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Immune-neuroendocrine patterning and response to stress. A latent profile analysis in the English longitudinal study of ageing

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ABSTRACT

Psychosocial stress exposure can disturb communication signals between the immune, nervous, and endocrine systems that are intended to maintain homeostasis. This dysregulation can provoke a negative feedback loop between each system that has high pathological risk. Here, we explore patterns of immune-neuroendocrine activity and the role of stress. Using data from the English Longitudinal Study of Ageing (ELSA), we first identified the latent structure of immune-neuroendocrine activity (indexed by high sensitivity C-reactive protein [CRP], fibrinogen [Fb], hair cortisol [cortisol], and insulin growth-factor-1 [IGF-1]), within a population-based cohort using latent profile analysis (LPA). Then, we determined whether life stress was associated with membership of different immune-neuroendocrine profiles. We followed 4,934 male and female participants, with a median age of 65 years, over a four-year period (2008–2012). A three-class LPA solution offered the most parsimonious fit to the underlying immune-neuroendocrine structure in the data, with 36 %, 40 %, and 24 % of the population belonging to profiles 1 (*low-risk*), 2 (*moderate-risk*), and 3 (*high-risk*), respectively. After adjustment for genetic predisposition, sociodemographics, lifestyle, and health, higher exposure to stress was associated with a 61 % greater risk of belonging to the *high-risk* profile (RRR: 1.61; 95 %CI = 1.23–2.12, $p = 0.001$), but not the *moderate-risk* profile (RRR = 1.10, 95 %CI = 0.89–1.35, $p = 0.401$), as compared with the *low-risk* profile four years later. Our findings extend existing knowledge on psychoneuroimmunological processes, by revealing how inflammation and neuroendocrine activity cluster in a representative sample of older adults, and how stress exposure was associated with immune-neuroendocrine responses over time.

1. Introduction

Communication between proinflammatory cytokines of the innate immune system with glucocorticoids and their analogs of the neuroendocrine system, is an active continuous process necessary to maintain homeostasis, even in healthy individuals (Shimba et al., 2021; O'Connor, 2008). Proinflammatory cytokines initiate a local inflammatory response that systemically passes through the bloodstream to endocrine and neural foci, where a number of neuroendocrine counterregulatory mechanisms are actuated, provoking a negative feedback loop (Taub, 2008). The received stimulatory signals are then transduced, leading to a complex hormonal and cytokine cascade (Chikanza and Grossman, 2000). This integrative network between the immune, nervous, and endocrine systems is known to control physiologic processes, such as cell growth and differentiation, metabolism, and human behaviour. Dysregulation of this network has negative implications in disease aetiology (Dantzer, 2017; Kany et al., 2019), with the development of a number of physical and mental ill-states, from cardiovascular disease (Iob and Steptoe, 2019) to depression (Iob et al., 2019), and even accelerated ageing (Wagner et al., 2016). The high rates of chronic conditions associated with inflammatory and neuroendocrine dysregulation, along with the advancing age of the population, has provided the impetus to

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identify modifiable factors that could be leveraged to mitigate disease genesis; stress is one such factor (Acabchuk et al., 2017).

An expansive literature has elucidated the role of chronic psychosocial stress (referred to as stress hereafter) as a determinant of morbidity and mortality (Acabchuk et al., 2017; Steptoe and Kivimäki, 2012; Batty, 2020; Hamer et al., 2019; Cohen et al., 2007). Equally, stress has been implicated as a modulator of immune and neuroendocrine activity via psychoneuroimmunological (PNI) pathways (Kiecolt-Glaser et al., 2002; Johnson et al., 2019); that is, the integrative network between the nervous, endocrine, and immune systems. Therefore, if causally related to morbidity and mortality, conceivably via immune-neuroendocrine mechanisms, stress may present as a plausible preventative target to improve population health across a number of physical and mental health domains. However, the dominant position that stress disrupts immune and neuroendocrine integrity is an oversimplification of this biological pathway that fails to account for the reciprocal regulation of these transducing systems (Chikanza and Grossman, 2000; Dantzer, 2017) and their variation among the population (Steptoe et al., 2007). Immune and neuroendocrine interactions may be intensified in the presence of stress (Kiecolt-Glaser et al., 2002; Hamilton et al., 2021), but individuals can have highly heterogeneous patterns of immune and neuroendocrine activity, which may conflate effects and give a partial explanation for the diverse and comorbid clinical outcomes associated with stress in the literature (Acabchuk et al., 2017; Steptoe and Kivimäki, 2012; Batty, 2020; Hamer et al., 2019; Cohen et al., 2007).

The lack of observational evidence on immune and neuroendocrine activity, as measured by their dysregulated responses, may be due to the complexity of the multidirectional exchange between these systems in response to stress (Ménard et al., 2017). Hormonal and neuropeptide mediators that provide the link between the immune and neuroendocrine systems constitute specific axes of interactions (Taub, 2008; Chikanza and Grossman, 2000; Ménard et al., 2017). It is, thus, important to determine from a population perspective how biomarkers representing these integral systems cluster together.

The purpose of the exchange between the immune and neuroendocrine systems is to return to the physiological *status quo ante*, but many studies examine the nature of this regulation at the systemic level without considering how stress interferes with this physiological exchange (Andreassen et al., 2012). These biological responses appear to depend on stress duration and intensity, but our interest here is chronic stress (Johnson et al., 2019; Segerstrom and Miller, 2004). Understanding is further obfuscated by research that treats the mediators of each system as homogeneous constructs, when variation among the population is highly likely (Chikanza and Grossman, 2000). Further, elevated inflammation and HPA-axis hyperactivity have similarities in context of stress and disease (Segerstrom and Miller, 2004), which is paradoxical given the contrasting utility of cytokines and glucocorticoids, and the pleiotropic and redundant action between each (Shimba et al., 2021).

Owing to interindividual and intraindividual variability in biomarkers (de Maat, 1996), genetic variation is another key consideration. As a major determinant of circulating immune and neuroendocrine function, genetic variation plays an important role in susceptibility to disease (Frank et al., 2020), and these biomarkers are of high polygenic heritability (Prins, 2017). It is, therefore, important that genetic markers are accounted for in analyses that explore immune and neuroendocrine traits.

Moreover, despite concerns of inflammaging and somatopause (i.e., age-related increases in plasma concentrations of inflammatory peptide biomarkers and the reduced expression of growth hormone secretion across age; Wagner et al., 2016) there remains a paucity of literature on stress and immune-neuroendocrine activity in older cohorts. This demographic group is increasingly relevant from a public health perspective because of the advancing age of the population. Furthermore, financial strain (Hamilton and Steptoe, 2022), caregiving (Kiecolt-Glaser, 2003), illness, disability (Rhode et al., 2012), divorce (Kiecolt-Glaser, 2018), and bereavement (Schultze-Florey, 2012), are common stressors among older adults. While the accumulated burden of life stress, coupled with limited protective resources, has been associated with worse biological, psychological, and quality of life outcomes (Steptoe and Marmot, 2003).

Latent profile analysis is an estimation and inference methodological development that presents an opportunity to take a precision medicine approach (Kosorok and Laber, 2019) by applying more specificity to population risk to improve treatment personalisation and clinical decision-making. It will address the 'one-size-fits-all' legacy that has infiltrated the literature and contributed to the underwhelming translation of observational findings in sub-populations to clinical trials with small, heterogenous patient samples (Miller and Raison, 2023).

Classifying complex and subtle patterns of immune and neuroendocrine activity in a population-based cohort of older adults through latent profile analysis could be beneficial for three reasons. First, it may help to elucidate uncertainty about immune and neuroendocrine patterning. Second, it could contribute to more targeted preventative treatments and novel therapeutic strategies, such as the identification of biomarkers that characterise patients into subgroups most likely to benefit from cytokine-mediated pharmacological treatments, or the design of more personalised clinical trials through targeted recruitment. Third, it could be a resource for the formulation of more robust hypotheses for future research exploring stress models in immune and neuroendocrine activity, and their subsequent roles in human health and behaviour.

We sought to address these issues in a UK cohort of community-dwelling older adults, to classify and quantify distinct immune and neuroendocrine profiles, and to determine the longitudinal association between psychosocial stress and the revealed profiles. To represent these interrelated, molecular pathways, we selected two positive acute-phase reactants (i.e., C-reactive protein [CRP] and fibrinogen) and two hormones; one catabolic (i.e., hair cortisol), the other anabolic (i.e., insulin-like growth factor-1 [IGF-1]). Each biomarker has been selected for its relevance to immune and neuroendocrine processes in older adults. Positive acute-phase proteins increase as part of the innate immune response to inflammation. CRP, a rapid acting acute-phase protein, activates complement and acts as an opsonin, while fibrinogen is a key but slow reacting coagulation acute-phase protein that influences the erythrocyte sedimentation rate (a non-specific clinical marker of disease activity; Gruys et al., 2005). By contrast, hormones are signalling molecules in the neuroendocrine system that represent the classic response to stress. Cortisol, produced by the adrenal glands in response to stress, helps to regulate various physiological processes, including metabolism and immune function. While IGF-1, implicated in ageing and longevity (Wagner et al., 2016), promotes cell growth, tissue repair, and development (Taub, 2008), so is particularly relevant to our study population (Junnila et al., 2013). We expected heterogeneous patterns of immune and neuroendocrine activity, with two to three subgroups emerging from the data. We also expected psychosocial stress to be longitudinally associated with more adverse immune and neuroendocrine patterns four years later.

2. Method

2.1. Study design

This prospective cohort study used fully anonymised data from the English Longitudinal Study of Ageing (ELSA; Steptoe et al., 2013), a nationally representative, multidisciplinary prospective observational study of the English population aged 50 years and older. To ensure the full age spectrum is maintained, the sample is periodically refreshed with younger participants. The present study used data from ELSA participants at wave 4 (2008), who were followed up four years later at wave 6 (2012). Data collection is performed in participants' homes, via computer-assisted personal interviews (CAPI) and self-completed questionnaires biennially, then nurse visits every 4 years for biological

samples. All participants provide written consent and ethical approval was granted by the National Research Ethics Service (London Multicentre Research Ethics Committee). Full data collection procedures have been reported, in full, by Steptoe and colleagues (2013). A total of 6,572 participants had complete measures and at least one biomarker at baseline. After exclusions of CRP values > 20 mg/L (n = 116), the sample was 6,456. Of these, 1,522 had missing genetic data, leaving an analytic sample of 4,934 (Figure S4).

2.2. Exposures

On the basis of risk identified in the prior (Hamilton and Steptoe, 2022; Kiecolt-Glaser, 2018; Rhode et al., 2012; Schultze-Florey, 2012; Kiecolt-Glaser, 2003), six psychosocial stressors were assessed. We considered only those that occurred at wave 4 (2008). These were measured as a composite score on a scale from no stressful life events to the experience of six stressors. Thus, we estimated an ordinal score as the summation of the presence of six binary stressors. Due to its skewed distribution, we dichotomised this score at the median (low [0–2] vs. high [3-6]) , rather than at the mean (1.51 ± 0.90). Despite this median split, there is an unequal distribution of participants in each group due to the limited number of integer values of this score (0–6):

1. **Financial Strain.** Binary:- the perceived chance of not having enough financial resources in the future to meet needs; categorised by 0; 1–39; 40–60; 61–99; 100 % and dichotomised at > 60 %. The higher the percentage, the higher the belief of having insufficient resources and, thus, the higher the stress experience.
2. **Care Giving.** Binary:- either being an informal caregiver in the past week to an adult who is sick/frail , or being a caregiver during the last month in receipt of Carer's Allowance.
3. **Disability.** Binary:- encounters more than one difficulty with mobility (i.e., walking 100 yards; sitting 2-hours; rising from chairs after sitting long periods; climbing stairs; stooping, kneeling, or crouching; reaching or extending arms above shoulders; pulling or pushing large objects; lifting or carrying objects over 10 lb; picking-up a 5p coin).
4. **Illness.** Binary:- has a longstanding illness or health condition that limits activity.
5. **Bereavement.** Binary:- experienced the death of a parent, spouse, or partner within the past two years.
6. **Divorce.** Binary:- experienced divorce or the breakdown of a long-term relationship within the past two years.

2.3. Outcomes

Immune and neuroendocrine biomarkers measured at wave 6 (2012) included high-sensitivity plasma C-reactive protein (CRP; mg/L), plasma fibrinogen (Fb; g/L), serum insulin-like growth factor-1 (IGF-1; mmol/L) and hair cortisol (cortisol; pg/mg). The complete immunoassay procedure can be found in Supplementary Materials (SM) 1. Blood samples deemed insufficient or unsuitable (e.g., haemolysed; received > 5 days post-collection) were discarded. Exclusion criteria for bloods included coagulation, haematological disorders, being on anticoagulant medication or having a history of convulsions (SM 1). A latent profile analysis (LPA) was then conducted on these immune and neuroendocrine biomarkers, as later described.

2.4. Covariates (Wave 4)

Factors likely to confound analyses were selected *a priori* (see Figure S1 for the Directed Acyclic Graph), including *demographic variables*: age (≥50 years); sex (male; female); *socioeconomic variables*: education (categorised into higher education; primary/secondary/tertiary education; or alternative/none); occupational social class (a three-category version of the National Statistics Socio-Economic Classification (ONS, 2010): managerial and professional; intermediate; routine and manual); *lifestyle variables*: smoking status (binary:- non-smokers/ex-smokers or smokers); alcohol consumption (binary:- low < 3 or high ≥ 3 day weekly); physical activity (binary:- sedentary or moderate/vigorous weekly activity); *genetic variables*: polygenic scores (PGS) for CRP, cortisol, and IGF-1 (methods later described) and 10 principal components to account for population stratification; *biomarkers*: baseline (wave 4) CRP, fibrinogen, and IGF-1 entered into the LPA; (Figures S2-3); *binary health indicators*: any self-reported physician diagnosis of chronic lung disease, coronary heart disease, abnormal heart rhythm, heart murmur, congestive heart failure, angina, hypertension, diabetes, cancer, Parkinson's, Alzheimer's, dementia, asthma, arthritis, osteoporosis, and psychiatric disorder.

2.5. Genetic data

Using PLINK and PRSice software, PGS for CRP, cortisol, and IGF-1 were calculated using summary statistics from genome-wide association studies (GWAS; see SM2; Ajnakina and Steptoe, 2020). A single p-value threshold of 0.001 was used for all PGSs to limit multiple testing, while maximising their potential predictive ability. PGSs were used to account for the proportion of the variability in the biological traits attributable to genetic factors.

Imputation. Missingness ranged from 0.00 to 52.26 %, with cortisol having the greatest proportion of missingness, and other variables having less than 37 % missing (Table S1). Importantly, unbiased results can be obtained from large proportions of missingness (up to 90 %; Madley-Dowd et al., 2019), provided that the missing data pattern is at least Missing at Random (MAR), which we assumed here (Little and Rubin, 2019). Given the possibility of bias in the complete case analyses (Sterne, 2009), missing values on exposures, covariates, and outcomes were imputed using missForest (Stekhoven and Bühlmann, 2012). This is an algorithm based on Random Forests, a machine learning iterative imputation method in R v.4.2.0: RStudio v.2022.02.2. We did not impute missing genetic data; participants without genetic information were excluded from the analyses, as detailed in the analytic sample formation (Figure S4). The imputation of the missing values yielded minimal error for continuous variables (Normalized Root Mean Squared Error = 0.02 %) and categorical variables (Proportion of Falsely Classified = 0.07 %). Imputed and observed data were comparable in terms of their summary distributions on participant characteristics (Table S1).

2.6. Statistical analyses

First, we reported baseline (wave 4) characteristics, expressed as means and proportions. Fibrinogen was normally distributed but logarithmic transformation was performed on CRP, Cortisol, and IGF-1 values because of their originally skewed distribution.

Second, we conducted an LPA to determine patterns of immune and neuroendocrine activity at both waves. The optimal number of profiles was identified using a stepwise approach. Starting with a single-profile model, additional profiles were added to determine whether it improved the model fit. Once the number of latent profiles was determined, each individual in the sample was then assigned to a cluster for which they had the largest posterior probability (i.e., the profile they most likely belonged to). The LPA model for observed variable A can be expressed as:

$$\sigma\frac{2}{A} = \sum_{t=1}^{T} \pi_t(\mu_{At} - \mu_A)^2 + \sum_{t=1}^{T} \pi_t \sigma_{At}^2$$

where μ_{At} and σ_{At}^2 denote (t) class-specific means and variances for variable A, and π_t show the proportion of N participants that belong to class t. The number of latent profiles was determined on the basis of the Akaike information criterion (AIC; Akaike et al., 1998) Bayesian information criterion (BIC; Schwarz, 1978), and adjusted Bayesian

information criterion (aBIC; Bozdogan, 1987). The information criteria and the likelihood ratio tests indicated the goodness of fit of different latent profile models, with the best model being the one with the lowest AIC, BIC, and aBIC values. The entropy statistic that provides the quality of the classification model, and the average posterior probabilities for each latent profile that indicates profile membership classification errors, were also taken into account (Celeux and Soromenho, 1996). The closer to 1 these indicators were, the better the classification quality (Morin et al., 2016). A common cut-off point for posterior probabilities is 0.70 or above (Nagin, 2009). An entropy of 0.80 or greater indicates clear profile separation (Kamata et al., 2018). Every profile must contain more than 5 % of participants and the profiles must be of good theoretical interpretability (Herle, 2020).

Third, we used multinomial logistic regressions to investigate the association between psychosocial stress at wave 4 (2008) and the probability of immune and neuroendocrine profile membership at wave 6 (2012). Results were presented as relative risk ratios (RRR), with standard errors (SE) and 95 % confidence intervals (95 % CI). Analyses were two-tailed. Models with different sets of covariates were fitted to understand their role in the association between stress and immune and neuroendocrine profiles. Model 1 was unadjusted. Model 2 adjusted for baseline immune and neuroendocrine profiles. Model 3 additionally adjusted for *demographic* and *genetic* variables because the predictive value of genetic information can vary by context, particularly age and sex (Jiang et al., 2021). Model 4 adjusted for all covariates. All data analyses were conducted in Stata 17.1 (StataCorp, TX, USA).

2.7. Sensitivity analyses

We conducted seven sensitivity analyses to examine the robustness of our findings. First, to ensure that associations were not dependent on the binary classification of stress, analyses were repeated using an ordinal score of stress (reported as unstandardized regression coefficients with SE). Second, to reveal any differences in stress exposure on profile membership, regressions were repeated using each of the six psychosocial stressors independently. Third, individuals who were disabled or with longstanding limiting illness were more likely to be immunosuppressed given anti-inflammatory prescriptions, thus altering immune and neuroendocrine activity. Therefore, we reconstructed our stress index excluding these measures, then reran our analyses to quantify the extent to which they could have biased our results. Fourth, due to the potentially confounding effects of inflammaging and somatopause (Wagner et al., 2016), along with known differences in stress associations across age (Steptoe et al., 2015), the moderating effect of age was tested (dichotomised by mean age [≥65 years]). Fifth, because of known sex differences in biomarker activity (Klein and Flanagan, 2016), effect modification by sex was tested. Sixth, we wanted to determine genetic variance explained independent of age and sex. Finally, we compared results from our imputed analyses with a complete case analysis (CCA) to understand the potential impact of different approaches to deal with missing data on the results. The analytical sample formation for CCA is illustrated in Figure S5.

3. Results

The final analytic sample was 4,934 (Figure S4). Participant characteristics of the analytic sample were materially unchanged from participants in the core sample (Table S1) and are shown in Table 1. CRP was linearly correlated with fibrinogen ($r = 0.706$); cortisol ($r = 0.273$); and IGF-1 ($r = -0.163$), as fibrinogen was with cortisol ($r = 0.176$; all at $p < 0.001$; Table S2). Participants, male (~45 %) and female (~55 %), with a median age of 65 years old (interquartile range: 59–72; M_{age} = 66.31 ±9.35; $_{range}$50-99) were followed over a four year period (2008–2012). Most were non-smokers (87.27 %) and consumed alcohol less than three days a week (64.27 %), and almost two thirds were sedentary (72.88 %). There was a fairly equal educational (Higher – 32.12 %; Primary/Secondary/Tertiary – 31.29 %; Alternative/None – 36.58 %) and occupational social class divide (Managerial/Professional – 36.28 %; Intermediate Occupations – 25.62 %; Routine/Manual – 38.10 %). There were 8,083 unique documented stress experiences (Figure S6; S7), with many participants experiencing more than one stress indicator. Of our sample, 12.48 % experienced a high level of stress, and this high stress group tended to be younger, female, smokers, who drank less than three alcoholic drinks a week (Table 1). As it

Table 1
Sample characteristics.

Variable		Baseline (N = 4,934) N / M (SD)	% / Range	*t*	χ^2
Age		66.31 (9.35)	50–99	<0.001	
Age (Binary)	< M	2,437	49.39		<0.001
	≥ M	2,497	50.61		
Sex	Male	2,235	45.30		<0.001
	Female	2,699	54.70		
Education	Higher	1,585	32.12		0.961
	Primary/ Secondary/ Tertiary	1,544	31.29		
	Alternative/ None	1,805	36.58		
Occupational Social Class	Managerial/ Professional	1,790	36.28		0.708
	Intermediate Occupations	1,264	25.62		
	Routine/ Manual	1,880	38.10		
Smoking Status	Non-smokers/ Ex-smokers	4,306	87.27		<0.001
	Smokers	628	12.73		
Alcohol Consumption	<3 days a week	3,171	64.27		0.004
	≥3 days a week	1,763	35.73		
Physical Activity	Moderately/ Vigorously Active	1,338	27.12		0.335
	Sedentary	3,596	72.88		
PGS for CRP	Low	3,945	79.96		0.421
	High	989	20.04		
PGS for Cortisol	Low	3,969	80.44		0.482
	High	965	19.56		
PGS for IGF-1	Low	3,929	79.63		0.180
	High	1,005	20.37		
Stress Score (Ordinal)		1.51 (0.90)	0–6	–	
Stress Score (Binary)	No	4,318	87.52		–
	Yes	616	12.48		
CRP* (mg/L; Baseline)		1.19 (0.68)	0.18–3.04	0.915	
CRP* (mg/L; Follow-up)		1.37 (0.73)	0.10–3.05	0.998	
Fibrinogen (g/L; Baseline)		3.38 (0.56)	1.30–5.90	0.728	
Fibrinogen (g/L; Follow-up)		3.12 (0.54)	1.50–5.80	0.984	
Cortisol* (pg/mg; Follow-up)		2.93 (1.34)	0.13–6.49	0.999	
IGF-1* (nmol/L; Baseline)		2.78 (0.34)	1.10–4.19	0.393	
IGF-1* (nmol/L; Follow-up)		2.78 (0.27)	1.61–4.06	0.309	

Notes: ELSA, waves 4–6 (2008/09–2012/13); N = observations; M = Mean; % = percentage frequencies; SD = standard deviations; *t* = *t*-test significance between the exposed and unexposed for continuous variables; χ^2 = Pearson Chi square test significance between the exposed and unexposed for categorical variables; < = less than; ≥ = greater than or equal to; OSC = occupational social class; CRP = C-reactive protein; IGF-1 = Insulin-growth factor-1; * Log-transformed variable; I-N = immune and neuroendocrine.

pertains to each independent stressor, 17.02 % of the sample experienced financial strain, 7.01 % were informal carers, 45.80 % had difficulty mobilising, 31.46 % had a limiting longstanding illness, 40.86 % were bereaved, and 9.18 % were divorcees (Figure S7).

3.1. Latent profile analysis of immune and neuroendocrine biomarkers

A three-profile model of immune and neuroendocrine biomarkers provided the most parsimonious fit to biomarker data at wave 6 (Table S3; Figures S8 [a-g]), after which there were limited returns in AIC and BIC value (Figure S9); entropy was above 0.80 (Figure S10); the mean posterior probabilities did not exceed 0.70; each profile comprised more than 5 % of participants (Figure S11; Table S3); and each profile was theoretically meaningful. The most common profile was 2 (40 %), followed by profile 1 (36 %), then profile 3 (24 %; Figure S12). Profile 1 (M_{age} = 64.16; ±7.77; 36 % of the sample) was defined as '*low-risk*' as it was characterised by those having low CRP, low fibrinogen, low cortisol, and high IGF-1. Profile 2 (M_{age} = 66.59; ±9.38; 40 % of the sample) was the modal group, and consisted of individuals with moderate CRP, fibrinogen, cortisol, and IGF-1 levels, which was defined as '*moderate-risk*'. Finally, profile 3 (M_{age} = 69.03; ±10.62; 24 % of the sample) was marked by a high probability of high CRP, high fibrinogen, high cortisol, and low IGF-1, so this group was defined as '*high-risk*' (Fig. 1).

4. Stress and profile membership of immune and neuroendocrine biomarkers

In the unadjusted model, greater stress was associated with the probability of being in the *high-risk* profile versus *low-risk* profile (Model [M] 1: RRR = 1.34, 95 % CI = 1.08–1.66, p = 0.008). This persisted after adjustment for baseline immune and neuroendocrine profiles (M2: RRR = 1.42, 95 % CI = 1.10–1.83, p = 0.007), further adjustment for *demographic* and *genetic variables* (M3: RRR = 1.80, 95 % CI = 1.39–2.35, p < 0.001), and in our fully adjusted model, the risk of a *high*-immune and neuroendocrine profile was 1.6 times higher in the group exposed to high levels of stress compared with participants with lower stress exposure (M4: RRR = 1.61, 95 % CI = 1.23–2.12, p = 0.001). In the fully adjusted model, however, stress was not associated with the probability of being in the *moderate-risk* profile versus *low-risk* profile (Model M4: RRR = 1.10, 95 % CI = 0.89–1.35, p = 0.401; Table 2). To understand the role of specific confounding factors with greater nuance, results with incremental model adjustment can be found in the supplement (Table S4). There was evidence of negative confounding by *demographic* and *genetic variables,* which increased the RRR by 38 % (M3: RRR = 1.80, 95 % CI = 1.39–2.35, p < 0.001), and by *health* variables, which increased the RRR by 20 % (M3c: RRR = 1.81, 95 % CI = 1.39–2.36, p < 0.001).

4.1. Sensitivity analyses

First, results were consistent when we used an ordinal classification of psychosocial stress. For each single increase in the stress score, individuals were 19 % more likely to be in the *high-risk* immune and neuroendocrine profile versus the *low-risk* profile in our fully adjusted model (M4: RRR = 1.19, 95 % CI = 1.23–2.12, p = 0.001; Table S5). Second, when individual stressors were tested against immune and neuroendocrine profile membership, we found that financial strain (M4: RRR = 1.59, 95 % CI = 1.25–2.01, p < 0.001), limiting longstanding illness (M4: RRR = 1.34, 95 % CI = 1.10–1.65, p = 0.005), and bereavement (M4: RRR = 1.26, 95 % CI = 1.04–1.52, p = 0.016) were each associated with belonging to the *high-risk* profile, as compared with the *low-risk* profile in fully adjusted models. Financial strain and

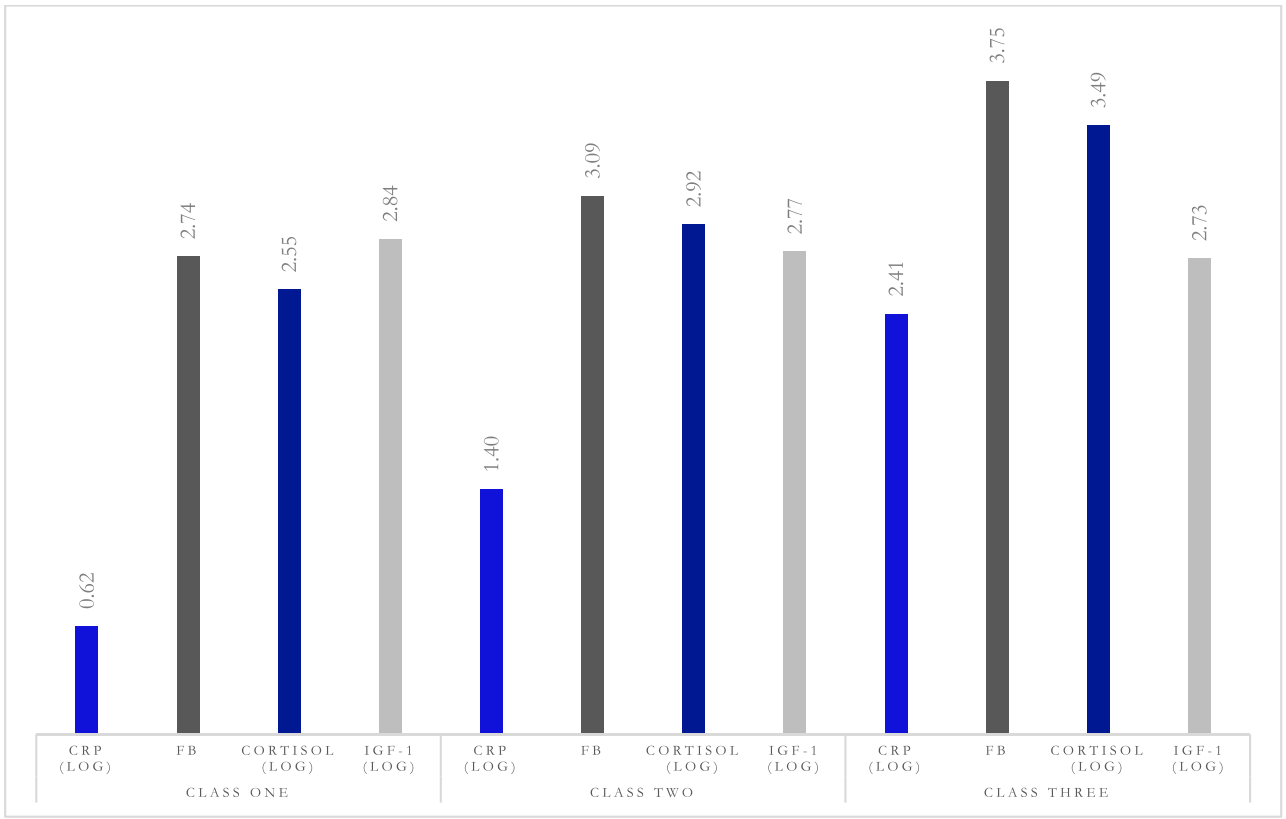

Fig. 1. The mean levels of immune and neuroendocrine biomarkers for a three-profile solution.

Table 2
Longitudinal associations of stress with immune and neuroendocrine biomarker profiles (N = 4,934).

Adjustments	Binary Stress Score				
	RRR	SE	95 % CI		*p*
***Moderate-risk* Profile**					
Model 1: *Unadjusted*	0.98	0.10	0.81	1.20	0.870
Model 2: *Model 1 + baseline biomarkers* [a]	1.01	0.11	0.83	1.24	0.898
Model 3: *Model 2 + demographics & genetics* [b]	1.14	0.12	0.93	1.41	0.213
Model 4: *Fully Adjusted* [c]	1.10	0.12	0.89	1.35	0.401
***High-risk* Profile**					
Model 1: *Unadjusted*	1.34	0.15	1.08	1.66	0.008
Model 2: *Model 1 + baseline biomarkers* [a]	1.42	0.18	1.10	1.83	0.007
Model 3: *Model 2 + demographics & genetics* [b]	1.80	0.24	1.39	2.35	<0.001
Model 4: *Fully Adjusted* [c]	1.61	0.22	1.23	2.12	0.001

Notes: The *low-risk* group is the reference; RRR = relative risk ratio; SE = standard errors; CI = confidence interval; *p* = significance value.
a Baseline biomarkers: C-reactive protein (CRP); fibrinogen; insulin-growth factor-1 (IGF-1).
b Demographic and genetic variables: age; sex; 10 principal components (PCs); CRP polygenic score (PGS); cortisol PGS; IGF-1 PGS.
c All variables: CRP; fibrinogen; IGF-1; age; sex; 10 PCs; CRP PGS; cortisol PGS; IGF-1 PGS; education; occupational social status; smoking status; alcohol consumption; physical activity; health (i.e., chronic lung disease; coronary heart disease; abnormal heart rhythm; heart murmur; congestive heart failure; angina; hypertension; diabetes; cancer; Parkinson's; Alzheimer's; dementia; asthma; arthritis; osteoporosis; psychiatric disorder).

bereavement showed gradients in risk, as each were associated with *high-* and *moderate-risk* profile membership. Caregiving and divorce were not associated with differences in profile membership, while disability was associated with a 30 % lower risk of belonging to the high-risk profile (Table S6[a-f]). Third, the stress index that excluded both disability and limiting long standing illness had higher relative risk coefficients than the primary composite score (M4: RRR = 1.71, 95 % CI = 1.32–2.22, $p < 0.001$), consistent with the previous observation with respect to disability (Table S7). Fourth, we found no evidence of differences in the association between stress and biomarker profile membership between younger and older age groups (interaction $p = 0.913$), although relative risk coefficients were substantially larger for those aged 65 and older (Table S8[a-b]). Fifth, similar to age, there was no interaction ($p = 0.239$) nor difference in the risk profile between the sexes when results were stratified by sex (Table S9[a-b]). Sixth, genetic variables accounted for 1 % of the variance explained for being in the *high-risk* immune and neuroendocrine profile (M3: RRR = 1.80, 95 % CI = 1.39–2.35, $p < 0.001$; Table S10). Finally, we observed similar mean levels of immune and neuroendocrine biomarkers for a three-profile solution in a CCA (Figures S13-14) as compared with the main imputed data (Figure S8c). Re-analysis of the association between stress and profile membership in the CCA sample yielded similar results (Table S11).

5. Discussion

In a large nationally representative sample of UK older adults, we used multiple biomarkers in a latent profile analysis to provide a comprehensive characterisation of physiological activity across the integrative network of the immune, nervous, and endocrine systems. We found longitudinal evidence of an overall association between stress and the risk of *high* versus *low* immune and neuroendocrine profile membership four years later. Associations remained significant after accounting for polygenic markers of immune and neuroendocrine activity, and a range of demographic, socioeconomic, lifestyle, and health factors. There was, however, no consistent gradient in risk as there was no significant difference in stress levels between *low-* and *moderate-risk* profiles, nor were there differences in the association between stress and immune-neuroendocrine profile activity by age or sex. Stress associated with financial strain was the strongest independent determinant of belonging to the *high-risk* immune and neuroendocrine profile, followed by limiting longstanding illness and bereavement. Furthermore, financial strain and bereavement showed gradients in risk. In contrast, disability was associated with a lower risk for *moderate-* and *high-risk* profile membership (vs *low-risk*), the reason for this is unclear, but there is plausible risk of reverse causality (Herle, 2020). Interestingly, our finding that the high-stress group tended to be those who drank less than three alcoholic drinks a week is not an unusual finding. Alcohol has a non-linear relationship with inflammation, where moderate consumption is associated with lower levels of inflammatory markers than with low alcohol consumption, while high consumption is associated with higher inflammatory levels (Pai, 2006; Romeo, 2007; Bektas et al., 2016).

As noted elsewhere (Dhabhar and Mcewen, 1997), the biological responses to stress exposure are multiphasic, where we see the stimulation or suppression of immune and neuroendocrine activity, or both simultaneously (Marshall, 1998), with the direction of effect depending on the biomarker being evaluated (Taub, 2008; Hamilton and Steptoe, 2022). We addressed the complexity of immune and neuroendocrine interconnectivity by using latent profile analyses to identify distinct typologies of activity. Variability was revealed within the derived profiles and highlights why the evaluation of single biomarkers can obfuscate understanding of stress exposure.

Though each biomarker has a unique role in maintaining health, functionally they are involved in proliferation, differentiation, migration, and apoptosis of targeted cells (Garbers, 2012). They are characterised by interrelated pleiotropic, synergistic, and redundant actions that have afferent and efferent functional components (Kany et al., 2019). When this dynamic process is dysregulated, it leads to varying concentrations of circulating biomarkers (Chikanza and Grossman, 2000) that can contribute to diversity in disease sequelae (Kiecolt-Glaser et al., 2002; Hamilton et al., 2021; Chung, 2009). This can make prediction more challenging and the interpretation of single biomarkers less intuitive, particularly because issues of multicollinearity mean that biomarkers are best modelled independently in regressions (Hamilton and Steptoe, 2022). Our latent variable modelling approach, similar to an earlier study of American adults (Yip, 2020), allowed for a synchronised assessment of a diverse set of biomarkers. However, these studies are not comparable because the selection of immune and neuroendocrine biomarkers differed. Even so, our derived profiles lend support to earlier experimental research that indicate symmetry between biomarkers of the immune, nervous, and endocrine systems (O'Connor, 2008; Taub, 2008; Ménard et al., 2017; Rivest, 2010), and our results confirm that biomarkers are, on average, temporally stable, despite individual trajectories varying widely (Hamilton and Steptoe, 2022).

The incremental rise in mean fibrinogen and cortisol levels from profile one to three, aligns with increases in mean CRP, which is consistent with earlier evidence on the synchronised physiological exchange between their respective systems to maintain homeostasis (Shimba et al., 2021). However, the unexpected moderate decline in IGF-1 between each of the derived profiles is notable. The reasons for this is unclear given the well documented covariance between each represented system in the LPA (O'Connor, 2008; Taub, 2008; Hamilton and Steptoe, 2022; Rajpathak, 2008). Specifically, that IGF-1 antagonises the effects of CRP (Liu, 2014), and IGF-1 alterations can impact both immunomodulation and immunosuppression (Shimba et al., 2021). As part of a coordinated systemic regulatory mechanism that facilitates a dynamic cellular microenvironment, proinflammatory cytokines can induce a state of resistance in hormonal secretion, including in IGF-1 (Taub, 2008). This can attenuate the mitogenic effect of IGF-1, but can also have anti-proliferative effects on IGF-1 (O'Connor, 2008), which should be reflected here. The reason for the blunted effect of IGF-1 seen in the present study, is conceivably because IGF-1 secretion is sensitive

to nutritional and endocrine control, such that hormonal resistance is rendered maladaptive by pharmacologic use and dietary choices (Witkowska-Sędek and Pyrżak, 2020); neither of which were measured here. In addition, O'Connor and colleagues (2008) suggest that cellular responses can vary tremendously depending on ligand origin and concentration, the number of cell receptors, and signalling kinetics post receptor activation, not to mention extracellular control of IGF-1, which is a second mode of regulation.

It is also clear from converging lines of evidence that different stressors have different predictive power (Cohen et al., 2007; Kiecolt-Glaser et al., 2002; Steptoe et al., 2007; Segerstrom and Miller, 2004). There was some evidence to support this in the present study, with the largest effect sizes observed following financial stress, but given the overlap of CI, there is not strong associative differentiation. Even so, Hamilton and Steptoe's (2022) recent observational study revealed idiosyncrasies in the role of different socioeconomic stressors in CRP, fibrinogen, IGF-1, and white blood cell count (WBCC/leukocytes). Part of the challenge is in establishing a '*hierarchy of stress*' to determine which psychosocial stressors are most problematic; distinguishing between rare acute stressors that have high clinical risk and everyday stressors that create chronic risk and contribute more to overall disease burden in the population. The present study takes a step toward this purpose, and while we used an LPA to look at immune and neuroendocrine patterning here, future study would benefit from a more comprehensive stress score that is also submitted to LPA to see how stress clusters in the population.

Our results extend previous evidence on psychoneuroimmunological processes (Kiecolt-Glaser et al., 2002; Steptoe et al., 2007; Segerstrom and Miller, 2004), by showing that stress exposure is associated with a greater probability of *high-risk* immune and neuroendocrine profile membership, irrespective of genetic propensity. This is an important feature of our study, and a methodological advance over previous research given that genetic factors can affect the magnitude of the immune and neuroendocrine response (Prins, 2017). Inter-individual variability in biomarker concentrations and their respective binding proteins are partly the result of polymorphic variations in respective genes, while genes encoding biomarkers are candidate loci for diseases with an inflammatory basis (Prins, 2017). Moreover, CRP (Su, 2008), fibrinogen (Su, 2008), cortisol (Sawyers, 2021), and IGF-1 (Franco, 2014) each have high heritability, which can be understood as the proportion of the total variation of the trait that can be attributed to unobserved genetic effects (Pankow, 2001). Therefore, while single nucleotide polymorphisms (SNPs) associated with each biomarker only explained a small proportion of the variance in our phenotypic associations, it is plausible that they confounded earlier evidence, such that their omission inflated effect sizes.

Our study has several strengths. To our knowledge this is the first study to explore how stress is related to immune and neuroendocrine profile membership. The application of a latent profile approach and the prospective nature of the study facilitated an exploration into the temporal direction of stress associations with population-level configurations of immune and neuroendocrine biomarker activity with increased specificity. LPA was chosen over other traditional clustering methods because it identifies subgroups of individuals with similar biomarker activity (Wang and Wang, 2012) thereby providing more specificity to population risk assessment. This offers the promise of improving epidemiological and clinical assessments. We show that 'one-size does not fit-all' when assessing risk, so scientific research and clinical trials should consider distinct samples with higher risk burdens. Dichotomising the ordinal stress score reduced the influence of its non-normality, quasi-continuous quality, and limited the chance of underestimated correlations and an inflation of Type II errors (i.e., false negatives). Therefore, it offered more meaningful results, despite the potential loss of power. In the presence of nonlinearity and interactions missForest outperforms prominent imputation methods, such as multivariate imputation by chained equations and k-nearest neighbours in all metrics (Stekhoven and Bühlmann, 2012). Another key strength is in our use of a well-powered, well-characterised cohort that offers precise estimates of objective, systematically measured, interrelated biomarkers (Steptoe et al., 2013). ELSA offers a rich selection of repeated biological measures, and while other biomarkers may be of interest for future study, here we have a narrow focus on immune and neuroendocrine biomarkers.

We do, however, note some important caveats. We cannot claim causality. Given the observational nature of the study, our results might be subject to residual confounding or over-adjustment. While the fibrinogen PGS was not available, a strong genetic correlation with CRP has been documented elsewhere (Su, 2008), and PGS for CRP was accounted for in analyses. Similarly, baseline cortisol was unavailable, although follow-up cortisol was correlated with CRP and fibrinogen (Table S2); both adjusted for at baseline. The self-reported nature of the stress score may have introduced some measurement error to the results, and there is an assumption in the stress measure that different exposures carry equal weight but this is typically not so. Given that ELSA participants are 99 % White, and ethnic groups are said to experience higher levels of stress (Thames et al., 2019), their absence in the present study is a considerable limitation. Crucially, immune and neuroendocrine activation involves a constellation of cells that interact and create a microenvironment that promotes disease, but here we include a relatively small number of biomarkers to represent this complex network.

6. Conclusion

The synergistic immune and neuroendocrine response to stress represents an important target for clinical intervention. Intervening on these processes could alter the course of disease (Walker, 2014). We examined multivariate biomarkers, including CRP, fibrinogen, cortisol, and IGF-1, using empirically derived data reduction techniques to uncover subgroup differences in how immune and neuroendocrine biomarkers pattern together. It proved an effective method to explore the complex series of reactions across the immune, nervous, and endocrine systems. Because stress was positively associated with the derived immune and neuroendocrine profiles, our results support that exposure to high levels of stress can actuate a cascade of complex central and peripheral physiological events that has previously been linked to pathology, sub-clinical illness, and debility.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Research Ethics Committee [MREC/01/2/91] nres.npsa.nhs.uk) granted ethical approval for each of the ELSA waves. All participants provided informed consent, and research was performed in accordance with research and data protection guidelines. **Contributorship.** Study funding was secured by AS. Conception and planning by OSH and AS. OSH and EI designed the statistical analysis plan. OSH and OA prepared the data. OSH performed data analyses, then interpreted results with input from all authors. All authors had access to the data, take responsibility for data integrity, the accuracy of analysis, and its interpretation. All authors act as guarantors, and critically appraised the manuscript drafted by OSH for submission.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2023.11.012.

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