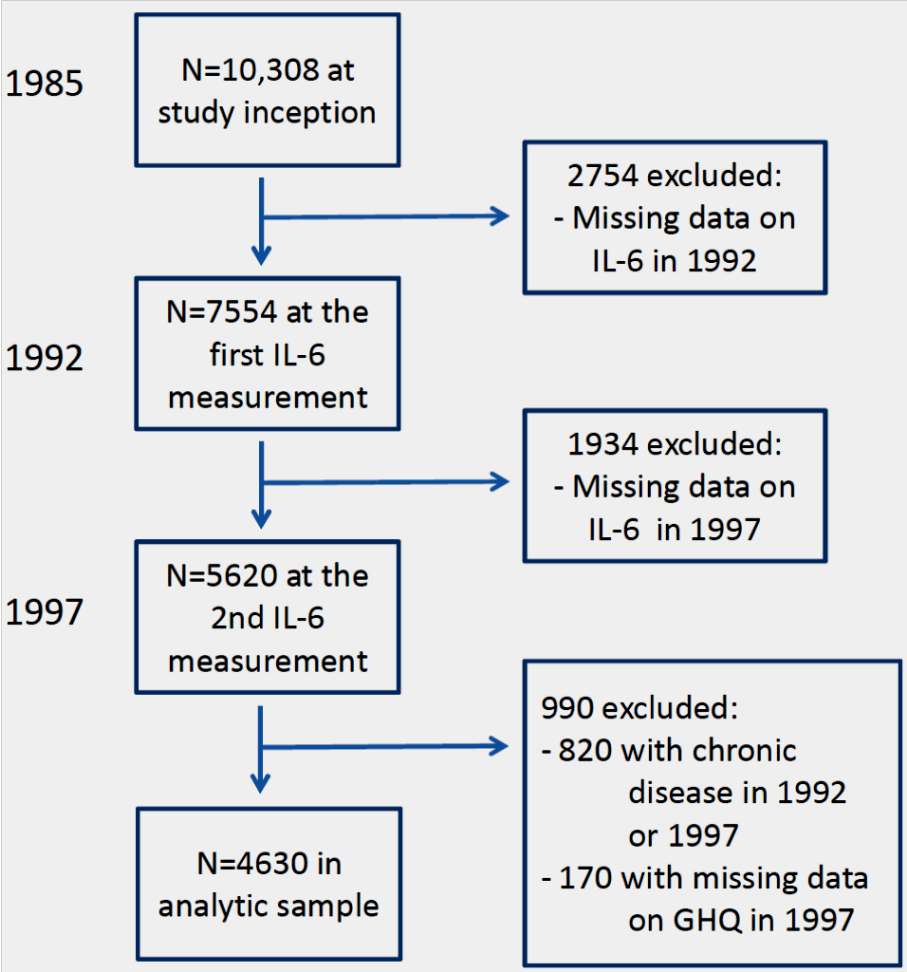
Study Population and Study Design

The Whitehall II study is a prospective cohort study of British civil servants, established in 1985. The target population was all London-based office staff, aged 35-55, working in 20 civil service departments when recruited to the study in 1985. With a response of 73%, the cohort consisted of 10,308 persons (6895 men and 3413 women). IL-6 was first measured in the study in 1992 with repeat measurements in 1997 and 2003. Common mental disorder was measured in 1997, 2003 and 2008 using the General Health Questionnaire (GHQ). Ethical approval for the Whitehall II study was obtained from the University College London Medical School committee on the ethics of human research; all participants provided written informed consent.

Four study designs, labeled from A to D, used in the analysis are described below with flow charts.

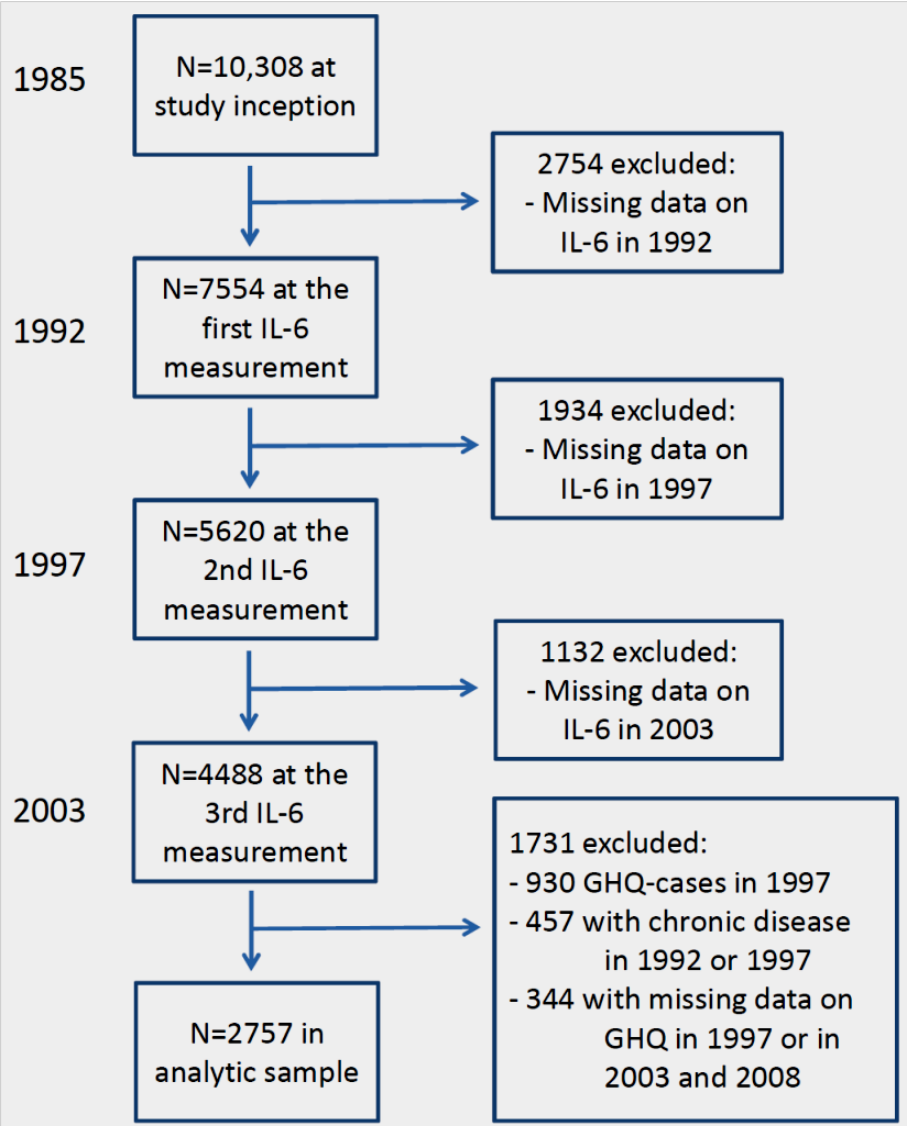
Study Design-A: To examine cross-sectional association between IL-6 and common mental disorder, we assessed odds ratio of GHQ-caseness in 1997 by category of IL-6 in 1997. The analytic sample after exclusions included 4630 men and women free of chronic disease (Figure S1).

Figure S1: Flow chart for sample selection in Study Design A (exclusions are sequential).



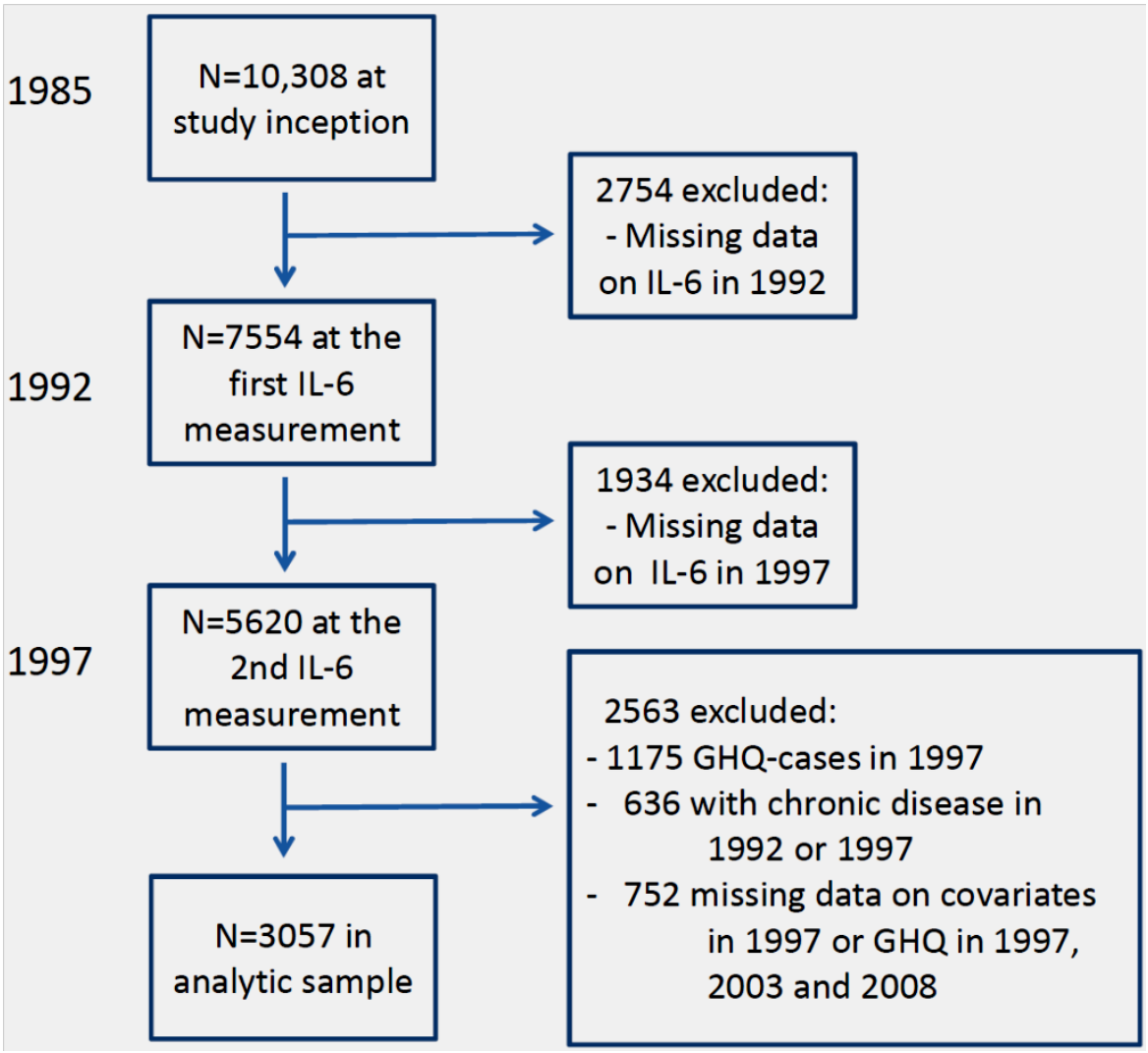
Study Design-B. To examine longitudinal association between IL-6 and 10-year cumulated risk of common mental disorder, we assessed odds ratio of new-onset GHQ-caseness in 2003 and/or 2008 by category of IL-6 in 1992, 1997 and/or 2003. The analytic sample after exclusions included 2757 participants without common mental disorder in 1997 (Figure S2).

Figure S2: Flow chart for sample selection in Study Design B (exclusions are sequential).



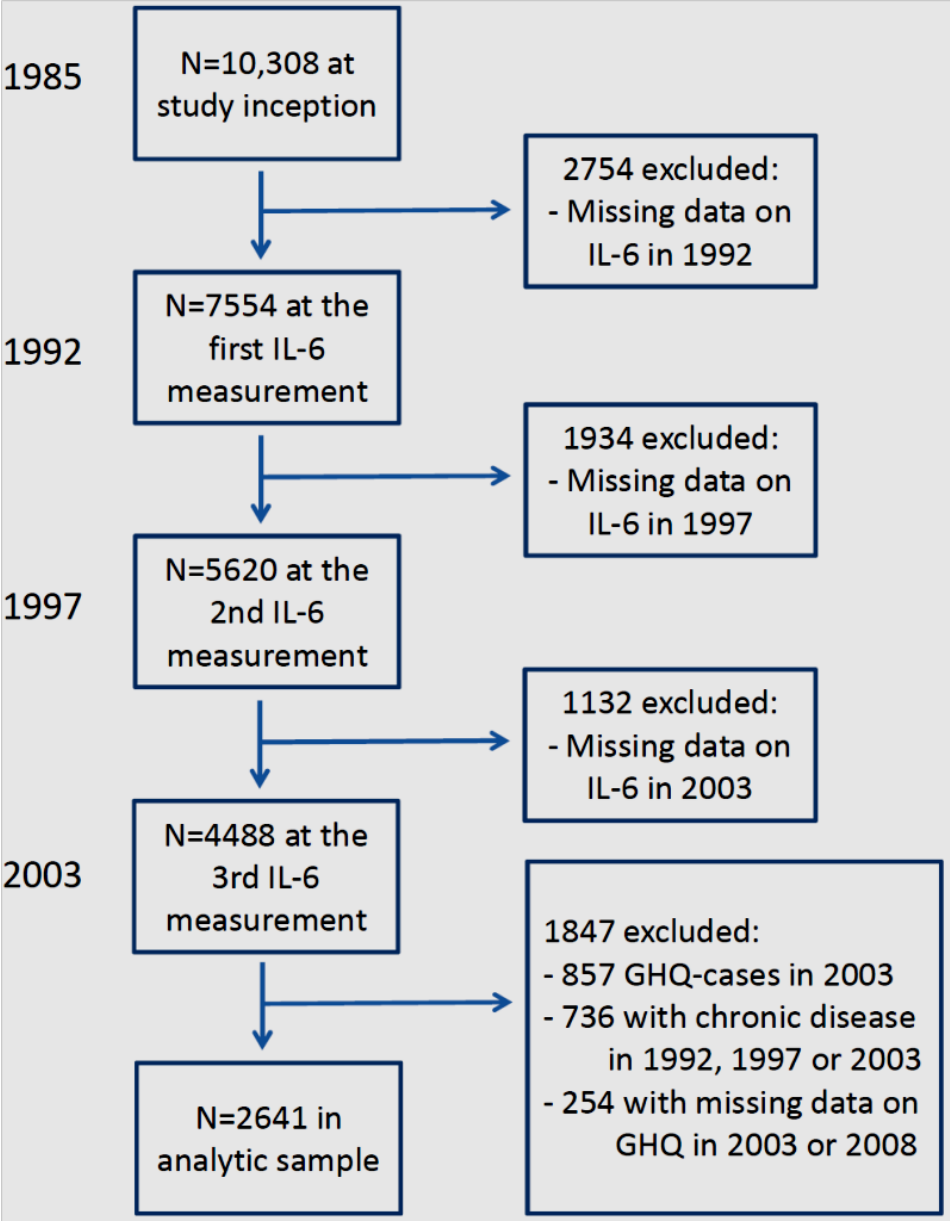
Study Design-C. To examine multivariable-adjusted longitudinal association between long-term IL-6 and 10-year cumulated risk of common mental disorder, we assessed the relationship of averaged 1992 and 1997 IL-6 levels to new-onset GHQ-caseness in 2003 and/or 2008. The analytic sample after exclusions included 3057 participants without common mental disorder in 1997 (Figure S3).

Figure S3: Flow chart for sample selection in Study Design C (exclusions are sequential).



Study Design-D. In this analysis, we examined 5-year (rather than 10-year) risk of common mental disorder among participants who did not have such disorder after three measurements of inflammation over 10 years. Thus, we assessed odds ratio of new-onset GHQ-caseness in 2008 by category of IL-6 in 1992, 1997 and/or 2003 in 2641 participants without common mental disorder in 2003 (Figure S4).

Figure S4: Flow chart for sample selection in Study Design D (exclusions are sequential).



Assessment of Interleukin-6

IL-6 was measured using a high-sensitivity ELISA assay (R&D Systems, Oxford, UK) in 1992, 1997, and 2003. At each examination, blood samples were collected between 8 am and 1 pm, stored at −80°C and were not thawed or refrozen during storage. Values below the detection limit (0.08 pg/mL) were assigned a value equal to half the detection limit. To measure short-term biological variation and laboratory error, a repeat sample was taken from a subset of 241 participants (average elapsed time between samples was 32 (SD =

10.5) days. Intra- and inter-assay coefficient of variation were 7.5% and 8.9%, respectively, and reliability between samples assessed with Pearson's correlation coefficients was $r = 0.61$.

Assessment of Common Mental Disorder

Participants responded to the self-administered 30-item General Health Questionnaire (GHQ) in 1997, 2003, and 2008. The GHQ is a screening instrument designed to detect common mental disorder, and is widely used in population-based surveys and trials.[1] Each questionnaire item enquires about a specific symptom; response categories are scored as either 1 or 0 to indicate presence of the symptom. A total score of 5 or more led to individuals being defined as GHQ-symptom "cases" and scores 0-4 as "non-cases".[2] A recent population-based study showed GHQ-caseness to be sensitive (84%) and specific (84%) in detecting dysthymia or major depressive disorder, as indicated by the Composite International Diagnostic Interview (CIDI).[3] The GHQ was also validated at baseline against a clinical interview schedule in the Whitehall II study, with acceptable sensitivity (73%) and specificity (78%).[2] In a more recent validation using a subgroup of 274 participants aged 58 to 70 in 2010, the sensitivity and specificity of GHQ-symptom caseness against diagnosed common mental disorder based on a structured psychiatric interview were 74% and 98%, respectively.[4]

Ascertainment of Other Characteristics

To exclude participants with physical illness, we assessed prevalent CHD (a history of myocardial infarction or angina), stroke, diabetes mellitus and cancer in 1992, 1997, and 2003. A history of angina was identified via questionnaire and was corroborated with medical records, abnormalities in a resting electrocardiogram (ECG), an exercise ECG, or a coronary angiogram. Non-fatal myocardial infarction was defined following the World Health Organization MONICA (Multinational Monitoring of Trends and Determinants in Cardiovascular Disease) criteria[5] and ascertained using data from medical examinations, hospital records of acute ECGs and use of cardiac enzymes.[6] A history of stroke was ascertained by self-reports ("Have you ever been told by a doctor that you have had a stroke or transient ischaemic attack?" Yes/No). Diabetes was defined as fasting glucose $\geq$7.0 mmol/L or a 2-hour postload glucose $\geq$11.1 mmol/L during the oral glucose tolerance test performed at the Whitehall screening, as physician-diagnosed diabetes or use of diabetes medication.[7] Cancers were ascertained through the National Health Service cancer registry.

The following clinical characteristics, used as covariates, were taken from 1997 examination: age, sex, body mass index (BMI), acute inflammation, smoking and medication use. BMI was defined as weight [in kg]/height squared [in m^2], obesity as BMI $\geq$30kg/m^2 and acute inflammation as a C-reactive protein level (based on high-sensitivity immunonephelometric assay in a BN ProSpec nephelometer, Dade Behring, Milton Keynes, UK) >10mg/L. At medical examination, participants provided details of current medications use (generic name, brand name, or both); these were subsequently coded using the British National Formulary to determine use of anti-inflammatory medication, oral contraceptives, and antidepressants and hormone replacement therapy.[8]

Statistical Analysis

All data analyses were performed with SAS version 9.2. The analyses combine men and women. We divided the distribution of IL-6 into three categories:$\leq$1.0pg/mL (low, N=1256 in 1997), 1.1-2.0pg/mL (intermediate, N=2200) and >2.0pg/mL (high, N=1174)(Study Design A). We used logistic regression, adjusted for age and sex to study the cross-sectional association of IL-6 category with common mental disorder (Study Design A).

In longitudinal analyses (Study Design B), we excluded those with common mental disorder in 1997 and computed the odds ratios for common mental disorder in 2003 and/or 2008 (approximately 10-year risk) for

high IL-6 in 1997 only, high IL-6 in 1992 and 1997 and in 1992, 1997 and 2003. Those with low IL-6 in 1997 were the reference category in these comparisons. To further examine dose-response, we ran an age- and sex-adjusted analysis with the number of times the participant had a high IL-6 as the exposure.

To examine the robustness of the association between long-term IL-6 and 10-y risk of common mental disorder with sufficient statistical power, we treated IL-6 as a continuous variable (Study Design C). We normalized the distribution of IL-6 by natural logarithmic transformation and used linear regression to assess the age- and sex-adjusted association between the $\log_e$ of the mean IL-6 across 1992 and 1997 levels as the exposure and new-onset common mental disorder in 2003 or 2008 as the outcome among participants without common mental disorder in 1997. We adjusted the analyses for BMI; acute inflammation; hormonal contraceptives and hormone replacement therapy; and antidepressant medication in 1997 in addition to age and sex. All these covariates were subsequently included in a model fully adjusted for multiple variables. In subgroup analyses, we examined the age-adjusted association separately in men and women and the age- and sex-adjusted association after sequential exclusion of participants with acute inflammation, obese individuals and those on medication (anti-inflammatory medication, oral contraceptives, hormone replacement therapy, antidepressants) in 1997.

Finally, in the analysis of chronic inflammation across all 3 phases among participants who had remained free of common mental disorder at the last measurement of inflammation, 5-year risk of common mental disorder at fourth examination was used as the outcome. Thus, we excluded those with common mental disorder in 2003 from longitudinal analyses of new-onset common mental disorder in 2008 and computed the odds ratios for high IL-6 in 2003 only, in 1997 and 2003, and in 1992, 1997 and 2003 compared to low IL-6 in 2003 as the reference category (Study Design D).

RESULTS

Table S1 shows that in terms of age any differences between the analytic samples and the population of the Whitehall II study at recruitment were small. Common mental disorder was more common in women (prevalence 27%) than men (19%, P<0.001, Study Design A). Thus, the proportion of women was higher in the sample used for cross-sectional analyses (Study Design A) than in longitudinal samples in which those with common mental disorder at baseline were excluded (Study Designs B to D). **Table S2** shows characteristics of participants in 1997. Mean age was 55 years and over 70% were men. The geometric mean of IL-6 was 1.5 pg/mL. One in five had common mental disorder.

There was no evidence to suggest that the associations of IL-6 and common mental disorder differed by sex (all P-values for the interaction term >0.80 for cross-sectional and >0.31 for the longitudinal analyses). Analyses based on Study Designs A to C show a significant influence of persistently elevated levels of IL-6 on the risk for development of a GHQ-based common mental disorder over a 10-year follow-up period.

More specifically, IL-6 among 4630 participants (Study Design A) was not cross-sectionally associated with common mental disorder in 1997 (991 prevalent cases), age- and sex-adjusted odds ratio for high vs. low IL-6=1.04 (95% confidence interval 0.85-1.27, *P*=0.69).

In analysis of the cumulative 10-year risk of common mental disorder among the 2757 participants without the disorder at the first GHQ-assessment in 1997 (Study Design B), those with low IL-6 in 1997 formed the comparison group (N=768). High IL-6 in 1997 (N=653) predicted common mental disorder in 2003 and/or 2008 (a total of 549 new cases over approximately 10 years), age- and sex-adjusted odds ratio=1.40 (1.07-1.82, *P*=0.01). However, those with high IL-6 at both the first and second measurements (1992 and 1997, respectively, N=281) had higher odds of a new-onset mental disorder in 2003 and/or 2008, 1.61 (1.14-2.28, *P*=0.007). This odds ratio was even higher, 1.75 (1.19-2.57, *P*=0.004) among those who had high IL-6 in 1992, 1997 and 2003 (N=224).

Table S3 shows that in analyses based on Study Design C the significant association between the 5-year average level of IL-6 and subsequent 10-year risk of common mental disorder was little affected by adjustments for acute inflammation, obesity, smoking and drug treatments. The relationship between IL6 and common mental disorder was significant in both men (odds ratio per doubling of IL-6 1.46, 1.19-1.78) and women (1.34, 1.00-1.79), but there was no statistical evidence of sex interaction (*P* for sex interaction=0.67).

Finally, analyses predicting new-onset mental disorder only in 2008 among participants free of this disorder at the third measurement of inflammation were based on 238 new cases over an approximately 5-year period (Study Design D). High IL-6 based on 2003 measurement (N=593) gave an age- and sex-adjusted odds ratio of 1.18 (0.77-1.82) and high IL-6 in both 1997 and 2003 (N=176) an odds ratio of 1.18 (0.71-1.97). Participants with three high IL-6 measures (N=138, 24 new GHQ-cases) had an odds ratio of 1.42 (0.78-2.57). This analysis may not be well powered given the small number of new GHQ-cases among those with chronic inflammation (24 new cases) and that those with high IL-6 levels in 1992, 1997 and 2003 and common mental disorder in 2003 were excluded by design.

Table S1. Demographic Characteristics of Participants in the Total Whitehall II Population and Those Included in Study Designs A to D.

Characteristic in 1985	Total population		Design A		Design B		Design C		Design C	
	No. of participants	Mean (SD) or %	No. of participants	Mean (SD) or %	No. of participants	Mean (SD) or %	No. of participants	Mean (SD) or %	No. of participants	Mean (SD) or %
Mean age, y	10308	44.4 (6.1)	4630	43.8 (5.9)	2757	44.0 (5.9)	2641	43.6 (5.8)	3057	44.0 (5.9)
Sex, % male	10308	66.9	4630	71.8	2757	75.4	2641	74.4	3057	74.5

Table S2. Characteristics of Participants without Chronic Conditions and with Assessment of Inflammation in 1997 (Study Design A), the Whitehall II Study.

Characteristic	No. of participants	Mean (SD) or %
Mean age, y	4630	55.3 (5.9)
Sex, % male	4630	71.8
Interleukin-6, pg/mL	4630	1.46 (0.59)[a]
Body mass index, kg/m^2	4626	25.8 (3.8)
Obesity, %	4626	12.1
Acute inflammation[b], %	4607	2.7
Anti-inflammatory medication, %	4630	7.1
Hormonal contraceptive or hormone replacement therapy (women only), %	1308	22.3
Antidepressant therapy, %	4625	2.4
Common mental disorder, %	4630	21.4

[a] The values presented are the geometric mean of interleukin-6 and the SD of the $\log_e$(IL-6) distribution.
[b] C-reactive protein >10mg/L.

Table S3. Association of Long-term Interleukin 6 Level[a] with Subsequent Onset of Common Mental Disorder Among Participants without Such Disorder at Start of Follow-up (Study Design D).

	Future Common Mental Disorder			
	N	N of cases	Odds ratio[b] (95% CI)	P-Value
Log_e IL-6 (per 1SD increment),				
Adjusted for				
Age, sex	3057	634	1.42 (1.21, 1.68)	<0.001
Age, sex, BMI	3057	634	1.42 (1.19, 1.68)	<0.001
Age, sex, acute inflammation	3057	634	1.46 (1.23, 1.73)	<0.001
Age, sex, anti-inflammatory medications	3057	634	1.41 (1.19, 1.66)	<0.001
Age, sex, hormonal contraceptives or HRT	3057	634	1.43 (1.21, 1.68)	<0.001
Age, sex, antidepressants medication	3057	634	1.42 (1.20, 1.67)	<0.001
Fully adjusted[c]	3057	634	1.45 (1.22, 1.73)	<0.001
Subgroups (age- and sex-adjusted)				
Men only	2276	445	1.46 (1.19, 1.78)	<0.001
Women only	781	189	1.34 (1.00, 1.79)	0.05
Excluding those with acute inflammation	2980	619	1.44 (1.22, 1.71)	<0.001
Excluding obese participants	2712	550	1.39 (1.16, 1.67)	<0.001
Excluding those with acute inflammation or on medication[d]	2617	513	1.48 (1.23, 1.79)	<0.001

[a] Average IL-6 level from measurements in 1991 and 1997. New-onset of common mental disorder measured in 2003 and/or 2008 in participants with no common mental disorder in 1997.
[b] Odds ratio associated with a doubling in average IL-6 level.
[c] After a further adjustment for smoking, the fully adjusted odds ratio is 1.43 (95% CI: 1.19, 1.70), p = <0.001 among the 3014 participants with no missing data on smoking or other covariates.
[d] Hormonal contraceptives, hormone replacement therapy, antidepressant or anti-inflammatory medication.

References in Supplement

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