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ORIGINAL ARTICLE

Association of C-reactive protein and interleukin-6 with new-onset fatigue in the Whitehall II prospective cohort study

H. J. Cho[1]*, M. Kivimäki[2], J. E. Bower[1] and M. R. Irwin[1]

[1] *Cousins Center for Psychoneuroimmmunology, UCLA Semel Institute for Neuroscience and Human Behavior, Los Angeles, CA, USA*
[2] *Department of Epidemiology and Public Health, University College London, UK*

Background. Although basic research on neuroimmune interactions suggests that inflammatory processes may play a role in the development of fatigue, population-based evidence on this association is limited. This study examined whether plasma C-reactive protein (CRP) and interleukin-6 (IL-6), biomarkers of systemic inflammation, predict fatigue onset.

Method. The Whitehall II study is a large-scale cohort study conducted in 20 civil service departments in London. Plasma CRP and IL-6 were measured in 4847 non-fatigued participants at phase 3 (1991–1993, aged 39–63 years). Fatigue was assessed using the Vitality subscale of the 36-item Short Form Health Survey (SF-36) at phase 3 and phase 4 (1995–1996).

Results. During a mean follow-up of 3.1 years, 957 new fatigue cases (19.7%) were identified using the pre-established cut-off score of $\leqslant$50 on the Vitality subscale. CRP values were dichotomized as low ($<$1.0 mg/l) or high ($\geqslant$1.0 mg/l) using the Centers for Disease Control/American Heart Association recommendations. Similarly, IL-6 values were also dichotomized as low ($<$1.5 pg/ml) or high ($\geqslant$1.5 pg/ml). After full adjustment for socio-demographic and biobehavioral covariates, the odds ratios for new-onset fatigue were 1.28 [95% confidence interval (CI) 1.09–1.49, $p=0.003$] for high CRP and 1.24 (95% CI 1.06–1.45, $p=0.008$) for high IL-6. Similar results were found when CRP and IL-6 were treated as continuous variables.

Conclusions. Plasma CRP and IL-6 were prospectively associated with new-onset fatigue, supporting the hypothesis that low-grade inflammation has a role in the development of fatigue.

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Key words: C-reactive protein, fatigue, inflammation, interleukin-6, prospective cohort study.

Introduction

Fatigue, i.e. a subjective sense of weariness, tiredness, lack of energy and low vitality, is a highly prevalent symptom with prevalence rates up to 38% in community-dwelling individuals and 43% among primary-care patients (Valdini, 1985; Pawlikowska *et al*. 1994; Cho *et al*. 2009*a*). Fatigue can be a co-morbid symptom for many major medical and psychiatric disorders [e.g. human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS), cancer, multiple sclerosis, chronic fatigue syndrome, major depression and schizophrenia] (Anderson & Ferrans, 1997; Curt, 2000; Amato *et al*. 2001), but it also occurs independently, in otherwise healthy individuals, and can lead to disability and cost for society. In the USA, for example, workers with fatigue are estimated to cost employers $136.4 billion annually in lost productivity (Ricci *et al*. 2007) – far higher compared with $61.2 billion for pain (Stewart *et al*. 2003*b*) and $44.0 billion for depression (Stewart *et al*. 2003*a*). The burden imposed by fatigue in clinical practice is also significant. It is often a difficult symptom to be managed because the available treatments for fatigue are at most of moderate effectiveness, e.g. cognitive behavioral therapy in chronic fatigue syndrome (White *et al*. 2011), amantadine in multiple sclerosis (Compston & Coles, 2002) and modafinil in major depression (Fava, 2006), and also because fatigue can be a side-effect of the treatments targeting the underlying disorders, such as anticancer (Miller *et al*. 2008), antidepressant (Zajecka, 2000) and anti-psychotic pharmacotherapy (Ritsner *et al*. 2002).

* Address for correspondence: H. J. Cho, M.D., Ph.D., Cousins Center for Psychoneuroimmunology, UCLA Semel Institute for Neuroscience and Human Behavior, 300 Medical Plaza, Suite 3156, Los Angeles, CA 90095, USA.
(Email: hjcho@mednet.ucla.edu)

Recently, inflammatory processes have been suggested as playing a role in fatigue through cytokine effects on the central nervous system (Dimsdale & Dantzer, 2007). Studies have shown that administration of pro-inflammatory cytokines such as interleukin (IL)-6 and interferon (IFN)-α induces fatigue in healthy men (Spath-Schwalbe *et al*. 1998) and patients with malignant melanoma (Capuron *et al*. 2002, 2007), respectively. Similarly, inflammatory challenge with endotoxin administration, which leads to an acute elevation of IL-6 and tumor necrosis factor (TNF)-α, increases fatigue level in healthy subjects (Eisenberger *et al*. 2010). However, these experimental strategies resulting in a highly robust and acute immune activation might not reproduce the effects of low-grade chronic inflammation which is thought to be responsible for many pathological processes (Danesh *et al*. 2000).

To date, research on the association between low-grade systemic inflammation and fatigue has yielded conflicting results represented by positive, null, and even negative – i.e. systemic inflammation being associated with lower risk of fatigue – correlations (summarized in Supplementary Table S1) (Chao *et al*. 1991; Buchwald *et al*. 1997; Cannon *et al*. 1997, 1999; Gupta *et al*. 1997; LaManca *et al*. 1999; Moss *et al*. 1999; Zhang *et al*. 1999; Giovannoni *et al*. 2001; Kashipaz *et al*. 2003; Flachenecker *et al*. 2004; Collado-Hidalgo *et al*. 2006; Heesen *et al*. 2006; ter Wolbeek *et al*. 2007; Vollmer-Conna *et al*. 2007). Furthermore, evidence is limited to a small number of cross-sectional or case–control studies conducted primarily in clinical populations, such as patients with cancer, multiple sclerosis and chronic fatigue syndrome (Chao *et al*. 1991; Buchwald *et al*. 1997; Cannon *et al*. 1997, 1999; Gupta *et al*. 1997; LaManca *et al*. 1999; Moss *et al*. 1999; Zhang *et al*. 1999; Giovannoni *et al*. 2001; Kashipaz *et al*. 2003; Flachenecker *et al*. 2004; Collado-Hidalgo *et al*. 2006; Heesen *et al*. 2006; ter Wolbeek *et al*. 2007; Vollmer-Conna *et al*. 2007). The design of these studies does not address the direction of causality, and the presence of severe medical co-morbidity may either compound or obscure the associations between inflammation and fatigue.

We have previously demonstrated that high levels of C-reactive protein (CRP), a biomarker of systemic inflammation, were associated with fatigue 5 years later in a general adult population, using data from the Coronary Artery Risk Development in Young Adults (CARDIA) study, a community-based prospective cohort study (Cho *et al*. 2009*b*). However, the only marker of systemic inflammation measured was CRP, an acute-phase reactant that does not cross the blood–brain barrier, and no pro-inflammatory cytokines were included in the study. Furthermore, the assessment of fatigue relied on a single item rather than a composite measure, hence not allowing a comprehensive evaluation of this construct. This limitation also impeded the identification of fatigue cases, a categorical classification approach that would assist in translating the research findings into the clinical context and in generating data on the incidence or new-onset of illnesses/symptoms.

Therefore, using data from the Whitehall II study, an ongoing large-scale occupational cohort study, we examined whether low-grade systemic inflammation, measured by CRP and IL-6, a pro-inflammatory cytokine, predicted the onset of fatigue 3 years later, assessed using the Vitality subscale of the 36-item Short Form Health Survey (SF-36), a valid and reliable four-item measure of energy–fatigue supported by both observational and experimental data (Ware, 1993). By adopting this measure, which has a pre-established cut-off score for well-being *versus* limitation/disability related to fatigue, we were able to identify cases of fatigue. Specifically, we first aimed to confirm the findings of our prior work (Cho *et al*. 2009*b*) by assessing prospective associations between inflammatory markers and fatigue as continuous variables, and then aimed to provide new information within the clinical context of case prediction by assessing prospective associations between inflammatory markers and new-onset cases of fatigue as categorical variables.

Method

Subjects

The Whitehall II study is a prospective cohort study of 10 308 civil servants (6895 men and 3413 women) working in 20 departments based in London, aged 35–55 years at study inception in 1985–1988 (phase 1). Since then, there have been seven further data collection phases. Odd-numbered phases include both a clinical examination and a self-administered questionnaire, while even-numbered phases are questionnaire only. Informed consent was obtained from all participants. Ethical approval was obtained from the University College London Medical School Committee on the Ethics of Human Research. Full details of the study design and methods have been published previously (Marmot & Brunner, 2005).

For the purpose of the present study, phase 3 (1991–1993) is considered the baseline, as it was the first clinical examination with a measurement of inflammatory markers. Phase 4 (1995–1996) is considered the follow-up. Initially, for the analysis of cross-sectional associations at baseline, 7509 participants were selected as they had inflammatory markers – either CRP or IL-6 – and fatigue assessed at

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baseline (aged 39–63 years). For the analysis of prospective associations, 819 participants were excluded as they did not have fatigue assessed at follow-up, hence leaving 6690 for the prospective analysis (mean follow-up time 3.1 years, range 1.9–4.6 years). These 6690 individuals were slightly older, more likely to be male, more likely to be white, and more likely to have high employment grades compared with those excluded (Supplementary Table S2). However, the differences were small, reaching statistical significance mostly due to the large sample size. In addition, given the focus of the study on fatigue onset, 1843 participants were excluded as they evidenced fatigue 'caseness' at baseline, with $\leqslant 50$ on the SF-36 Vitality subscale (Ware, 1993). Hence, the remaining 4847 non-fatigued individuals were considered for the analysis of the associations between inflammatory markers and new-onset fatigue. Specifically, 4822 were included in the analysis of CRP and 4786 in the analysis of IL-6. Compared with the rest of the sample, these 4847 non-fatigued individuals were older, included a higher proportion of males, and had higher employment grades (Supplementary Table S3).

Inflammatory markers

Venous blood was taken in the fasting state or at least 5 h after a light, fat-free breakfast. CRP was measured using a high-sensitivity immunonephelometric assay in a BN ProSpec nephelometer (Dade Behring, UK). IL-6 was measured using a high-sensitivity enzyme-linked immunosorbent assay (ELISA) (R&D Systems, UK). Values lower than the detection limit (0.154 mg/l for CRP and 0.08 pg/ml for IL-6) were assigned a value equal to half the detection limit. To measure short-term biological variation and reproducibility of the assessment, a repeated sample was taken from a subset of 150 participants for CRP and 241 for IL-6 (average time between samples 32 days). Reliability (Pearson's r) between samples was $r = 0.77$ for CRP and $r = 0.61$ for IL-6 (Gimeno *et al*. 2011).

Given the skewed distribution of CRP and IL-6, the values were log-transformed. Initially, log-transformed CRP and IL-6 as continuous variables were used for the analyses of cross-sectional and prospective associations between inflammatory markers and fatigue without excluding fatigue cases at baseline. Subsequently, in order to investigate the prediction of new-onset fatigue by inflammatory markers, both CRP and IL-6 were categorized. CRP values were dichotomized as low (<1.0 mg/l) or high ($\geqslant 1.0$ mg/l) using the Centers for Disease Control/American Heart Association (CDC/AHA) criteria, originally recommended for the risk assessment of cardiovascular disease (Pearson *et al*. 2003). Using 1.0 mg/l as the cut-off, Liukkonen *et al*. (2006) previously found a significant association between CRP and depression in a large cohort. In the current sample, this cut-off approximately corresponded to the median and defined 54.6% of the initially selected 7509 participants as having low CRP. The categorization of CRP into three groups using the CDC/AHA criteria (<1.0 mg/l, 1.0–3.0 mg/l, and >3.0 mg/l) yielded almost identical results, supporting the use of dichotomized CRP (Supplementary Table S4). Similarly, IL-6 values were also dichotomized as low (<1.5 pg/ml) or high ($\geqslant 1.5$ pg/ml). As there is no available guideline for the categorization of IL-6, we chose the cut-off that would roughly reproduce the distribution of participants determined by the CRP categorization. Consequently, the cut-off of 1.5 pg/ml approximately corresponded to the median and defined 55.3% of the initially selected 7509 participants as having low IL-6. Finally, to explore the combined effect of CRP and IL-6, we generated a composite variable of both inflammatory markers with the following four categories: low CRP and low IL-6 (i.e. absence of inflammation); high IL-6 and low CRP (i.e. acute inflammation that is not of sufficient magnitude or duration to induce CRP); low IL-6 and high CRP (i.e. indicative of inflammation that may have been initiated but is no longer sustained by activation of IL-6); and high IL-6 and high CRP. IL-6 induces CRP; hence elevated levels of both IL-6 and CRP are indicative of activation of proximal as well as distal components of systemic inflammation, and might be indicative of an ongoing and persistent state of inflammation (Pepys & Hirschfield, 2003; Cole *et al*. 2011).

Main outcome measure

Fatigue was measured using the Vitality subscale of the SF-36, referring to the past 4 weeks (Ware *et al*. 1996). The SF-36 Vitality subscale is a valid and reliable four-item measure of energy–fatigue supported by both observational and experimental data (O'Connor, 2004). Moreover, the SF-36 Vitality subscale is one of the most frequently used measures of energy–fatigue in a variety of subject groups including arthritis patients, cancer survivors and the general population, and correlates highly with other fatigue measures such as the Chalder Fatigue Questionnaire and the Piper Fatigue Scale (Andrykowski *et al*. 1998; O'Connor, 2004; Wolfe, 2004; Dagfinrud *et al*. 2005). The standardized scores range from 0 to 100, higher scores reflecting higher vitality, i.e. less severe fatigue, and scores greater than the midpoint of 50 representing well-being, whereas scores of $\leqslant 50$ represent limitations or disability related to fatigue (Ware, 1993). This variable was first used as a continuous variable

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and then dichotomized using a cut-off of 50, therefore defining fatigue caseness as a score of $\leqslant 50$.

Potential biobehavioral confounders

Potential biobehavioral confounders to be included in the multivariable analysis were obtained from phase 3. Sociodemographic variables included age, sex, ethnicity (white or non-white), and socio-economic position based on each participant's last known civil service employment grade, categorized as high (administrators), middle (executives, professionals and technical staff) and low (clerical and office support staff) (Kumari *et al*. 2004). Biomedical variables, measured according to standard protocols, included body mass index (BMI in kg/m^2), systolic blood pressure (mmHg), presence of common medical conditions (diabetes, diagnosed heart disease, or respiratory illness), and use of prescription medications that could affect systemic inflammatory status or fatigue severity (lipid-lowering drugs, aspirin, oral steroids, oral contraceptives, hormone replacement therapy, antidepressants and hypnotics). Health-related behaviors included current smoking status (yes or no) and alcohol consumption (units of alcohol in the last week) (Kumari *et al*. 2004). Fatigue-related symptoms included symptoms of psychological distress (i.e. symptoms of depression and anxiety as measured by the 30-item General Health Questionnaire) (Goldberg, 1972; Stansfeld & Marmot, 1992) and sleep difficulty (presence or absence in the last 14 days).

Analysis

First, the cross-sectional associations between inflammatory markers and fatigue at baseline were examined by performing multivariable linear regression analyses, using these variables as continuous. To facilitate comparison across models, standardized regression coefficients (β) were calculated, which express the change in standardized fatigue score per 1 standard deviation in log-transformed CRP or IL-6 concentration. Similarly, the prospective associations between baseline inflammatory markers and fatigue at follow-up were examined by performing multivariable linear regression analyses, again using these variables as continuous. Subsequently, the prospective associations between inflammatory markers and newly onset cases of fatigue were examined by performing multivariable logistic regression analyses, using these variables as categorical. Covariates were selected based on external clinical judgment rather than predetermined p-value criteria; the latter approach, which selects factors for inclusion in a multivariable model only if the factors are 'statistically significant' in bivariate screening, is considered less optimal (Steyerberg *et al*. 2000). All covariates were assessed at baseline. Models were adjusted for age, sex and ethnicity, since they influence the distribution of the inflammatory markers (Wener *et al*. 2000). Further multivariable models also included each of the following sets of variables in turn: socio-economic position (employment grade), biomedical factors (BMI, systolic blood pressure, common medical conditions, and use of prescription medication that could affect systemic inflammatory status), health-related behaviors (smoking and alcohol consumption) and fatigue-related symptoms (depression/anxiety and sleep difficulty). Finally, the analysis was repeated with simultaneous adjustment for all the above covariates. Age, sex and ethnicity were tested for potential effect modification. Analyses were performed using Stata version 12.0 (StataCorp LP, USA).

Results

Baseline characteristics

Table 1 describes the characteristics of 7509 participants who had data on either of the inflammatory markers and fatigue at baseline by sex. Levels of CRP and IL-6 were higher in women than men. Women had lower vitality and consequently were more likely to be classified as fatigue cases. Overall, regardless of sex, 27.9% of 7509 participants were classified as fatigue cases. Women were older, included a higher proportion of non-white participants, and were more likely to be from the low employment grade. Women had higher BMI and higher likelihood of using prescription medications that could affect systemic inflammatory status or fatigue severity but had lower blood pressure and lower prevalence of common medical conditions. Women had a better profile of health-related behaviors, smoking less and drinking less. Women presented with a higher level of psychological distress (i.e. depression/anxiety) and reported more sleep difficulty than men. CRP and IL-6 levels were positively correlated ($r = 0.37$, $p < 0.0001$).

Cross-sectional associations between inflammatory markers and fatigue

At baseline, there were significant cross-sectional associations between inflammatory markers (CRP and IL-6) and fatigue, both treated as continuous variables, after adjustment for age, sex and ethnicity (Table 2). More specifically, higher levels of inflammatory markers were associated with lower levels of vitality (as represented by negative values of β), hence higher levels of fatigue. These associations remained statistically significant in the subsequent multivariable

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Table 1. *Characteristics of the participants at baseline* ($n = 7509$[a])

Variable	Men ($n = 5219$)	Women ($n = 2290$)	p[b]
Main variables			
Median CRP, mg/l (interquartile range)	0.85 (0.43–1.72)	1.01 (0.47–2.36)	<0.001
Median IL-6, pg/ml (interquartile range)	1.35 (0.99–1.93)	1.57 (1.11–2.45)	<0.001
Mean SF-36 Vitality score (S.D.)	63.9 (17.4)	57.9 (19.8)	<0.001
Fatigue, *n* (%)	1267 (24.3)	829 (36.2)	<0.001
Sociodemographics			
Mean age, years (S.D.)	49.3 (6.0)	50.2 (6.1)	<0.001
White ethnicity, *n* (%)	4822 (92.4)	1951 (85.2)	<0.001
Low employment grade, *n* (%)	346 (6.6)	909 (39.7)	<0.001
Biomedical variables			
Mean BMI, kg/m² (S.D.)	25.1 (3.2)	25.7 (4.7)	<0.001
Mean systolic blood pressure, mmHg (S.D.)	121.8 (13.2)	117.7 (14.1)	<0.001
Presence of common medical conditions[c], *n* (%)	883 (16.9)	327 (14.3)	0.005
Use of prescription medications[d], *n* (%)	218 (4.2)	348 (15.2)	<0.001
Health-related behaviors			
Current smoking, *n* (%)	651 (12.7)	368 (16.5)	<0.001
Mean alcohol consumption, units in last week (S.D.)	12.3 (13.8)	5.3 (7.4)	<0.001
Fatigue-related symptoms			
Mean depression/anxiety – GHQ-30 score (S.D.)	2.8 (4.9)	3.4 (5.4)	<0.001
Sleep difficulty, *n* (%)	1314 (25.2)	759 (33.2)	<0.001

CRP, C-reactive protein; IL-6, interleukin-6; SF-36, 36-item Short Form Health Survey; S.D., standard deviation; BMI, body mass index; GHQ-30, 30-item General Health Questionnaire.

[a] Participants with data on inflammatory markers (either CRP or IL-6) and fatigue at baseline.

[b] p Value from χ^2 test, t test, or Wilcoxon rank-sum test, respectively, for proportion, mean or median.

[c] Diabetes, diagnosed heart disease or respiratory illness.

[d] Use of prescription medications that affect systemic inflammatory status or fatigue severity, including lipid-lowering drugs, aspirin, oral steroids, oral contraceptives, hormone replacement therapy, antidepressants and hypnotics.

Table 2. *Cross-sectional associations between circulating inflammatory markers* (*CRP and IL-6*) *and fatigue at baseline*

	CRP as predictor			IL-6 as predictor		
Adjustment[a]	*n*	β	*p*	*n*	β	*p*
Age, sex, ethnicity (model A)	7476	−0.055	<0.001	7422	−0.044	<0.001
A + socio-economic position	7471	−0.056	<0.001	7417	−0.046	<0.001
A + biomedical factors	7408	−0.033	0.006	7358	−0.031	0.010
A + health-related behaviors	7328	−0.050	<0.001	7274	−0.037	0.002
A + fatigue-related symptoms	7466	−0.049	<0.001	7412	−0.040	<0.001
Fully adjusted	7249	−0.032	0.003	7199	−0.027	0.013

CRP, C-reactive protein; IL-6, interleukin-6; β, standardized regression coefficient expressing the change in standardized fatigue score per 1 standard deviation in log-transformed CRP or IL-6 concentration.

[a] Biomedical factors include body mass index, systolic blood pressure, presence of common medical conditions, and use of prescription medications that could affect systemic inflammatory status. Health-related behaviors include smoking and alcohol consumption. Fatigue-related symptoms include psychological distress and sleep difficulty at baseline.

models, including the fully adjusted model, which further controlled for socio-economic position, BMI, systolic blood pressure, presence of common medical conditions, use of prescription medications that could affect systemic inflammatory status or fatigue severity, smoking, alcohol consumption, symptoms of depression/anxiety and sleep difficulty (adjusted $\beta = -0.032$ for CRP, $p = 0.003$; adjusted $\beta = -0.027$ for IL-6, $p = 0.013$) (Table 2). In practical terms, $\beta = -0.032$ means that, for every 1 standard deviation increase of log-transformed CRP, there was a decrease of 0.59 in the SF-36 Vitality subscale.

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Table 3. *Prospective associations of circulating inflammatory markers (CRP and IL-6) at baseline with fatigue at follow-up*

	CRP as predictor			IL-6 as predictor		
Adjustment[a]	*n*	*β*	*p*	*n*	*β*	*p*
Age, sex, ethnicity (model A)	6658	−0.052	<0.001	6614	−0.042	0.001
A + socio-economic position	6654	−0.052	<0.001	6610	−0.042	0.001
A + biomedical factors	6600	−0.033	0.011	6559	−0.031	0.013
A + health-related behaviors	6532	−0.047	<0.001	6488	−0.035	0.005
A + fatigue-related symptoms	6649	−0.046	<0.001	6605	−0.039	0.001
Fully adjusted	6465	−0.025	0.048	6424	−0.025	0.044

CRP, C-reactive protein; IL-6, interleukin-6; *β*, standardized regression coefficient expressing the change in standardized fatigue score per 1 standard deviation in log-transformed CRP or IL-6 concentration.

[a] Biomedical factors include body mass index, systolic blood pressure, presence of common medical conditions, and use of prescription medications that could affect systemic inflammatory status. Health-related behaviors include smoking and alcohol consumption. Fatigue-related symptoms include symptoms of depression/anxiety and sleep difficulty at baseline.

Prospective associations between inflammatory markers and fatigue

Table 3 describes the prospective associations of baseline inflammatory markers (CRP and IL-6) with fatigue at follow-up, both treated as continuous variables. High plasma concentrations of inflammatory markers at baseline predicted fatigue at follow-up about 3 years later when adjusted for age, sex and ethnicity. These associations remained statistically significant in the subsequent multivariable models, including the fully adjusted model (adjusted $\beta = -0.025$, $p = 0.048$ for CRP; adjusted $\beta = -0.025$, $p = 0.044$ for IL-6). In practical terms, −0.025 means that, for every 1 standard deviation increase of log-transformed CRP, there was a decrease of 0.49 in the SF-36 Vitality subscale.

Among the covariates, the fully adjusted model with CRP indicated depression/anxiety as having the strongest effect on fatigue (adjusted $\beta = -0.237$, $p < 0.001$). Similarly, the fully adjusted model with IL-6 also indicated depression/anxiety as having the strongest effect on fatigue (adjusted $\beta = -0.237$, $p < 0.001$).

Prospective associations between inflammatory markers and new-onset fatigue

Of 4847 participants free of fatigue at baseline, 957 (19.7%) developed new fatigue at follow-up. As shown in Table 4, after adjustment for age, sex and ethnicity, those with high CRP at baseline had 35% higher odds of developing fatigue compared with those with low CRP [odds ratio (OR) 1.35, 95% confidence interval (CI) 1.17–1.56, $p < 0.001$], and those with high IL-6 at baseline had 27% higher odds of developing fatigue compared with those with low IL-6 (OR 1.27, 95% CI 1.10–1.47, $p = 0.001$). Table 4 also describes the contribution of the four sets of covariates to the associations between inflammatory markers at baseline and new-onset fatigue at follow-up. The full adjustment indicated that both CRP and IL-6 were significant independent predictors of new-onset fatigue. After adjusting for age, sex, ethnicity, socio-economic position, BMI, systolic blood pressure, presence of common medical conditions, use of prescription medications, smoking, alcohol consumption, depression/anxiety and sleep difficulty, the respective ORs for CRP and IL-6 were 1.28 (95% CI 1.09–1.49, $p = 0.003$) and 1.24 (95% CI 1.06–1.45, $p = 0.008$). No effect modification was observed for age, sex, ethnicity or education.

The categorization of CRP into three groups using the CDC/AHA criteria (<1.0 mg/l, 1.0–3.0 mg/l and >3.0 mg/l) yielded almost identical results (Supplementary Table S4). A similar pattern was observed when IL-6 was categorized into three groups using the cut-offs that would roughly reproduce the distribution of participants determined by the CRP categorization (<1.5 pg/ml, 1.5–2.5 pg/ml and >2.5 pg/ml) (Supplementary Table S4).

Combined effect

Additional analyses explored whether the combination of elevated CRP and IL-6 had additive predictive effects on fatigue caseness. Having both inflammatory markers at low levels was defined as the reference category, and this included 40.0% of the participants. The category of low CRP and high IL-6 included 16.0% of the participants and that of high CRP and low IL-6 included 16.4%. The last category with both markers at high levels included 27.6% of participants. As shown in Table 5, after adjustment for age, sex and ethnicity, only membership in the last category was significantly associated with increased

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Table 4. *Prospective associations of circulating inflammatory markers (CRP and IL-6) at baseline with new-onset fatigue cases at follow-up*

	CRP as predictor				IL-6 as predictor			
Adjustment[a]	Low[b]	Total[b]	Odds ratio[c] (95% CI)	*p*	Low[b]	Total[b]	Odds ratio[c] (95% CI)	*p*
Age, sex, ethnicity (model A)	2708	4822	1.35 (1.17–1.56)	<0.001	2700	4786	1.27 (1.10–1.47)	0.001
A + socio-economic position	2706	4819	1.34 (1.16–1.55)	<0.001	2700	4783	1.27 (1.09–1.47)	0.002
A + biomedical factors	2684	4782	1.34 (1.15–1.56)	<0.001	2768	4747	1.25 (1.07–1.46)	0.004
A + health-related behaviors	2673	4737	1.31 (1.13–1.51)	<0.001	2658	4701	1.24 (1.07–1.44)	0.005
A + fatigue-related symptoms	2705	4816	1.34 (1.16–1.56)	<0.001	2699	4780	1.29 (1.11–1.50)	0.001
Fully adjusted	2644	4689	1.28 (1.09–1.49)	0.003	2635	4654	1.24 (1.06–1.45)	0.008

CRP, C-reactive protein; IL-6, interleukin-6; CI, confidence interval.

[a] Biomedical factors include body mass index, systolic blood pressure, presence of common medical conditions, and use of prescription medications that could affect systemic inflammatory status. Health-related behaviors include smoking and alcohol consumption. Fatigue-related symptoms include symptoms of depression/anxiety and sleep difficulty at baseline.

[b] Number of individuals in low categories and total number.

[c] CRP and IL-6 were dichotomized using the respective cut-off points of 1.0 mg/l and 1.5 pg/ml. Odds ratios were calculated taking low categories (CRP <1.0 mg/l and IL-6 <1.5 pg/ml) as the reference.

Table 5. *Prospective associations of the composite variable of CRP and IL-6 at baseline with new-onset fatigue cases at follow-up*

Adjustment	Level[a]	*n*	Odds ratio (95% CI)	*p*
Age, sex, ethnicity	Low CRP and low IL-6	1906	1	
	Low CRP and high IL-6	761	1.07 (0.86–1.34)	0.526
	High CRP and low IL-6	780	1.15 (0.93–1.43)	0.198
	High CRP and high IL-6	1314	1.51 (1.26–1.81)	<0.001
Fully adjusted[b]	Low CRP and low IL-6	1863	1	
	Low CRP and high IL-6	741	1.07 (0.85–1.35)	0.544
	High CRP and low IL-6	759	1.10 (0.88–1.37)	0.419
	High CRP and high IL-6	1267	1.45 (1.19–1.77)	<0.001

CRP, C-reactive protein; IL-6, interleukin-6; CI, confidence interval.

[a] The respective cut-off points for CRP and IL-6 were 1.0 mg/l and 1.5 pg/ml.

[b] Adjusted for age, sex, ethnicity, socio-economic position, body mass index, systolic blood pressure, presence of common medical conditions, use of prescription medications that could affect systemic inflammatory status, smoking, alcohol consumption, symptoms of depression/anxiety and sleep difficulty.

risk of developing fatigue compared with the reference category (OR 1.51, 95% CI 1.26–1.81, $p<0.001$). This association remained significant after the full adjustment for age, sex, ethnicity, socio-economic position, BMI, systolic blood pressure, presence of common medical conditions, use of prescription medications, smoking, alcohol consumption, depression/anxiety and sleep difficulty (OR 1.45, 95% CI 1.19–1.77, $p<0.001$).

Discussion

In a large sample of British civil servants, higher levels of circulating inflammatory markers, CRP and IL-6, were cross-sectionally and prospectively associated with fatigue. Furthermore, higher levels of circulating CRP and IL-6 predicted new-onset fatigue about 3 years later. These associations were independent of a series of risk factors such as sociodemographic characteristics, BMI, systolic blood pressure, presence of common medical conditions, use of prescription medications, smoking, alcohol consumption, symptoms of depression/anxiety and sleep difficulty. Additionally, when the data were analysed using CRP and IL-6 as a combined variable rather than separately, participants with both markers at high levels were at a significant risk of developing fatigue, while those who had only one of the markers at high levels

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were not. Elevated levels of both of these inflammatory markers are more likely in the setting of ongoing (e.g. elevations of IL-6) and persistent (e.g. elevations of CRP) inflammation rather than temporarily heightened inflammation.

Comparison with other studies

Although an association between systemic inflammation and fatigue has been reported in cancer survivors, the implications of those data for the non-medical general adult population is unknown due to the confounding influence of cancer diagnosis and related treatments (for reviews, see Schubert *et al*. 2007; Miller *et al*. 2008; Saligan & Kim, 2012). Among persons with chronic fatigue syndrome (for a review of recent work, see Klimas *et al*. 2012), overproduction (Chao *et al*. 1991; Buchwald *et al*. 1997; Cannon *et al*. 1997, 1999; Gupta *et al*. 1997; Moss *et al*. 1999), reduced production (ter Wolbeek *et al*. 2007) and no difference (LaManca *et al*. 1999; Zhang *et al*. 1999; Kashipaz *et al*. 2003; Vollmer-Conna *et al*. 2007) of pro-inflammatory cytokines have been reported as compared with controls, with similar conflicting results in patients with multiple sclerosis (Giovannoni *et al*. 2001; Flachenecker *et al*. 2004; Heesen *et al*. 2006) (see Supplementary Table S1 for a summary). In a correlational study of 40 healthy young adults, no association of fatigue with TNF-α or CRP was found, although this could have been due to limited statistical power (Corwin *et al*. 2002). Our previous analysis using the CARDIA study data overcame the limitations of the previous studies; however, it was still limited because the assessment of fatigue relied on a single item rather than a composite measure and CRP was the only marker of systemic inflammation measured (Cho *et al*. 2009*b*).

Strengths and limitations

Derived from a large prospective cohort study, the current data largely overcome the limitations of prior studies and suggest that low-grade systemic inflammation plays a role in the development of fatigue. The main outcome was assessed using a valid and reliable composite measure of fatigue supported by both observational and experimental data (Ware, 1993). The current study employed two systemic inflammatory markers involved in different steps of the inflammation process, a pro-inflammatory cytokine and an acute-phase reactant, respectively corresponding to a proximal and a distal step of the cascade. By including only fatigue-free participants at baseline, the prediction of new-onset fatigue was evaluated. Given that the findings were obtained from a non-medical occupational cohort, it does not appear that fatigue is simply a byproduct of medical disorders and related inflammation. Lastly, as noted above, the association between inflammatory markers and fatigue was independent of a series of confounding variables such as obesity, depression/anxiety, sleep difficulty, use of prescription medications and presence of common medical conditions.

The following limitations should be considered. First, this was not an incidence study, as the study design only allowed the identification of fatigue present during the 4 weeks prior to the follow-up assessment. It is possible that some participants may have had transient fatigue sometime between the baseline and the follow-up, and hence not identified by the study. For this reason, we could not estimate any incidence and purposely used the term 'new-onset' instead of 'incident'. Second, although we carefully performed multivariable analyses considering a series of potential risk factors, it is still possible that there is some residual confounding since this was not a randomized controlled trial, the only approach that can eliminate the confounding effect entirely. There could be unmeasured confounding variables accounting for some of the association between inflammatory markers and fatigue. Hence, although this study suggests a possible causal link between low-grade systemic inflammation and fatigue, no definite causality can be established. Third, we measured inflammatory markers only at one point in time. Further research is needed to examine whether duration of inflammation, based on repeat data, is associated with the risk of new-onset fatigue in a dose–response manner.

Possible mechanisms

The mechanisms that drive increases of inflammation and symptoms of fatigue in a non-clinical adult population such as the current sample are unknown. Experimental studies suggest that physical and psychological stressors activate the peripheral immune system, mounting an inflammatory response with the release of pro-inflammatory cytokines and acute-phase proteins ('signal generated') (Black, 2002). These peripheral inflammatory signals are then transduced to the brain through specific pathways across the blood–brain barrier such as vagal nerve afference and IL-1 receptors located on endothelial cells of brain venules ('signal received'), and the brain finally may produce sickness behaviors including fatigue ('response to signal') (Dantzer *et al*. 2008). While extensive research efforts have accumulated mechanistic evidence on the 'generation' and 'reception' of inflammatory signals (Black, 2002), the specific

mechanisms of how the brain 'responds' to these signals producing the symptom of fatigue are still to be elucidated. To date, basal ganglia hypermetabolism – hence altered dopaminergic activities – has been related to physical fatigue and anterior cingulate activation to mental fatigue during IFN-α therapy of patients with malignant melanoma (Capuron *et al*. 2005, 2007). The current findings extend the existing mechanistic knowledge of inflammatory biology to the study of fatigue.

Conclusion and implication

Population-based evidence on a robust association of CRP and IL-6 with new-onset fatigue is consistent with the hypothesis that low-grade systemic inflammation may play an important role in the development of fatigue. No validated prediction algorithms or specific pharmacotherapies with demonstrated effectiveness are currently available for fatigue. These findings suggest that inflammatory markers might provide an incremental component to risk prediction models for fatigue.

Supplementary material

For supplementary material accompanying this paper visit http://dx.doi.org/10.1017/S0033291712002437.

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Declaration of Interest

None.

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