The Physiometrics of Inflammatory Proteins and Implications for Medical and Psychiatric Research: Investigation of Empirically-informed Inflammatory Composites

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Abstract

Much inflammation research examines individual proteins; however, some studies have used summed score composites of all available inflammatory markers without first investigating dimensionality. Using three different samples (MIDUS-2: N = 1,255 adults, MIDUS-R: N =863 adults, and ACE: N = 315 adolescents), this study investigates the dimensionality of eight inflammatory proteins (C-reactive protein (CRP), interleukin (IL)-6, IL-8, IL-10, tumor necrosis factor-α (TNF-α), fibrinogen, E-selectin, and intercellular adhesion molecule (ICAM)-1) and compares the resulting factor structure to a) an "a priori" factor structure in which all inflammatory proteins equally load onto a single dimension (a technique that has been used previously) and b) proteins modeled individually (i.e., no latent variable) in terms of model fit, replicability, reliability, temporal stability, and their associations with medical history and depression symptoms. A hierarchical factor structure with two first-order factors (Factor 1A: CRP, IL-6, fibrinogen; Factor 2A: TNF-α, IL-8, IL-10, ICAM-1, IL-6) and a second-order general inflammation factor was identified in MIDUS-2 and replicated in MIDUS-R and partially replicated in ACE (which unfortunately only had CRP, IL-6, IL-8, IL-10, and TNF-α but, unlike the other two, has longitudinal data). Both the empirically-identified structure and modeling proteins individually fit the data better compared to the one-dimensional "a priori" structure. Results did not clearly indicate whether the empirically-identified factor structure or the individual proteins modeled without a latent variable had superior model fit. Modeling the empirically-identified factors and individual proteins (without a latent factor) as outcomes of medical diagnoses resulted in comparable conclusions, but modeling empirically-identified factors resulted in fewer results "lost" to correction for multiple comparisons. Importantly, when the factor scores were recreated in a longitudinal dataset, none of the individual proteins, the "a priori" factor, or the empirically-identified general inflammation factor significantly predicted

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concurrent depression symptoms in multilevel models. However, both empirically-identified first-order factors were significantly associated with depression, in opposite directions. Measurement properties are reported for the different aggregates and individual proteins as appropriate, which can be used in the design and interpretation of future studies. These results indicate that modeling inflammation as a unidimensional construct equally associated with all available proteins does not fit the data well. Instead, empirically-supported aggregates of inflammation, or individual inflammatory markers, should be used in accordance with theory. Further, the aggregation of shared variance achieved by constructing empirically-supported aggregates might increase predictive validity compared to other modeling choices, maximizing statistical power.

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Introduction

Inflammation is a popular immunological construct in medical and psychiatric research. It is commonplace to measure several inflammatory proteins and test them all as independent or dependent variables, which results in several complications. First, many theories and hypotheses are described in terms of "inflammation" (e.g., Dooley et al., 2018; Miller et al., 2009; Moriarity et al., 2018; Slavich, 2020), not individual proteins, underscoring a disconnect between theory and analysis. Further, there are concerns about the degree to which any individual protein can be considered a true "biomarker" of inflammation (Konsman, 2019). This approach also invites concerns with multiple comparisons that could be addressed using composite variables. Thus, some studies (e.g., Moriarity, Ng, Titone, et al., 2020) have used "a priori" composite variables consisting of the sum or average of several standardized inflammatory proteins in a dataset to create a measure of general inflammation. However, this decision risks aggregating proteins that do not load strongly onto the same dimension of inflammatory physiology (e.g., pro- vs. anti-inflammatory processes) and makes the unlikely assumption that all inflammatory proteins are equally associated with a higher-order inflammation construct. Assuming unidimensionality is particularly worrisome with multifaceted constructs like inflammation. If different dimensions have different associations with outcomes of interest, aggregating these dimensions could result in inconsistency of the size and direction of effects. Additionally, data aggregation uninformed by investigating dimensionality can result in increased measurement error, which, on average, attenuates effect sizes and reduces power. Consequently, thoughtful investigation and consideration of biological plausibility is necessary to evaluate the best way to create an inflammatory composite.

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There is theoretical rationale for believing that different inflammatory proteins might be influenced by shared underlying processes. For example, they are all known to be broadly involved in the initiation and maintenance of, or recovery from, inflammatory activity (hence their classification as "inflammatory" proteins) in what is sometimes referred to as the inflammatory cascade (Cavaillon & Adib-Conquy, 2002). However, given the many component processes of inflammation (e.g., the acute phase response and upregulation of pro-inflammatory proteins, activation of the vascular and endocrine systems, neutrophil migration to the site of injury, fibrinolysis, apoptosis, coagulation, and the induction of regulatory anti-inflammatory processes to return the system to homeostasis (Gruys et al., 2005)), it is plausible that a unidimensional model might not best represent the complexity of this system. It is also important to note that many inflammatory proteins are pleotropic and can contribute to different, even opposing (e.g., pro- and anti-inflammatory), processes under certain conditions. For example, interleukin (IL)-6 typically is described in terms of proinflammatory functions (mediated by classic signaling), but also has some anti-inflammatory functions (mediated by trans-signaling) (Scheller et al., 2011). Further, it is also possible that the resting concentrations of various proteins are partially determined by shared cellular sources (i.e., proteins produced by myokines might have higher intercorrelations than proteins produced by different types of cells).

To our knowledge, only one other study has tested the dimensionality of inflammatory proteins in a non-medical sample. Egnot and colleagues (2018) tested the dimensionality of several inflammatory and coagulatory proteins (C-reactive protein (CRP), IL-6, fibrinogen, intercellular adhesion molecule (ICAM)-1, D-dimer, Lipoprotein (Lp)-a, and pentraxin (PTX)-3). It was concluded that CRP, IL-6, and fibrinogen loaded onto an inflammatory factor and D-

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dimer and PTX-3 loaded onto a factor indicative of a thrombogenic process and the concomitant vascular perturbation. ICAM-1 and Lp-a did not load onto any observed factors.

A few other studies have conducted factor analyses of inflammatory proteins in medical samples, which may or may not generalize to community samples. In a sample of acute coronary syndrome patients, Tziakas et al. (2007) tested the dimensionality of CRP, fibrinogen, HDL cholesterol, IL-10, IL-18, and ICAM-1 and found three inflammatory factors: a "systemic inflammation" factor consisting of CRP and fibrinogen, a "local inflammation—endothelial dysfunction" factor consisting of IL-18 and ICAM-1, and an "anti-inflammatory factor" comprised of IL-10 and HDL cholesterol. Koukkunen et al. (2001) conducted an exploratory factor analysis (EFA) on CRP, fibrinogen, IL-6, tumor necrosis factor-α (TNF-α), troponin T, and creatine kinase MB mass in a sample of adults with unstable angina pectoris. This study described two factors: an "inflammation factor" including CRP, fibrinogen, and IL-6, and an "injury" factor including TNF-α, troponin T, and creatine kinase MB mass. Finally, Sakkinen and colleagues (2000) conducted an EFA on 21 different biological characteristics (including several inflammatory proteins) in participants with insulin resistance syndrome. Six biomarkers (fibrinogen, CRP, plasmin-α_2-antiplasmin, Factor VIIIc, Factor IXc, and fibrin fragment D-dimer) loaded onto what was interpreted as an inflammation factor. HDL cholesterol was tested, but was not found to load onto a factor with other inflammatory markers, as was found by Tziakas et al. (2007).

Some notable similarities between these studies emerge; specifically, 1) CRP, fibrinogen, and IL-6 frequently load onto the same factor (potentially due to their interactive role in the acute phase reaction) when these proteins are included in the dataset and 2) TNF-α and ICAM-1 never loaded onto the same factor as CRP, fibrinogen, and IL-6. Thus, these studies do not support the

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use of unidimensional composites created by summing standardized values of inflammatory proteins in a dataset (consequently, giving all of the proteins equal weight in the composite) without investigating the empirical structure of the data first. McNeish and Wolf (2020) provide a thorough review of concerns about using sum scores in this manner when the structure of the data is more complex. Additional work must be done to test and replicate the structure of inflammation in populations of interest (e.g., community samples, cancer patients, individuals with HIV/AIDs) to determine the best way to aggregate different inflammatory proteins (and whether this is appropriate). It is also worth noting that two of these four studies found multiple inflammation factors, suggesting that inflammation might be best represented as a multidimensional construct. Additionally, none of these studies tested the same panel of inflammatory proteins. Thus, the direct replicability of the structure of an array of inflammatory proteins has never been tested.

The Present Study

This study sought to investigate the dimensionality of several inflammatory proteins and compare the empirically-identified factor structure to an "a priori" factor structure computed by summing the z-standardized values of all available proteins in the dataset (a technique used in previously published studies (e.g., Moriarity, Ng, Titone, et al., 2020)). First, the structure of eight inflammatory proteins was investigated in a sample of adults with a mean age of 55.42 years. Replicability of this structure was tested in a second sample of similarly aged adults and model fit was compared to the "a priori" data structure. Structural equation modeling also was used in this replication dataset to compare 1) the empirically-identified structure, 2) the "a priori" structure, and 3) modeling the inflammatory proteins individually (without a latent variable) as outcomes of several different medical criterion variables (i.e., heart disease, diabetes, asthma,

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tuberculosis, thyroid disease, peptic ulcer disease, and arthritis). Because these datasets were cross-sectional in nature, a longitudinal sample of adolescents (mean age of 16.44 years) was used for prospective analyses. Specifically, the model fit of a short-form (this dataset only included five of the proteins available in the adult datasets) version of the empirically-identified factors and the "a priori" composite were estimated and compared to establish developmental generalizability. Then, 1) the empirically-identified factors, 2) the "a priori" composite, and 3) individual proteins were tested as predictors of depression (the primary outcome of the adolescent sample's study and a psychiatric outcome associated with inflammation (Moriarity, Kautz, et al., 2020)). Reliability estimates for these inflammatory modeling options are reported as appropriate.

Methods

Participants and Procedure

Participants were selected from three pre-existing datasets: Midlife in the United States (MIDUS)-2, MIDUS-R, and Project Adolescent Cognition and Emotion (ACE). MIDUS-2 (Ryff et al., 2017) consisted of 1,255 (Mage = 55.42 years, 50% female, 78% White) participants between the ages of 25 and 75 who were fluent in English and volunteered to participate in a biomarker collection that included a sera assessment of eight inflammatory proteins (C-reactive protein (CRP), interleukin (IL)-6, IL-8, IL-10, tumor necrosis factor-α (TNF-α), fibrinogen, E-selectin, and intercellular adhesion molecule (ICAM)-1). MIDUS-R (Weinstein et al., 2017) was designed to parallel the procedure of the MIDUS-2 study, and included 863 adults (Mage = 53.53 years, 50% female, 87% white).

Project ACE (Alloy et al., 2012) is a longitudinal study of adolescent depression. Inclusion criteria for the original study were that: (i) adolescents were aged 12-13 years, (ii) their

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mothers were willing to participate, and (iii) adolescents self-identified as Caucasian, African-American, or biracial (examining racial differences in depression was a goal of Project ACE). Exclusion criteria were if the adolescent or mother had: (i) insufficient English reading/speaking skills to complete the assessments, or (ii) a psychotic, developmental, medical, or learning disorder. Complete details on recruitment and sample characteristics have been published (Alloy et al., 2012). A subsample nested within ACE (n = 315, Mage at first blood draw = 16.44 years, 53% female, 42% White, 53% Black, 5% Biracial) agreed to participate in a supplementary sera assessment of inflammation (CRP, IL-6, IL-8, IL-10, TNF-α). Blood draws were offered as an optional part of the protocol annually. The Children's Depression Inventory (CDI: Kovacs, 1985) was collected at these sessions as well. The dataset included a total of 866 observations with complete inflammation and CDI data across seven waves (Ns = 313, 226, 165, 101, 46, 13, 2, chronologically).

Measures

MIDUS-2 and MIDUS-R

Inflammatory proteins. Fasting blood draws were collected between 6:00 and 8:30 am for both MIDUS-2 and MIDUS-R. Blood was centrifuged and stored in a -60 °C to -80 °C freezer. Samples were shipped to the MIDUS Biocore Lab on dry ice, where they were stored at -65 °C until they were assayed. C-reactive protein (CRP) originally was analyzed in plasma via BNII nephelometer (Dade Behring Inc.). Samples falling below the assay range for this method were re-assayed using immunoelectrochemiluminescence using a high-sensitivity array kit (Meso Scale Diagnostics (MSD)). Comparisons of these two methods showed results to be highly correlated. Beginning in 2016, all participants (150 from MIDUS-R) had CRP assayed using the MSD platform using serum. Corrections to account for these changes were applied

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before the data were made publicly available. Fibrinogen was measured using the same BNII nephelometer. E-Selectin and Intercellular Adhesion Molecule (ICAM)-1 both were measured using ELISA assays (R&D Systems, Minneapolis, MN). Lot-to-lot changes in both E-Selectin and ICAM-1 assays were made throughout the course of the study and adjusted for prior to the data being made available to the public. Cytokines (interleukin (IL)-6, IL-8, IL-10, and tumor necrosis factor alpha (TNF-α)) were quantified by V-plex Custom Human Cytokine Kit (MSD, Rockville, MD), MSD Sulfo-tag, and MSD Sector Imager. E-Selectin and ICAM-1 values outside of the detectable range (LLOD = <.1 ng/mL and <45 mg/L, respectively) were set at .09 ng/mL and 44 ng/mL, respectively. MIDUS documentation indicates that none of the other proteins had values outside of the detectable range. Assay ranges and variability for all proteins can be found in the MIDUS documentation available online. Bivariate correlations between the proteins in MIDUS-2 and MIDUS-R can be found in Supplemental Tables 1 and 2, respectively.

C-reactive protein. CRP is a pentameric protein, generalized marker of inflammation, and acute phase reactant upregulated by IL-6 (Davidson, 2013). CRP is primarily synthesized by the liver and activates the complement system, promoting phagocytosis.

Fibrinogen. Fibrinogen is a glycoprotein complex and acute phase protein made in the liver and is upregulated by IL-6. It is involved in creating blood clots, regulating thrombin, and influencing leukocyte migration (Amrani, 1990; Davidson, 2013). Additionally, it influences the induction of cytokine/chemokine expression (e.g., IL-6 and TNF-α) via MAC-1 signaling. Breakdown products of fibrinogen (e.g., D-dimers) stimulate the release of several inflammatory proteins including CRP and IL-6 (Davidson, 2013).

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E-selectin. E-selectin is a selectin cell adhesion molecule expressed by endothelial cells and activated by cytokines. Local release of IL-1 and TNF-α induces over-expression of E-selectin, which then recruits leukocytes to the site of injury (Imhof & Dunon, 1995).

Intracellular Adhesion Molecule-1. ICAM-1 concentrations increase rapidly in response to TNF-α and IL-1 and influence neutrophil adhesion (Divietro et al., 2001) and the recruitment of macrophages. It is expressed by the vascular endothelium, macrophages, and lymphocytes. There is also evidence that ICAM-1 is involved in the secretion of TNF-α (Etienne-Manneville et al., 1999).

Interleukin-6. IL-6 is responsible for stimulating acute phase protein synthesis in the liver and production/trafficking of neutrophils (Davidson, 2013; Fielding et al., 2008). It is produced by a wide variety of cells including liver cells, macrophages, osteoblasts, and monocytes (Davidson, 2013). In addition to its pro-inflammatory roles, it also has anti-inflammatory properties and is involved in the regulation of TNF-a and IL-10 (Scheller et al., 2011).

Interleukin-8. IL-8 (also known as neutrophil chemotactic factor) is a chemokine produced by macrophages and other types of cells (e.g., epithelial cells, smooth muscle cells in the airway, and endothelial cells) (Hedges et al., 2000). It is one of the most important proteins in neutrophil adhesion (both in terms of adhesion promotion and inhibition) and migration toward injury sites via chemotaxis, and also stimulates phagocytosis (Divietro et al., 2001; Dixit & Simon, 2012; Luscinskas et al., 1992).

Interleukin-10. IL-10 is predominantly produced by monocytes and lymphocytes, and primarily, is an anti-inflammatory cytokine that regulates pro-inflammatory proteins (e.g., TNF-

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a, IL-8, IL-6) as well as enhancing B-cell survival and antibody production (Kessler et al., 2017; Sun et al., 2009).

Tumor necrosis factor alpha. TNF-α is a predominantly proinflammatory cytokine primarily released by macrophages (in addition to other cells such as lymphoid cells, adipose tissue, and mast cells) to recruit other cells to activate immune processes (Olszewski et al., 2007). Many of the proinflammatory functions of TNF-α are apoptotic (promotes programmed cell death) in nature (Gough & Myles, 2020). TNF-α also influences migration of neutrophil to injury sites (Smart & Casal, 1994) and induces ICAM-1 (Burke-Gaffney & Hellewell, 1996).

Medical Status. Participants' medical history was assessed on the day of the study visit via interview. This interview found that 11.5/9.3 % of the sample (MIDUS-2/MIDUS-R, respectively) reported a history of heart disease, 12.4/10.9% reported a history of diabetes, 12.6/18.1 % reported a history of asthma, 0.6/0.9% reported a history of tuberculosis, 12.4/12.1% reported a history of thyroid disease, 43.4/36.4% reported a history of arthritis, and 5.4/5.2% reported experiencing a peptic ulcer.

Project ACE

Inflammatory Proteins. Blood samples were obtained via antecubital venipuncture by a certified phlebotomist into a 10 mL vacutainer designed for freezing plasma separated from the cell fraction within the vial (BD Hemogard with K2 EDTA). Vacutainers were stored in an ultracold freezer at -80 ^{o}C, and later thawed on the day of assay. Four cytokines were quantified by multi-cytokine array (IL-6, IL-8, IL-10, and TNF-α), and high-sensitivity CRP was determined in a singleplex assay, using an electrochemiluminescence platform and a QuickPlex SQ 120 imager for analyte detection (MSD, Gaithersburg, MD). Each specimen was assayed in duplicate and the intra-assay coefficients of variation averaged 5.36 and 2.29 for the cytokines

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and CRP, respectively. Plasma was diluted 1:2 for the cytokine assay and 1:1000 for the CRP assay. CRP is present in blood at higher concentrations, and thus, plasma was diluted to correspond to the standard curve. Values were calculated with respect to a standard curve generated from 7 calibrators with known concentrations. The lower limit of detection (LLOD) for the cytokines was 0.1 pg/mL, with a large dynamic range up to 2000 pg/mL. The LLOD for CRP was 0.1 mg/L. Values below the LLOD were set at the LLOD. Values were converted to mg/L units to be consistent with the clinical literature (Breen et al., 2011; Dabitao et al., 2011). See above for a brief description of the inflammatory proteins. Bivariate correlations between these proteins can be found in Supplemental Table 3.

Depression Symptoms. Symptoms of depression were measured using the Children's Depression Inventory (CDI; Kovacs, 1985). It consists of 27 items reflecting affective, behavioral, and cognitive symptoms of depression. Items are rated on a 0 to 2 scale and total scores range from 0 to 54. The CDI has been demonstrated to be a reliable and valid measure of depressive symptoms in youth samples (Klein et al., 2005) and has strong measurement invariance over time (Stumper et al., 2019), making it a good option for longitudinal analyses with adolescents. Internal consistency ranged from $\alpha = .84 - .92$ across each time point. The CDI was administered at all annual assessments during the same visit as the blood draws. All items in the scale were summed (after reverse scoring several items, in accordance with the manual) to create the total CDI score.

Analyses

All analyses were conducted in R 3.6.2 (R Core Team, 2013). All proteins were z-standardized for the purposes of all analyses. Outliers were identified using the median absolute deviation (MAD)-median rule and winsorized. To avoid biasing estimates toward participants

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with more blood draws in ACE, only the first blood draw (which had the most participants) was used to identify cutoffs for outliers. No proteins were skewed following winsorization.

Exploratory Factor Analysis

"Bass-ackwards" exploratory factor analyses (Goldberg, 2006) were conducted with all eight proteins in MIDUS-2 using the Geomin rotation to allow for a hierarchical factor structure and correlated factors using *efaUtilities* (Zhang et al., 2020). Parallel analysis was used to determine the number of factors to retain using *EFA.dimensions* (Connor, 2020). Unlike other factor retention methods, parallel analysis allows for correction for the effects of sampling error. Eigenvalues were generated using permutations of the raw dataset to create eigenvalues that could be compared to the eigenvalues produced by the factor analysis on the dataset. When an eigenvalue generated from the factor analysis is higher than that generated from the parallel analysis, it can be assumed that the eigenvalue represents a real factor that accounts for more variance than a factor based on the permutated data (Horn, 1965). Thus, the number of factors retained was determined by the number of eigenvalues for which the eigenvalue for the factor analysis is higher than the eigenvalue of the parallel analysis using the permutations of the dataset. Complex loadings (i.e., items loading onto more than one factor) were allowed due to the pleotropic nature of these biomarkers. Loadings at or above .30 were considered to load onto a factor; however, if a protein was close to this threshold (above .20), it was included in an alternative CFA (described below) and model fits were compared. Proteins that did not meet this threshold for any factor were removed and the EFA re-estimated. Factor scores were calculated using the regression method. Models were estimated using maximum likelihood.

Confirmatory Factor Analysis/Structural Equation Modeling

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Confirmatory factor analyses (CFAs) were estimated in MIDUS-R for the factor structure found in the EFA from MIDUS-2. CFAs were conducted in *lavaan* (Rosseel, 2012). The variance of latent variables was set to 1 and all factor loadings, unless otherwise noted in the results, were freely estimated. Alternative models with proteins with factor loadings between .20 and .30 also were estimated and model fit was compared to select the final "empirically-identified" factor structure. Next, model fit of the empirically-identified factor structure was compared to the "a priori" unidimensional factor structure with the factor loadings of all proteins constrained to equality. In addition to interpretation of standard goodness-of-fit indices (i.e., good fit indicated by comparative fit index [CFI] ≥ .95, root mean square-error of approximation [RMSEA] ≤ .06, and standardized root-mean-square residual [SRMR] ≤ .08, and non-significant chi-square test; Hu et al., 2009), AIC and BIC will be compared between models. Unlike most other fit indices, AIC and BIC can be directly compared, with lower values indicating preferable model fit. Additionally, it is important to note that the chi-square test of significance is over-powered in large sample sizes (Bollen, 1989). SEM models also were conducted in MIDUS-R comparing the empirically-identified structure, "a priori" structure, and individual inflammatory proteins as outcomes of several medical criterion variables (heart disease, diabetes, asthma, tuberculosis, thyroid disease, peptic ulcer disease, and arthritis) and model fit was compared again. Finally, CFAs of the empirically-identified and "a priori" models were estimated in ACE to test generalizability to a more racially-diverse, adolescent sample. Three of the proteins used in MIDUS were not measured in ACE, so this is analogous to testing a short form of a self-report measure. Missing data was handled with maximum likelihood estimation.

Predictive Validity—Depression Symptoms

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Mixed linear models using *lme4* (Bates et al., 2015) tested the predictive validity of i) the empirically-identified weighted composites, ii) the "a priori" composite, and iii) the individual proteins for depression symptoms at the time of blood draw in random intercept, fixed slope models. Multilevel modeling was chosen over standard regression because the data were clustered within individuals, which would result in greater probability of Type I error and less efficient estimates of coefficients compared to multilevel modeling. Models were estimated using restricted maximum likelihood. These analyses used a total of 866 observations across seven waves (Ns = 313, 226, 165, 101, 46, 13, 2, chronologically).

Reliability and Temporal Stability

Factor reliability was quantified via coefficient ω (Raykov, 2001) using the R package *semTools* (Jorgensen et al., 2021) in MIDUS-2. Reliability for higher-order latent variables will be quantified using $\omega_{partial}$, which indicates the proportion of observed variance explained by a higher-order factor after partialing out the lower-order factors. Coefficient ω was selected over Cronbach's α because a) ω makes fewer and more realistic assumptions compared to α and b) problems with misestimation of reliability are far less likely. For a more in-depth argument for the adoption of ω over α as the new field standard refer to Dunn et al. (2014).

Temporal stability was calculated two ways: Pearson's *r* and intra-class correlation coefficients (ICCs). Importantly, temporal stability estimates are only informative for the time between data points they were estimated under. Time between blood draws in Project ACE were highly variable due to the optional nature of the blood draw (.13 – 70.70 months), so observations only were chosen that fell into "waves" of data collection at increments of 11.5 months (the modal duration between observations) +/- 3 months. This resulted in a number of observations being excluded due to not fitting in these waves. Pearson correlations were

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calculated between the first two waves (N = 97). ICCs were calculated using random intercept, fixed slope hierarchical linear models with weighted composite scores across seven waves (Ns = 187, 97, 96, 76, 48, 15, 6, chronologically). Pearson correlations reflect stability of ordinal rankings, whereas ICCs indicate the within-person variance relative to between-person variance.

Results

Exploratory Factor Analysis

The initial parallel analysis indicated two lower-order factors (i.e., two factors with eigenvalues greater than the corresponding factors in the random data) in MIDUS-2. The initial EFA found that E-selectin did not load onto any factors, and thus, it was removed (EFA results can be found in Table 1). The new parallel analysis still indicated two factors. CRP, IL-6, and fibrinogen loaded onto Factor 1A above the cutoff of .30. TNF-α, IL-8, IL-10, and ICAM-1 loaded onto Factor 2A above .30 (Table 1). IL-6 had a subthreshold (.22) cross-loading on Factor 2A. These factors accounted for 20% and 16% of the total variance, respectively. In comparison, a single factor model only accounted for 24% of the total variance. The two-factor model also fit the data better than a single factor model (AIC/BIC = 42027.253/42145.153 vs 42323.654/42431.302, respectively). Both first order factors strongly correlated with a higher-order factor (Factor 1B, loadings = .84 and .53, respectively), suggesting a multidimensional, hierarchical factor structure (Loehlin & Goldberg, 2014).

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Table 1. Exploratory Factor Analyses (EFAs) in MIDUS-2

	Initial EFA		Without E-selectin	
	Factor 1A	Factor 2A	Factor 1A	Factor 2A
CRP	.78	.00	.78	.00
Interleukin-6	.58	.22	.58	.22
Tumor Necrosis Factor-α	.03	.75	.02	.78
Interleukin-8	.02	.34	-.02	.32
Interleukin-10	.05	.52	-.05	.50
Fibrinogen	.68	.06	.67	-.06
Intercellular Adhesion Molecule-1	.11	.30	.11	.30
E-selectin	.17	.11	x	x
Correlation Between Factors	.33		.33	
Proportion of Variance Explained	.18	.14	.20	.16
Proportion of Variance Explained by Single Factor	.22		.24	

Note: x = not included in EFA

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Confirmatory Factor Analyses/Structural Equation Modeling

First, the factor structure obtained using a .30 loading threshold for the exploratory analyses was compared to an identical model except with IL-6 loading onto both first-order factors in MIDUS-R (Figures 1a and 1b, respectively). In all CFA models, factor loadings between the two first-order factors (Factors 1A and 2A) and the second-order factor (Factor 1B) were constrained to be equal because a latent variable needs 3 indicators to be properly identified and constraining the loadings to equality frees up degrees of freedom to identify the model. The model with IL-6 loading onto both first order factors had lower AIC/BIC (Table 2: 15909.725/16018.839 vs 15943.590/16047.960, respectively), suggesting better model fit. The chi-squared difference test that compared model fit found that this difference in model fit was significant ($\Delta\chi^2 = 35.865$, $p < .001$). This model demonstrated good model fit with a CFI = .970, RMSEA = .055 (90% CI: .037-.073), and SRMR = .036. The chi-square test was significant ($\chi^2(12) = 42.331$, $p < .001$), but this is uninformative with sample sizes this large. Additionally, the EFA was well-replicated at the factor loading level as well. All of the originally estimated factor loadings were between the 95% confidence intervals for the factor loadings in the replication models (Table 3).

Second, this empirically-identified factor structure was compared to the "a priori" factor structure with all proteins having an equal weight on a single factor (Figure 1c). AIC/BIC are not comparable for models with different numbers of indicators; thus, E-selectin was not included in this model despite this decision being informed by the results of the original EFA. Model fit for the "a priori" model was poor: CFI = .601, RMSEA = .155 (90% CI = .142-.167), and SRMR = .119. The chi-square test was significant ($\chi^2(12) = 425.386$, $p < .001$). The empirically-identified

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model also had a lower AIC/BIC (15909.73/16018.84 vs 16276.780/16347.940, respectively), indicating worse fit for the "a priori" model.

Third, several medical conditions associated with inflammation (heart disease, diabetes, asthma, tuberculosis, thyroid disease, peptic ulcer disease, and arthritis) were modeled as predictors of a) the empirically-identified factors (Figures 2a and 2b), b) the "a priori" factor (Figure 2c), and c) individual proteins (modeled without a latent variable; Figure 2d) in separate models. Note that the first-order and second-order factors in the empirically-identified model could not be entered as outcomes in the same model because Factor 1B was entirely composed of variance from Factors 1A and 2A; thus, two models had to be estimated (the only difference being which level of the structure was predicted by the medical diagnoses). The first-order and second-order empirically-identified models (Table 2: AIC/BIC = 14617.221/14789.709; 14612.368/14752.223, respectively) and individual protein models (AIC/BIC = 14586.231/14977.827) all outperformed the "a priori" model (AIC/BIC = 14958.274/15060.834). Both empirically-identified models had better BIC, but worse AIC than the individual protein model, suggesting no clear answer as to which fit the data better. Because the individual protein model was just-identified, no other fit statistics were generated. Model fit for both the first-order and second-order empirically-identified models were generally good (RMSEA = .046 (90% CI = .036-.056), SRMR = .034; RMSEA = .044 (90% CI = .034-.053), SRMR = .036, respectively), except the CFI was slightly below the ideal cutoff of .95 (CFI = .926 and .924, respectively). As expected with this sample size, both chi-square tests were significant ($\chi^2(47) = 124.989$, $p < .001$; $\chi^2(54) = 131.136$, $p < .001$, respectively). Associations between the inflammation variables and medical conditions are described below in the section "Predictive Validity".

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Finally, the empirically-identified and "a priori" factor structures were partially recreated (using just CRP, IL-6, IL-8, IL-10, and TNF-α) in the more racially-diverse, longitudinal dataset of ACE adolescents. This is analogous to validating a "short-report" version of a self-report questionnaire. Note that, because Factor 1A only had two indicators (CRP and IL-6), these loadings had to be constrained to equality to fit the model in this dataset. Similar to the MIDUS-R dataset, the empirically-identified model outperformed the "a priori" model (Table 2: AIC/BIC = 4290.608/4350.649 vs. 4405.040/4446.319). Model fit for the "a priori" model was poor: CFI = .395, RMSEA = .209 (90% CI = .178-.241), SRMR = .148, $\chi^2(9) = 132.672$, $p < .001$. The empirically-identified dataset fit the data well with a nonsignificant chi-square test ($\chi^2(4) = 8.239$, $p = .083$), CFI = .979, RMSEA = .058 (90% CI = .000-.115), and SRMR = .033. The only parameter suggesting potentially poor fit was the upper bound of the RMSEA 90% CI was slightly above the ideal cutoff of .100. However, despite global indicators of good fit, the original factor loading estimates did not consistently fall within the 95% CIs for the CFA. Given general support for the empirically-identified model found in MIDUS-2, the predictive validity and temporal stability of these factors were tested in ACE.

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Figure 1a. Empirically-identified Structure without IL-6 Cross-loading

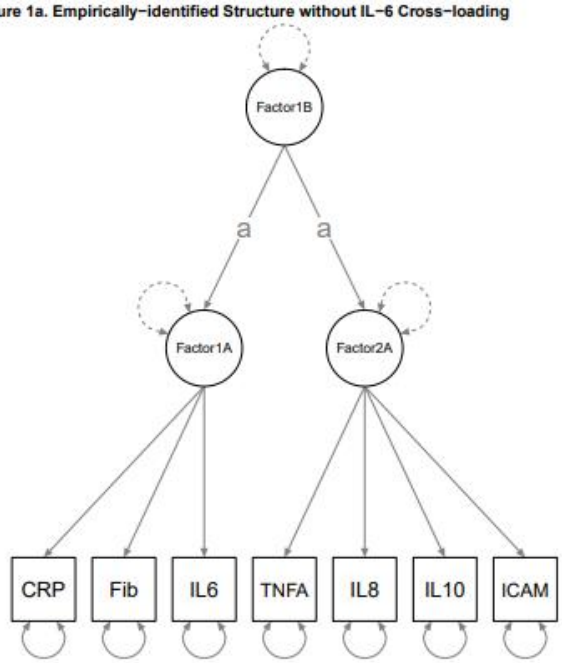


Figure 1b. Empirically-identified Structure with IL-6 Cross-loading

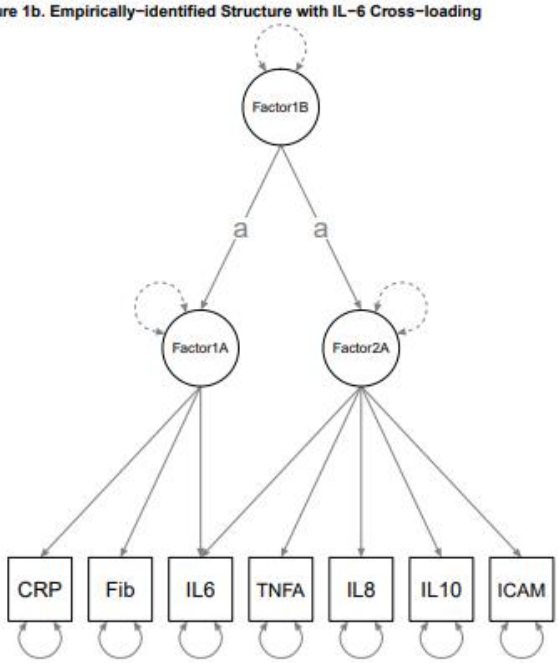
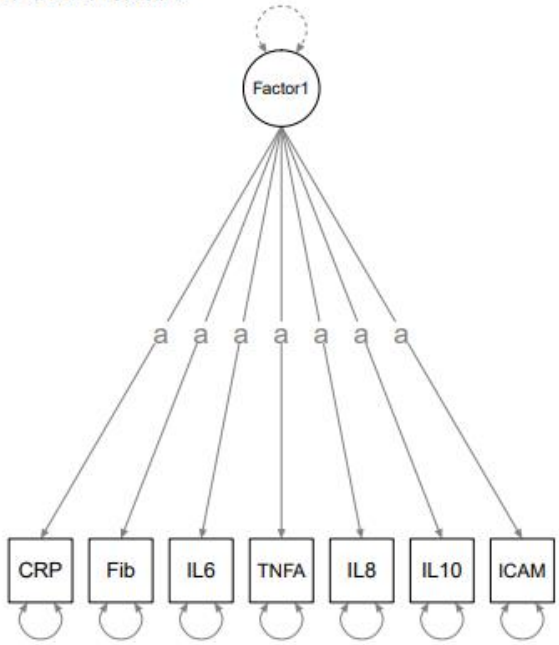


Figure 1c. A Priori Structure



Note: "a" denotes loadings constrained to equality, z= z-standardized, CRP = C-reactive Protein, Fib=fibrinogen, IL = interleukin, TNFA= Tumor Necrosis Factor-α, ICAM = Intracellular Adhesion Molecule-1.

Figure 2a. Empirically–identified Structure: Medical Predictors of 1st Order Factors

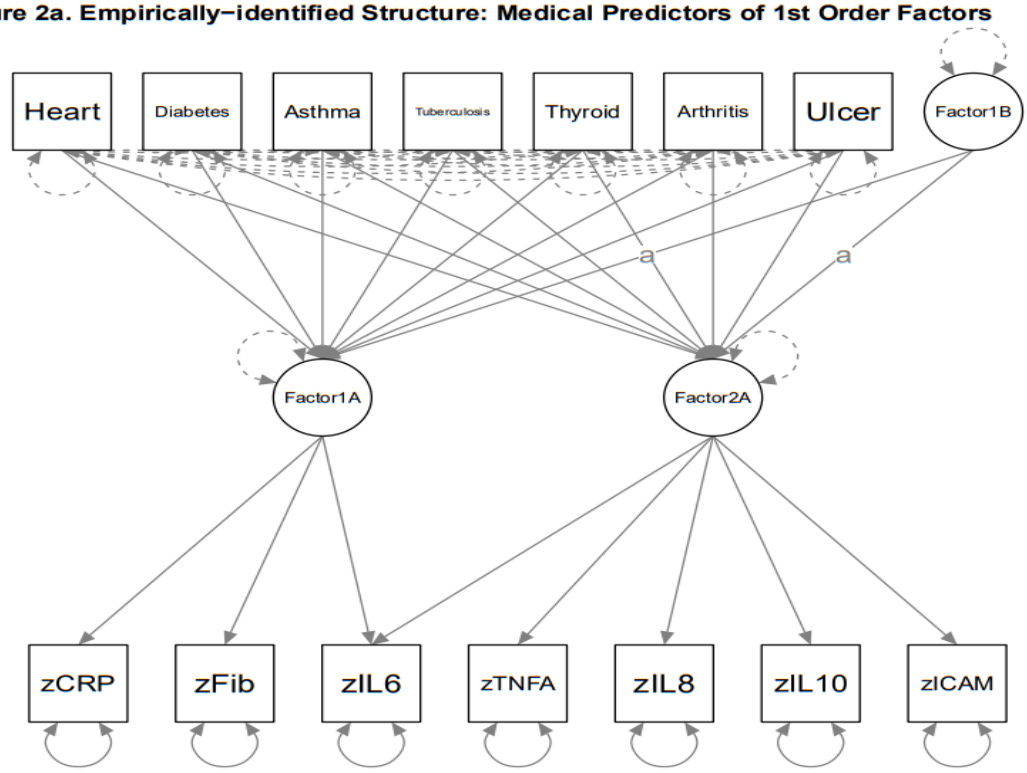
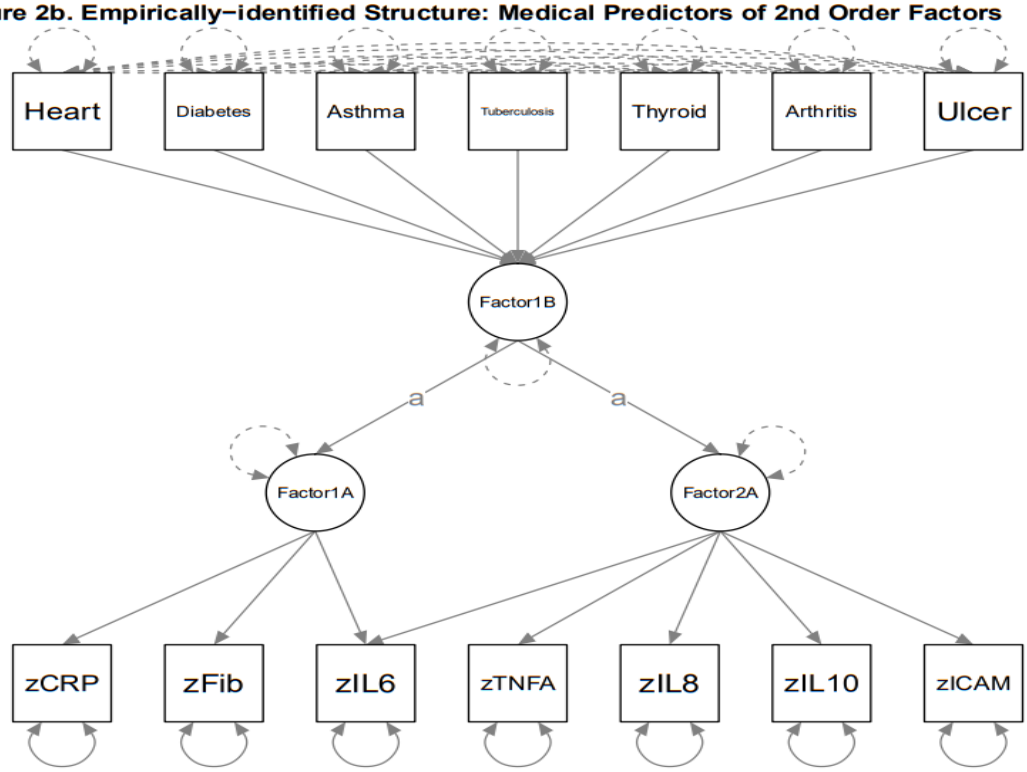


Figure 2b. Empirically–identified Structure: Medical Predictors of 2nd Order Factors



Note: "a" denotes loadings constrained to equality, z= z-standardized, CRP = C-reactive Protein, Fib=fibrinogen, IL = interleukin, TNFA= Tumor Necrosis Factor-α, ICAM = Intracellular Adhesion Molecule-1.

Figure 2c. Empirically–identified Structure: Medical Predictors of Individual Proteins

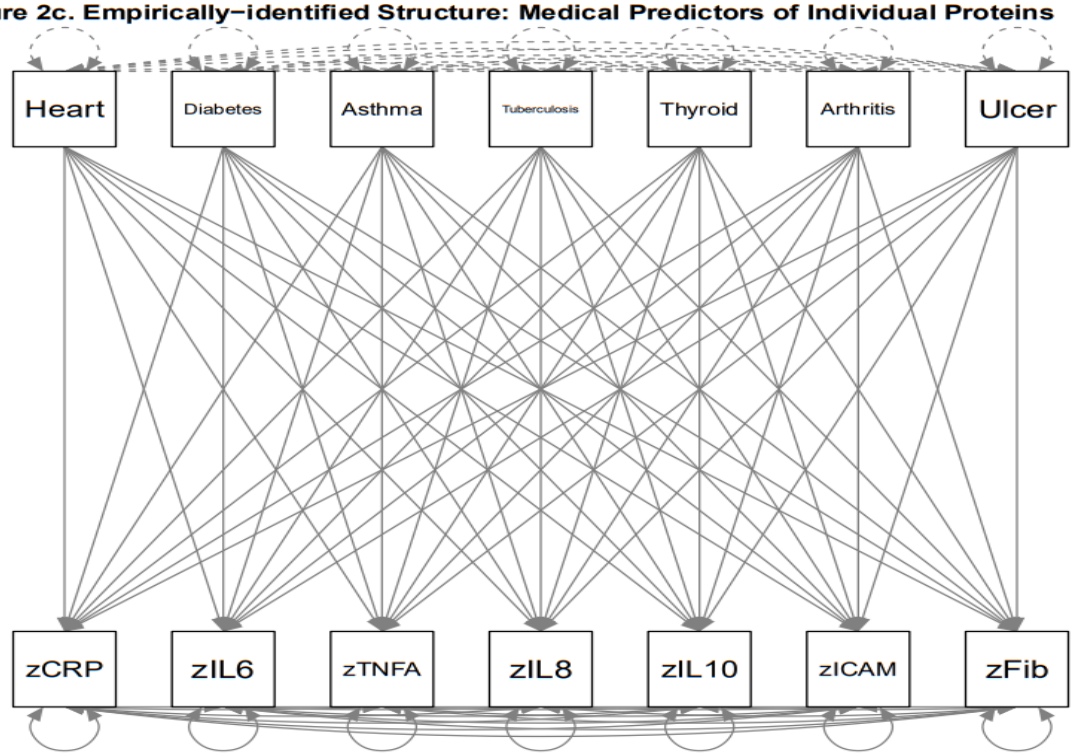
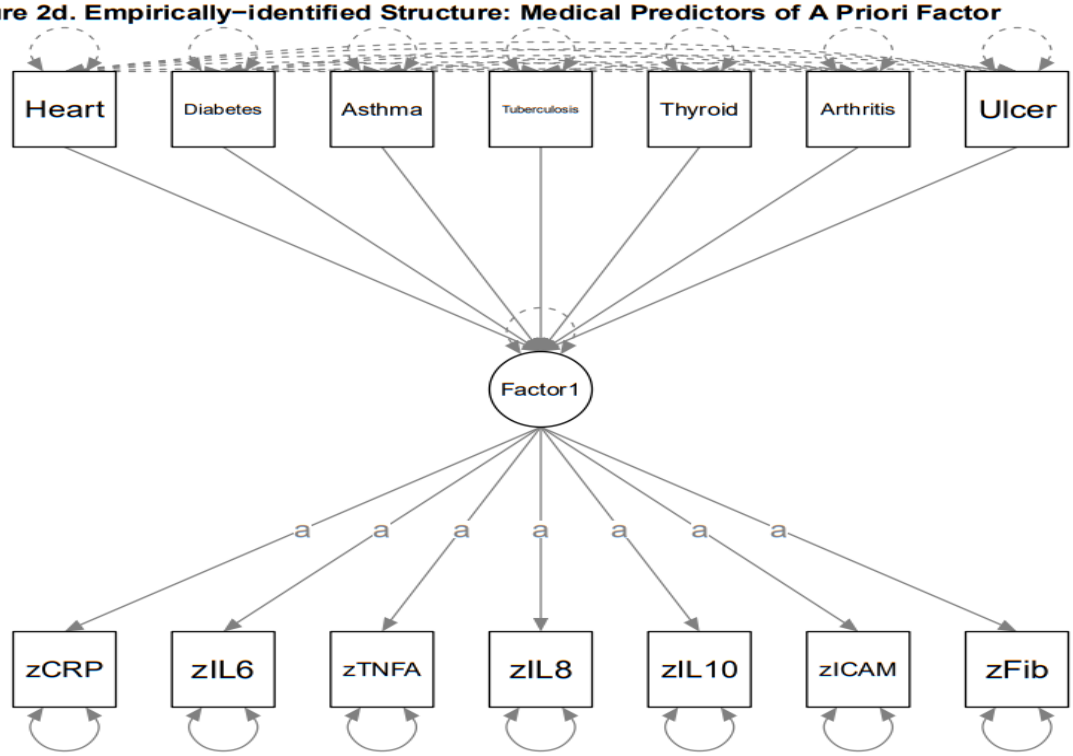


Figure 2d. Empirically–identified Structure: Medical Predictors of A Priori Factor



Note: “a” denotes loadings constrained to equality, z = z-standardized, CRP = C-reactive Protein, Fib=fibrinogen, IL = interleukin, TNFA= Tumor Necrosis Factor-α, ICAM = Intracellular Adhesion Molecule-1.

Table 2. Fit Statistics of Different Inflammatory Models

	χ^2	χ^2 df	p_{χ^2}	CFI	RMSEA	90% CI RMSEA	SRMR	AIC	BIC
MIDUS-R CFAs									
A priori	425.386	20	.001	.601	.155	.142-.167	.119	16276.780	16347.940
Empirically-identified (no IL-6 cross-loading)	78.196	13	.001	.936	.077	.061-.094	.042	15943.590	16047.960
Empirically-identified	42.331	12	.001	.970	.055	.037-.073	.036	15909.725	16018.839
MIDUS-R SEMs with Medical Criteria									
A priori	496.042	62	.001	.588	.095	.087-.102	.073	14958.274	15060.834
Empirically-identified: 1st order	124.989	47	.001	.926	.046	.036-.056	.034	14617.221	14789.709
Empirically-identified: 2nd order	131.136	54	.001	.924	.044	.034-.053	.036	14612.368	14752.223
Individual proteins	0.000	0	N/A	1.000	0.000	0.000	0.000	14586.231	14977.827
ACE CFAs									
A priori	136.672	9	.001	.395	.209	.178-.241	.148	4405.040	4446.319
Empirically-identified	8.239	4	.083	.979	.058	.000-.115	.033	4290.608	4350.649

Note: χ^2= Chi-squared, df = degrees of freedom, p = p-value, CFI = Comparative Fit Index, RMSEA = Root Mean Square Error of Approximation, CI = Confidence Interval, SRMS = Standardized Root Mean Square Residual, AIC = Akaike Information Criterion, BIC = Bayesian Information Criterion, MIDUS-R = Midlife in the United States-Replication, ACE = Adolescent Cognition and Emotion, CFA= Confirmatory Factor Analysis, SEM = Structural Equation Model, IL-6 = Interleukin-6

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Table 3. Protein Loadings

	MIDUS-2 EFA		MIDUS-R CFA		ACE CFA	
	Factor 1A	Factor 2A	Factor 1A	Factor 2A	Factor 1A	Factor 2A
CRP	.78		**.78** **90% CI = .71-.85**		**.71*** **90% CI = .62-.80**	
Interleukin-6	.58	.22	**.59** **90% CI = .52-.67**	**.23** **90% CI = .16-.31**	.71* 90% CI = .62-.80	**.18** **90% CI = .05-.31**
Tumor Necrosis Factor-α		.78		**.80** **90% CI = .71-.90**		.59 90% CI = .41-.77
Interleukin-8		.32		**.34** **90% CI = .27-.42**		.04 90% CI = -.10-.18
Interleukin-10		.50		**.47** **90% CI = .40-.55**		.75 90% CI = .54-.96
Fibrinogen	.67		**.69** **90% CI = .62-.76**		x	x
Intercellular Adhesion Molecule-1		.30		**.31** **90% CI = .23-.39**	x	x

Note: * = constrained to equality, x = unavailable in dataset. Loadings < .20 not depicted. CFA confidence intervals that include the original EFA estimate are bolded. MIDUS = Midlife in the United States, EFA = Exploratory Factor Analysis, CFA = Confirmatory Factor Analysis, CI = Confidence Interval, ACE = Adolescent Cognition and Emotion

Predictive Validity

Because of poor model fit (Table 2), the associations between the medical conditions and the "a priori" inflammation variable are not reported. The associations between the medical conditions and a) the empirically-identified factors and b) the individual proteins are reported in Table 4. All three empirically-identified inflammatory factors were significantly associated with a history of diabetes (Factor 1A b =.936, SE = .166, p < .001; Factor 2A b =.596, SE = .174, p = .001; Factor 1B b =1.355, SE = .260, p < .001) and arthritis (Factor 1A b =.256, SE = .106, p = .016; Factor 2A b =.284, SE = .113, p = .012; Factor 1B b =.470, SE = .151, p = .002). Additionally, Factor 1A was associated with asthma (b =.272, SE = .129, p = .035) and both Factor 2A and 1B were associated with a history of thyroid disease (Factor 2A b =.333 SE = .161, p = .038; Factor 1B b =.417, SE = .207, p = .044); however, these three results (33% of significant results) were no longer significant after family-wise (grouped by independent variable) Benjamini-Hochberg false-discovery rate corrections (BH-FDR).

In the model with the medical conditions predicting individual proteins, diabetes was significantly associated with CRP (b =.556, SE = .114, p < .001), IL-6 (b =.566, SE = .113, p < .001), TNF-α (b =.335, SE = .115, p = .004), and IL-8 (b =.613, SE = .112, p < .001) and arthritis was associated with IL-6 (b =.235, SE = .075, p = .002), IL-8 (b =.340, SE = .074, p < .001), and fibrinogen (b =.208, SE = .075, p = .005). These seven results were robust to BH-FDR corrections. The following seven associations (50% of significant results), were no longer significant after BH-FDR corrections: heart disease was associated with IL-6 (b =.257, SE = .127, p = .042), both asthma and tuberculosis were associated with CRP (b =.183, SE = .092, p = .048 and b =.791, SE = .371, p = .033, respectively), thyroid disease was associated with IL-8 (b =.214, SE = .106, p = .043), arthritis was associated with TNF-α (b =.156, SE = .076, p = .041),

and ulcers were associated with IL-6 and ICAM-1 (b =.378, SE = .159, p = .018 and b =.340, SE = .164, p = .038, respectively).

Four hierarchical linear models were estimated with inflammation predicting contemporaneous depression in Project ACE. Following the same logic described above, separate "first-order" and "second-order" models were estimated for the empirically-identified structure. The model with the five available proteins modeled as separate predictors in a single model had no significant associations with depression (CRP b = .224, SE = .199, p = .261; IL-6 b = .186, SE = .211, p = .380; IL-8 b = -.212, SE = .191, p = .268; IL-10 b = -.417, SE = .218, p = .056; TNF-α b = -.101, SE = .219, p = .628). Neither the unidimensional "a priori" aggregate nor the second order (Factor 1B) aggregate were significantly associated with depression (a priori b = -.065, SE = .061, p = .289; Factor 1B b = -.023, SE = .134, p = .864). Both first-order factors were significantly associated with depression, in opposite directions (Factor 1A b = .355, SE = .172, p = .039; Factor 2B b = -.391, SE = .149, p = .009).

Table 4. Medical Disorders Predicting Inflammatory Outcomes

	Heart	Diabetes	Asthma	Tuberculosis	Thyroid	Arthritis	Ulcer
	Coefficient (SE) *p*	Coefficient (SE) *p*	Coefficient (SE) *p*	Coefficient (SE) *p*	Coefficient (SE) *p*	Coefficient (SE) *p*	Coefficient (SE) *p*
Empirically-identified Factors							
Factor 1A	.241 (.178) .177	**.936 (.166)** **<.001**	**.272 (.129)** **.035**	.593 (.519) .254	.156 (.150) .298	**.256 (.106)** **.016**	.057 (.224) .801
Factor 2A	.243 (.188) .197	**.596 (.174)** **.001**	.041 (.136) .764	-.760 (.550) .167	**.333 (.161)** **.038**	**.284 (.113)** **.012**	.255 (.239) .287
Factor 1B	.423 (.244) .083	**1.355 (.260)** **<.001**	.284 (.176) .105	-.078 (.697) .911	**.417 (.207)** **.044**	**.470 (.151)** **.002**	.260 (.304) .391
Proteins Modeled Individually							
zCRP	.087 (.128) .497	**.556 (.114)** **<.001**	**.183 (.092)** **.048**	**.791 (.371)** **.033**	.093 (.108) .389	.085 (.075) .258	-.04 (.161) .803
zIL-6	**.257 (.127)** **.042**	**.566 (.113)** **<.001**	.123 (.091) .179	-.051 (.368) .890	.153 (.107) .153	**.235 (.075)** **.002**	**.378 (.159)** **.018**
zTNF-α	.195 (.129) .130	**.335 (.115)** **.004**	.058 (.093) .535	-.287 (.374) .443	.158 (.109) .146	**.156 (.076)** **.041**	.063 (.162) .063
zIL-8	.216 (.125) .085	**.613 (.112)** **<.001**	-.042 (.091) .644	-.426 (.364) .242	**.214 (.106)** **.043**	**.340 (.074)** **<.001**	-.034 (.158) .828
zIL-10	-.062 (.131) .637	.143 (.117) .223	-.038 (.095) .685	-.665 (.381) .081	.177 (.111) .110	.051 (.077) .514	.043 (.165) .796
zICAM-1	-.168 (.130) .199	.081 (.116) .485	.045 (.094) .635	-.020 (.379) .959	.144 (.110) .189	-.065 (.077) .400	**.340 (.164)** **.038**
zFib	.149 (.126) .238	.599 (.113) <.001	.153 (.091) .093	-.099 (.367) .788	096 (.106) .368	**.208 (.075)** **.005**	-.129 (.159) .415

Note: **bolded** = significant at an alpha-level of .05 (uncorrected), slashed cell = Benjamini-Hochberg correction shifted significant result to non-significant, SE = standard error, *p* = *p*-value, z = z-standardized, CRP = C-reactive Protein, IL = interleukin, TNF-α= Tumor Necrosis Factor-α, ICAM-1 = Intracellular Adhesion Molecule-1, Fib=fibrinogen.

Reliability and Temporal Stability

According to standard interpretive thresholds, reliability was questionable for the "a priori" factor structure (ω = .690). It was acceptable for Factor 1A (ω = .750) and poor for both Factor 2A (ω = .579) and Factor 1B ($\omega_{partial}$ = .579). Pearson correlations found the 8.5-14.5-month rank order stability to be consistently higher for all three empirically-identified factors compared to the "a priori" factor (a priori *r* = .242, Factor 1B *r* = .314, Factor 1A *r* = .471, Factor 2A *r* = .286). Rank order stability was variable for the individual proteins (CRP *r* = .473; IL-6 *r* = .374; IL-8 *r* = .205; IL-10 *r* = .363; TNF-α *r* = .384). Longitudinal ICCs for Factor 1A = .534, Factor 2A = .235, Factor 1B = .405, the "a priori" factor = .317, CRP = .457, IL-6 = .450, IL-8 = .164, IL-10 = .343, and TNF-α = .222.

Discussion

Most studies investigating inflammation in relation to medical or psychiatric illnesses test a variety of individual proteins as predictors and/or outcomes. This invites problems with multiple comparisons and creates a disconnect between theories about generalized inflammation and the analyses conducted. Alternatively, some studies use composite variables created without first investigating the appropriateness of this decision in what we describe as "a priori" composites. Results suggested that seven of the eight proteins available in the MIDUS datasets were best organized into a two-level factor structure with a general "inflammation" factor and two lower-order subfactors. In all comparisons made, this structure fit the data better than an "a priori" structure in which all inflammatory proteins are equally associated with a single factor, an approach that has been used previously (e.g., Moriarity, Ng, Titone et al., 2020). Compared to the "a priori" composite, empirically-identified factors and modeling proteins individually

(without an aggregate variable) consistently fit the data better (in fact, model fit was not acceptable for the “a priori” composite in all models tested).

Interestingly, fit criteria diverged on whether the empirically-identified structure fit the model with external criteria better than modeling the proteins individually. Specifically, BIC supported the empirically-identified structures and AIC supported individual markers. Unfortunately, because all variables in the individual protein model were observed variables, it is impossible to evaluate other fit statistics. Given the large sample size and the fact that BIC, but not AIC, is a consistent metric (i.e., the probability of selecting the true model approaches 100% as the sample size increases), it might be that the empirically-identified factors are preferable (see Dziak et al., 2020 for a more thorough comparison of information criteria). However, these findings are far from conclusive and future research is necessary. Additionally, careful consideration should be given to whether variable aggregation is a theoretically-appropriate course of action (i.e., if specific biomarkers are theorized to drive effects rather than generalized inflammation). Further, this study adopted a latent modeling approach to form aggregates; it is possible that other types of causal modeling (e.g., causal indicator models, network models, multiple-indicator multiple-cause (MIMIC) models) might be more suitable for inflammatory proteins.

In the empirically-identified structure, the inflammatory factor including CRP, IL-6, and fibrinogen was interpreted to reflect proteins involved in acute phase reaction processes localized in the liver. Both CRP and fibrinogen are acute phase reactants synthesized in the liver and upregulated by IL-6 (some of which is also made by liver cells, although it is produced by a variety of cell types) in response to acute stress and inflammatory challenges (Amrani, 1990; Davidson, 2013). Further, breakdown products of fibrinogen (e.g., D-dimers) can upregulate

both IL-6 and CRP, creating a positive feedback loop (Davidson, 2013). Additionally, this factor might represent chronic inflammatory processes (the temporal stability of this factor was higher than any other inflammatory variable assessed). Interestingly, these three proteins also consistently loaded onto the same factor in prior research in a community sample of older adults (Egnot et al., 2018) and a sample of adults with unstable angina pectoris (Koukkunen et al., 2001). Additionally, although IL-6 was not measured, CRP and fibrinogen also loaded onto the same factor in a sample of patients with insulin resistance syndrome (Sakkinen et al., 2000) and acute coronary syndrome (Tziakas et al., 2007). Also consistent with this study, ICAM-1 was measured, but did not load onto this factor in Egnot et al. (2018) or Tziakas et al. (2007). Similarly, TNF-α also was measured, but did not load onto this factor in Koukkunen et al. (2001). Consequently, there is evidence that this grouping is not an artifact of the specific array of proteins available in this study or other study-specific methods, demonstrating both internal replicability in this study and external replicability with previous studies.

The second inflammatory factor, consisting of IL-6, TNF-α, IL-8, IL-10 and ICAM-1 was interpreted to reflect a more general dimension of inflammation that might be related to more acute processes (both pro- and anti-inflammatory) particularly associated with neutrophil activity (Korthuis et al., 1994). IL-6 is a key regulator of neutrophil trafficking (Fielding et al., 2008), IL-8 and ICAM-1 can influence neutrophil adhesion (Divietro et al., 2001), and both TNF-α and IL-8 help neutrophils migrate to injury sites (Dixit & Simon, 2012; Smart & Casal, 1994). IL-6 also is involved in the regulation of TNF-α and IL-10, which might account for some of the shared variance between these indicators. Similarly, TNF-α often is co-released with IL-6. TNF-α also induces ICAM-1 (Burke-Gaffney & Hellewell, 1996), which, in turn, might increase TNF-α secretion (Etienne-Manneville et al., 1999) in a positive feedback loop. IL-10 serves as an

important mediator of neutrophil activity via regulation of proinflammatory cytokines (e.g., TNF-a and IL-6), and CXC keratinocyte-derived chemokine molecules (e.g., IL-8; Kessler et al., 2017; Sun et al., 2009). Further, given the different functions these factors are interpreted to reflect and differences in their temporal stability over time, it might be that this factor reflects more acute processes compared to the CRP, IL-6, and fibrinogen factor. Future research testing changes in these inflammatory factors in response to acute inflammatory challenges is necessary to investigate this possibility further.

It is critical to underscore the pleotropic nature of many inflammatory proteins and the complexity of inflammatory processes. For example, IL-6, commonly conceptualized as a "pro-inflammatory" protein, has several anti-inflammatory functions that are most likely dependent on classic-signaling via the membrane-bound non-signaling α-receptor IL-6R (Scheller et al., 2011). Given the contextual functioning of the immune system, shared variance analyses might be more informative in the context of acute inflammatory activity compared to the resting data used in this study. Truly, due to the complexity of this system and comparable model fit to the empirically-indicated factor structure, it might be more appropriate to analyze specific proteins and plan around the inherent complications of doing so. However, should the decision be made to use aggregate variables of inflammation, the results of this study consistently discourage the use of aggregates in which all proteins are equally weighted (throughout referred to as an "a priori" composite). Instead, standard aggregate measure-building procedures should be utilized (for more detailed concerns about the use of straight sum scoring to create aggregates when not all component variables are equally associated with the construct of interest, see McNeish & Wolf (2020)). Until a greater understanding of how to model inflammation is achieved, research

might benefit from simultaneously testing multiple scales of inflammatory measurement (Moriarity & Alloy, 2020).

If a choice between modeling individual proteins and empirically-supported composites is made, this decision should be made prior to data collection to facilitate complementary study design (e.g., sample size, time between observations in longitudinal studies) with the physiometrics of the variables to be used in mind. Armed with this information, researchers can design studies that require fewer resources (money, participants, repeated measures), with greater odds of finding replicable effects, and the research to intervention pipeline can be optimized (Moriarity & Alloy, 2021).

These empirically-identified factors, individual proteins, and the “a priori” factor were also tested in models with medical and psychiatric outcomes of interest. The associations between medical conditions and the “a priori” factor were not interpreted because of poor model fit. After using BH-FDR corrections, the same two medical conditions (diabetes and arthritis) predicted inflammatory outcomes in both the 1) empirically-identified factors and 2) individual proteins models. However, the use of the empirically-identified factors resulted in fewer results shifting from significance to non-significance post-correction (33% vs. 50%). When predicting depression symptoms, none of the individual proteins, the “a priori” composite, or the 2nd order empirically-identified composite (Factor 1B) were significantly associated with depression symptoms. However, the two empirically-identified first-order factors were associated with depression symptoms in opposite directions. Thus, aggregating these sources of variance would wash each other out, increasing the risk for false negatives and missing nuances in the relationship between inflammation and depression. Specifically, the “short-form” Factor 1A (CRP and IL-6) was positively associated with concurrent depression and “short-form” Factor

2A (IL-8, IL-10, TNF-α, and IL-6) was negatively associated with depression. Should these factors represent more chronic vs. acute inflammatory processes, these opposing associations would coincide with theory that inflammatory risk for depression symptoms is characterized by chronic low-grade inflammation (i.e., Factor 1A) as opposed to acute-reactions (which are a necessary part of biological reactivity to illness and injury). Further, all of the proteins in the "short-form" Factor 2A (i.e., IL-10, TNF-α, IL-6, IL-8) have functions that regulate pro-inflammatory processes (Kessler et al., 2017; Luscinskas et al., 1992; Scheller et al., 2011; Zakharova & Ziegler, 2005), which could explain an inverse association with depression symptoms.

This study has several important strengths. First, multiple sizeable datasets were used for replication featuring a combination of cross-sectional and longitudinal data allowing for a more thorough physiometric characterization of the variables under study. Second, the samples featured important differences in sample characteristics (i.e., age, race) supporting the generalizability of the empirically-identified factor structure to different demographic groups. Third, the fact that all three samples were community samples maximizes generalizability to other non-medical samples. Fourth, these three datasets allowed for both "complete" replication and a "conceptual replication" in which study methods differed. Indeed, MIDUS-R specifically was designed as a replication dataset for MIDUS-2, maximizing comparability of methods. This was complemented by the generally good replication in ACE, which did not use the same methods to measure biomarkers, suggesting that the results are not completely contingent on study methods.

However, this study must be interpreted in the context of several limitations. First, although eight proteins are more than most psychoneuroimmunology studies, there are many

inflammatory markers that were not included (e.g., IL-1β, T-cells, B-cells). Thus, the empirically-identified structure might look different when a broader array/different proteins are used. However, the finding that CRP, IL-6, and fibrinogen loaded onto one factor (which did not include ICAM-1 or TNF-α) is consistent with previous investigations of the dimensionality of inflammation in medical and community samples (e.g., Egnot et al. (2017)), supporting the generalizability of this factor. Second, the number of identifiable latent factors is constrained by the number of indicator variables. Consequently, a study with more than eight indicator variables might find a more multidimensional factor structure. Third, although a hierarchical structure of inflammation is theoretically plausible, it was impossible to statistically compare a hierarchical structure with two lower-order factors to a model with just two lower-order factors because they are equivalent models with alternative parameterizations. Fourth, the adolescent dataset used to test temporal stability and predictive validity only had five of the eight proteins included in the MIDUS datasets, forcing the investigation of a "short-form" aggregate. This concern is ameliorated somewhat by generally good model fit for the subset of inflammatory proteins. Additionally, despite good model fit, the original factor loadings (particularly IL-8 onto Factor 2A) in MIDUS-2 did not consistently fall in the confidence intervals of the CFA in ACE. It is important to note that this level of comparison might be more sensitive to missing two proteins (one from each first-order factor). These differences also could be due to differences in sample characteristics (e.g., developmental (Hager et al., 1994) or racial differences (Mayr et al., 2008)). Future research should conduct structural invariance tests to rule out these possibilities. Additionally, the medical criterion variables were measured via self-report of a *history* of a diagnosis. These results would be more informative if current medical conditions were known. Finally, many inflammatory proteins have diurnal variations (Dominguez-Rodriguez et al., 2009)

and Project ACE did not consistently restrict times for blood sampling (although efforts were made to ensure the majority of blood draws happened in the morning or early afternoon), unlike MIDUS-2 and MIDUS-R. However, patterns of model fit were comparable between the MIDUS projects and ACE, demonstrating that this methodological inconsistency did not render these samples incompatible for this analysis.

Conclusion

This study used standard aggregate measure building procedures to investigate the structure of eight inflammatory proteins. Results support the use of an empirically-identified hierarchical factor structure of inflammation or the modeling of individual proteins over an "a priori" aggregate in which all inflammatory proteins equally load onto a single dimension. Use of empirically-identified inflammatory factors as outcomes for medical history resulted in comparable conclusions to using individual proteins while losing fewer significant results to correction for multiple comparisons. When predictive validity to depression symptoms was compared, only the two first-order empirically-identified factors predicted depression, and the associations were in opposite directions, underscoring the potential benefits of this modeling approach and the increased risk of Type II error associated with falsely assuming unidimensionality. To facilitate other researchers in designing studies and analyzing data, reliability and temporal stability of the aggregates and proteins were described as appropriate. By building a strong physiometric foundation, research on inflammation can become more replicable, cost-effective, and clinically-impactful.

Supplemental Table 1. *Bivariate Correlations of Proteins in MIDUS-2*

Protein	1.	2.	3.	4.	5.	6.	7.	8.
1. CRP	—							
2. IL-6	.51	—						
3. TNF-α	.22	.33	—					
4. IL-8	.03	.15	.24	—				
5. IL-10	.10	.18	.37	.19	—			
6. Fibrinogen	.51	.42	.13	.07	.05	—		
7. E-selectin	.16	.17	.11	.11	.12	.13	—	
8. ICAM-1	.18	.17	.28	.07	.13	.12	.05	—

Note: CRP = C-reactive protein; IL- = Interleukin; TNF-α = tumor necrosis factor alpha; ICAM= intracellular adhesion molecule

Supplemental Table 2. *Bivariate Correlations of Proteins in MIDUS-R*

Protein	1.	2.	3.	4.	5.	6.	7.	8.
1. CRP	—							
2. IL-6	.52	—						
3. TNF-α	.21	.34	—					
4. IL-8	.06	.19	.28	—				
5. IL-10	.06	.17	.39	.04	—			
6. Fibrinogen	.53	.46	.18	.16	.03	—		
7. E-selectin	.22	.30	.29	.10	.19	.19	—	
8. ICAM-1	.19	.18	.23	.08	.18	.16	.39	—

Note: CRP = C-reactive protein; IL- = Interleukin; TNF-α = tumor necrosis factor alpha; ICAM= intracellular adhesion molecule

Supplemental Table 3. *Bivariate Correlations of Proteins in Project ACE*

Protein	1.	2.	3.	4.	5.
1. CRP	—				
2. IL-6	.54	—			
3. TNF-α	.14	.22	—		
4. IL-8	-.05	-.09	.10	—	
5. IL-10	.13	.28	.44	.01	—

Note: CRP = C-reactive protein; IL- = Interleukin; TNF-α = tumor necrosis factor alpha

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