Modelling stress constructs with biomarkers: the importance of the measurement model

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Modelling Stress Constructs with Biomarkers:

The Importance of the Measurement Model

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Abstract

Background

The transactional model of stress describes a psychological and physiological stress response that is elicited when an environmental demand is perceived to outweigh the ability to cope with the demand. With perception at its core, this theory explains large variances in human stress responses. A frequently cited measure of stress perception is the Perceived Stress Scale (PSS). A two-step approach to structural equation modelling (SEM) necessitates that a valid measurement model for stress be first established through confirmatory factor analysis (CFA), and then the impact of stress on the biomarkers be assessed.

Methods

The aims of this study were to explore and confirm the factorial structure of the 10-item PSS (PSS-10) in a sample of healthy Australians \( n = 194 \) and to cross-validate it in an independent sample \( n = 117 \) of healthy Australians with chronic work stress, and to explore the impact of stress on the production of the \textit{ex vivo} stimulated secretion of proinflammatory cytokines, interleukins IL-1\( \beta \), IL-6 and tumour necrosis factor (TNF\( \alpha \)), using SEM. As only Sample 1 had data on both PSS scores and proinflammatory cytokine secretion, SEM would be confined to Sample 1.
Results

Perceived Stress was found to have different factorial structures in the different samples. In Sample 1, it was best represented by three correlated factors; Overwhelmed (items 2, 10, 6, 8), Coping (items 4, 7, 5), and Emotional Reactivity (items 1, 9, 3). This three-factor model for was not cross-validated in Sample 2, which fit the two-factor model reported in the literature. The SEM suggested that the three factors predicted significant differential effects on proinflammatory cytokines that were less evident for the two-factor model.

Conclusions

These findings demonstrate that if the measurement model does not adequately represent the relationship structures within the data, differential effects on biomarkers may be diluted by larger measurement errors. This analysis has highlighted the importance of testing the assumptions of the measurement model used to represent stress in human populations. Further research is required to determine whether the construct known as perceived stress comprises two or three inherent factors in population norms.

Keywords

Psychological stress, stress appraisal, perceived stress scale (PSS), transactional model, factor analysis, structural equation modelling (SEM), stress measurement, proinflammatory cytokines, stress biomarkers.

Abbreviations

IL – interleukin
IFN – interferon
TNF – tumour necrosis factor
NKC – natural killer cells
df – degrees of freedom
χ² – chi-square
GOF – Goodness of Fit
TLI – Tucker-Lewis Index
RMSEA – Root Mean Square Error of Approximation
PSS – Perceived Stress Scale
EFA – exploratory factor analysis
CFA – confirmatory factor analysis
SEM – structural equation modelling
Background

‘Stress is a short, emotionally charged word for something that otherwise takes too many words to say … ’ [1]. According to the cognitive appraisal theory, stress results when and if the appraisal of an environmental demand results in the perception that there are insufficient coping resources to meet the demand. Stress perception, then, stimulates the brain (hypothalamus) to activate the biological response to stress, known as the fight-or-flight response [2]. Lazarus and Folkman [3] were instrumental in advancing this theory into a transactional model, a more holistic model that describes stress as a dynamic interaction between an individual and their environment over time, taking into account the role of previous experiences and adaptation processes to influence future perceptions and outcomes.

Research in the neurobiology of stress has highlighted the relationships between the mind and the body via the brain and the immune system; that emotional and psychological perceptions - via brain circuitry, nervous and endocrine systems - readily influence the health of the body via altered immune function. Further, it is now well established that communication between the brain and immune system is bi-directional [4]. The immune system, brain and nervous systems communicate via various mechanisms mediated by a wide group of signalling molecules, of which the most prominent are collectively known as cytokines. The immune system has been described as a sensory system and itself a stressor, capable of eliciting the biological stress response [5]. Short-term bursts of self-limiting psychological stress fuel the body with the necessary physiology for an adaptive response to environmental demands. Such transient enhancement of certain aspects of cognition, particularly attention, and immune function may even strengthen the organism and improve future survival prospects.

Exposure to chronic or repeated stress, however, increases susceptibility to impairment of neuroendocrine and immune function. Traditionally it was believed that the glucocorticoids (predominately cortisol in humans) themselves mediated neuroendocrine dysfunction. However, current thinking increasingly implicates the proinflammatory cytokines, predominately interleukin 1 (IL-1β), IL-6 and tumour necrosis factor (TNFα), as an important underlying mechanism linking stress with disease susceptibility. Mental health disorders are increasingly being associated with raised circulating proinflammatory biomarkers [6].

Cytokines are chemical messengers that coordinate the whole immune system during challenges to homeostasis; shaping an adaptive immune response by shifting the balance from predominately humoral to cellular during stress to optimise the chances of survival. These messenger molecules, however, are not restricted to communication between immune cells but are actually secreted by, and communicate with, every biological system including the brain. Consequently, these messenger molecules may be a vital link in the ‘mind-body
connection’, the molecular link between the perceptions of stress and altered immune function. Exploring the impact of stress on cytokines may provide vital information about the mechanisms by which mental and emotional states translate into physical disease and wellbeing states.

**Stress is proinflammatory**

Selective aspects of immune function, especially humoral immunity, are suppressed while aspects of cell-mediated (adaptive) immunity are increased. This is accomplished by a redistribution of blood leukocytes. Leukocyte trafficking and accumulation involves surface adhesion molecules on the leukocytes and on endothelial cells. Percentages of T and B cells, natural killer cells (NKCs) and monocytes are reduced in the general circulation and spleen but are increased in lymph nodes, bone marrow, and skin [7]. These transient changes are believed to be adaptive. For instance, a tear in the skin during the fight or flight response has the resources for quick repair. If the leukocytes accumulated in the lymph nodes receive a signal from cytokines of nearby injury they are readily released to infiltrate the tissue, and the inflammation cascade has commenced [8]. Therefore, the adaptive response to stress is to induce a proinflammatory state of physiological; hyper-vigilance.

Psychosocial and emotional stress has been associated with the increased secretion of proinflammatory cytokines [9-12]. Perceived stress induced the production of proinflammatory cytokines in 38 medical students under examination conditions. Maes et al [12] demonstrated significant increases in self-reported stress on the Perceived Stress Scale and anxiety (State-Trait Anxiety Scale) from baseline ($p = 0.002$ and $p < 0.0001$, respectively). Not surprisingly, the changes over time between stress and anxiety were strongly correlated ($r = 0.84$, $p < 0.0001$). In the group as a whole, the stress condition was characterised by increases in the whole blood ex vivo stimulation of proinflammatory cytokines IFN-$\gamma$ ($p = 0.0001$), TNF-$\alpha$ ($p = 0.0002$), IL-6 ($p = 0.05$), IL-1 receptor agonist ($p = 0.01$) and the immunoregulatory cytokine IL-10 ($p = 0.0003$) compared with the neutral condition (the average of neutral-pre and neutral-post conditions).

When students were divided into higher and lower stress perception, only those reporting higher stress perception had significantly higher stimulated production of IFN-$\gamma$ ($p = 0.0003$), TNF-$\alpha$ ($p = 0.0001$), IL-6 ($p = 0.028$) compared with baseline. Those reporting higher levels of stress perception had significantly higher IFN-$\gamma$ ($p = 0.01$), TNF-$\alpha$ ($p = 0.0007$), and IL-1 receptor agonist ($p = 0.006$) during stress than did those reporting lower levels of perceived stress. Higher scores on the PSS were correlated with higher levels of IFN-$\gamma$ ($r = 0.42$, $p = 0.007$), TNF-$\alpha$ ($r = 0.38$, $p = 0.01$), IL-1 receptor agonist ($r = 0.41$, $p = 0.008$) and IL-6 ($r = 0.32$, $p = 0.04$). When the groups were divided on high and low anxiety scores, those with high anxiety had higher levels of stimulated IFN-$\gamma$ production during stress compared with those with low self-reported anxiety ($p =$
0.03). During stress, those with high anxiety had lower stimulated production of IL-10 \((p = 0.04)\) and IL-4 \((p = 0.03)\) than those reporting low levels of anxiety. The increased Th-1 cytokine IFN-\(\gamma\) together with the decreased Th-2 cytokines IL-10 and IL-4 effectively skewed the Th-1/Th-2 ratio towards a Th-1 dominated proinflammatory state.

**Stress from a functional perspective**

There has been a suggestion of a new emerging paradigm in the understanding of stress, that it should be viewed from a functional perspective [13]. From this perspective, emotions may be viewed as mechanisms that adapt the neuroendocrine response to match the challenge. For instance, it has been proposed that an anger-driven motivational response prepares for confrontation or *fight*, where a fear-based response prepares for withdrawal or *flight*. Moons et al [13] demonstrated in 183 university students and staff members (71 men, 112 women), that IL-6 was significantly raised in states of fear \((p = 0.003)\) but not anger \((p = 0.93)\) immediately after an acute stressor (the Trier Stress Test). Moons et al speculated that proinflammatory cytokines are known to induce sickness behaviour and therefore promote withdrawal. However, they also conceded that this process would take time and therefore may not apply to the type of acute stress they measured (30 minutes after the commencement of the stressor).

Other studies, in contrast, have shown increased proinflammatory cytokine secretion during anger. Suarez et al [14] demonstrated in a sample of 62 men (aged 18 - 45 years) that anger was associated with increased *LPS*-stimulated TNF expression. They used the Buss–Perry aggression questionnaire. Raised TNF was associated with the total score \((p = 0.007)\), in addition to the subscales for hostility \((p = 0.013)\), physical aggression \((p = 0.010)\), and verbal aggression \((p = 0.034)\). They speculate that anger is a known predictor of coronary heart disease, and that this relationship could be mediated by TNF.

**Modelling perceived stress**

One of the few empirically tested multiple-item global stress appraisal scales is the Perceived Stress Scale (PSS) [15]. The original scale developed by Cohen et al [16] had 14 items, but Cohen and Williams [17] subsequently developed a 10-item and 4-item scale, and demonstrated that the 10-item scale had the highest internal consistency. This was recently cross-validated by Remor [18]. Scores on the PSS have been correlated with life events scores, depressive and physical symptomatology, social anxiety, utilisation of health services and maintenance of smoking-reduction [19].

The PSS-10 consists of ten questions designed to tap into how overloaded, unpredictable and uncontrollable an individual perceives their current life
circumstances. As distinct from scales that measure responses to specific stressors, the PSS was designed as a global measure of life stress. As such, the scale is sensitive to both current stressful circumstances and also background extraneous stressors. Six of the items are concerned with the experience of strain, while four items relate to perceptions of coping capacity over the past month. The total score for the PSS reflects the extent of perceived stress, is derived by summing across the ten items. This method of scoring assumes all items are equal indicators of the construct.

There are three published papers reporting on the factorial structure of the PSS-10 in non-clinical samples. A two-factor exploratory model for the PSS-10 was reported by Cohen et al [17] at the time of the development of the scale, with the four coping-related items (Qs 4, 5, 7, 8) loading on one factor and the six strain-related items on the other (Qs 1, 2, 3, 6, 9, 10). They conducted an exploratory factor analysis (EFA), using principle components and varimax rotation, and found the two factors accounted for 41.6% of the variance in the scale, with good scale reliability (Cronbach’s alpha = 0.75).

Although Cohen and Williamson had not cross-validated the exploratory two-factor model with a confirmatory factor analysis (CFA) on independent data, this has since been performed by Roberti, Harrington & Storch [20] in 285 American undergraduate students and by Wongpakaran and Wongpakaran [21] in 479 Thai medical students. The results of these two studies were, however, not consistent. Wongpakaran and Wongpakaran confirmed that the two-factor confirmatory model was a good representation of their data, given by the non-significant model chi-square statistic ($\chi^2_{(26)} = 35.035, p < 0.111$), but the same two-factor model in Roberti et al’s data was not such a good representation, given by a significant chi-square ($\chi^2_{(34)} = 121.78, p < 0.001$) and confirmed by the high relative ratio (chi-square/degrees of freedom > 2).

The aims of the current study were to (i) to confirm the two-factor structure as reported in the literature, using an Australian sample ($n = 194$). If the model was not adequately representative of the relationship structures inherent within the current data, then; (ii) to explore and confirm de novo the factor structure of the PSS-10 in the current data; (iii) to cross-validate the resulting measurement model for the PSS-10 with independent data ($n = 117$), and; (iv) explore the impact of the stress factors as represented by the measurement model on the production of proinflammatory cytokines.

**Methods**

**Participants and procedure**

Two samples were drawn from the general population of Australians. The recruitment of the first sample ($n = 194$) was conducted in 2007 and was
described in detail elsewhere [22]. Briefly, this sample was part of a larger cross-sectional study designed to model the impact of dietary fatty acids on cytokine production and stress outcomes in the general population. During sampling, participants completed stress questionnaires and gave a blood sample for cytokine and fatty acid analysis. Questionnaires were in paper format. The responses were then entered into an electronic spreadsheet by the researchers. This sample had prior ethics approval from two universities (University of Queensland and Southern Cross University) and all participants provided informed consent prior to sampling procedures.

The second sample \((n = 117)\) was recruited as part of a screening process for a clinical trial on the effects of a nutritional supplement in work stress in 2011. The recruitment advertisements called for people with high work stress and without any concurrent inflammatory diseases. Potential participants that passed a brief phone screen were sent an invitation via email to Qualtrics, an online survey software site, where they could complete the questionnaire. The responses were collected in an electronic spreadsheet by Qualtrics.

**Instruments**

The Perceived Stress Scale (PSS) [16] is a validated and reliable subjective measure of the amount of psychological or emotional stress an individual perceives. The PSS-10 consists of 10 questions, which ask about the individual’s appraisal of the stressful circumstances in addition to the appraisal of available coping resources. The questions ask respondents about thoughts and feelings they have experienced during the last month. Items were answered on a five-point Likert scale from 0 = never to 4 = very often. This questionnaire is included here as Appendix 1.

**Cytokine analysis**

Fasting blood samples were collected between 7 – 9am. Whole blood was stimulated \textit{ex vivo} with lipopolysaccharide (LPS) from \textit{E. Coli}. After 4h incubation at 37°C, the supernatant culture was harvested and stored at -80°C until completion of data collection, when all samples were analysed as a single batch. Cytokines were quantified by Becton-Dickenson bead array methods.

**Modelling**

The procedure for the statistical modelling started in Sample 1 using confirmatory factor analysis of the two-factor model from the literature. If found unrepresentative, exploratory factor analysis (EFA) and then confirmatory factor analysis (CFA) would be applied to explore and confirm the factor structure of the PSS-10 for the current sample. The resulting confirmatory model would then be applied to Sample 2 for cross-validation.
The EFA was conducted using SPSS v18. Exploratory Factor Analysis used Maximum Likelihood (ML) extraction. ML was used in conjunction with an oblique rotation method (Direct Oblimin, delta = 0) as the factors were likely to be correlated. The output from the rotated analysis provides pattern and structure matrices that aid in the interpretation of the factorial structure. The decision for the number of factors to extract was guided by: (i) eigenvalues > 1; (ii) inspection of the Scree plot; (iii) parallel analysis; (iv) GOF statistical non-significance, and; (v) consideration of the proportion of significant residuals (> 0.05) in the reproduced correlation matrix.

The CFAs and structural equation modelling (SEM) were conducted with MPlus, v6.1, using the Maximum Likelihood (ML) estimator to provide a chi-square ($\chi^2$) and associated goodness of fit (GOF) statistics. Model fit was considered adequate after consideration of the following criteria: (i) the chi-square statistic was not significant ($p > 0.05$); (ii) the chi-square to degrees of freedom ratio was < 2; (iii) Root Mean Square Error of Approximation (RMSEA) < 0.08; (iv) Tucker-Lewis Index (TLI) > 0.90, and; (v) consideration of model parsimony.

Results and Discussion

Descriptive and preliminary statistics

Sample 1 consisted of 194 adults predominately (94.9%) from an academic population, both university staff and students, comprising 74% females and 26% males. The overall Sample 1 mean for the total score on the PSS-10 was 15.9 (SD = 5.9). Sample 2 consisted of 117 adults with high work stress, comprising 68% female and 32% male. The overall sample mean was 23.2 (SD = 5.2). There were no significant differences in means scores by sex. As illustrated in Table 1, there was a much fuller range of stress scores in Sample 1, where Sample 2 stress scores were limited to the higher end of stress. Three multivariate outliers were removed from Sample 1, subsequent analysis was conducted on $n = 191$. There were no outliers in Sample 2.

Table 1 Descriptive statistics for the PSS-10 by sample and sex

<table>
<thead>
<tr>
<th>Subgroups</th>
<th>Mean</th>
<th>SE</th>
<th>Median</th>
<th>SD</th>
<th>Min</th>
<th>Max</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>16.3</td>
<td>.49</td>
<td>16.0</td>
<td>5.89</td>
<td>2</td>
<td>30</td>
<td>143</td>
</tr>
<tr>
<td>Male</td>
<td>14.9</td>
<td>.84</td>
<td>15.0</td>
<td>5.99</td>
<td>3</td>
<td>28</td>
<td>51</td>
</tr>
<tr>
<td>Sample 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>23.2</td>
<td>.61</td>
<td>23.0</td>
<td>5.51</td>
<td>11</td>
<td>33</td>
<td>80</td>
</tr>
<tr>
<td>Male</td>
<td>23.0</td>
<td>.75</td>
<td>22.0</td>
<td>4.60</td>
<td>16</td>
<td>37</td>
<td>37</td>
</tr>
</tbody>
</table>
Modelling stress constructs with biomarkers

Confirmatory Factor Analysis

The two-factor model from the literature was tested in Sample 1. This model had the four reverse-scored items (Qs 4,5,7,8 labelled Perceived Self-Efficiency) loading one factor and all the others loading on the other, called Perceived Helplessness. In Sample 1 this two-factor model was not a close fit to the data, given by the significant chi-square and RMSEA > .08 ($\chi^2_{(34)} = 80.78$, $p \leq .0005$, RMSEA = .09). Interestingly, when the factor labelled Perceived Helplessness was fitted with three correlated errors, the model was significantly improved ($\chi^2_{(31)} = 50.86$, $p = 0.01$, RMSEA = 0.06), as given by the chi-square difference test ($\chi^2_{(3)} = 29.92$, $p \leq 0.0005$).

Tellingly, the degrees of freedom were much lower in the Wongpakaran and Wongpakaran model (df = 26) suggesting the presence of unreported correlated errors accounting for the closer model fit to their data. Correlated measurement errors suggest that there may be factors unique to the items that are missing from the model. In this case, the error correlations were between items 1 and 3, 1 and 9, and 10 and 6, suggesting that the Perceived Helplessness factor could better be represented by two smaller factors. Further, the inter-factor correlation in this two-factor model was very high (-0.92), undermining the discriminate validity of the factors in the two-factor model.

Exploratory Factor Analysis (EFA)

An EFA was conducted de novo in the current data ($n = 191$). The suitability of the strength of the correlations for factor analysis was confirmed by a significant Bartlett’s Test of Sphericity ($\chi^2_{(45)} = 70.3$, $p \leq 0.0005$). The factorability of the items was confirmed by a Kaiser-Meyer-Olkin Measure of Sampling Adequacy = 0.9, well over the recommended minimum of 0.6. There was one Eigenvalue > 1, accounting for 48.7% of the total variance among the items. The Goodness of Fit (GOF) statistic was highly significant, indicating a poor model fit for the one-factor model ($\chi^2_{(35)} = 88.09$, $p \leq 0.0005$). In addition, the reproduced correlation matrix indicated that almost 40% of the residuals were significant (> 0.05), further evidence of a poor model fit to the data. However, a Monte Carlo PCA parallel analysis with a randomly generated dataset of a similar size (10 variables, 191 observations, 100 replications) suggested one valid factor. While the Scree plot (Figure 1) indicated one clear factor, it also suggested that there could be up to three distinct factors.
The EFA was subsequently reanalysed specifying a three-factor solution. The three factors accounted for 67.2% of the total variance. The GOF statistic signified a good model fit ($\chi^2_{(18)} = 22.4, p = 0.215$) and there was an acceptable proportion of large residuals (6%). Comparison of the model fit statistics and number of residuals in the reproduced covariance matrix between the one, two and three factor exploratory models determined by the EFA demonstrated that the three-factor model was clearly the best representation of the relationship structure contained within the data (Table 2).

Table 2 Model fit statistics and residuals in reproduced covariance matrix for the three exploratory factor models of the PSS-10

<table>
<thead>
<tr>
<th>EFA Model</th>
<th>Goodness-of-fit Test</th>
<th>Sig. residuals(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chi-Square</td>
<td>df</td>
</tr>
<tr>
<td>1 Factor</td>
<td>88.091</td>
<td>35</td>
</tr>
<tr>
<td>2 Factor</td>
<td>53.193</td>
<td>26</td>
</tr>
<tr>
<td>3 Factor</td>
<td>22.398</td>
<td>18</td>
</tr>
</tbody>
</table>

Table 2 legend: \(^1\) Significant residuals ( > 0.5) in the reproduced covariance matrix; * signifies a significance at the $p < .05$ level
The pattern matrix (Table 3) determined that after rotation, only one item was significantly (> .3) cross-loaded on two factors (Q8), however three other items had cross-loadings over .25. Apart from these cross-loadings, the pattern matrix revealed a clear factor structure for a three-factor solution.

**Table 3** Pattern matrix for two models extracted by exploratory factor analysis for the ten items of the PSS-10.

<table>
<thead>
<tr>
<th>Item</th>
<th>Three factor EFA</th>
<th>Two factor EFA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Q1</td>
<td>-0.89</td>
<td>-0.93</td>
</tr>
<tr>
<td>Q2</td>
<td>0.39</td>
<td>0.30</td>
</tr>
<tr>
<td>Q3</td>
<td>-0.56</td>
<td>0.38</td>
</tr>
<tr>
<td>Q4R</td>
<td>0.28</td>
<td>0.53</td>
</tr>
<tr>
<td>Q5R</td>
<td>-0.56</td>
<td>0.38</td>
</tr>
<tr>
<td>Q6</td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td>Q7R</td>
<td>-0.31</td>
<td>0.27</td>
</tr>
<tr>
<td>Q8R</td>
<td>0.45</td>
<td>0.37</td>
</tr>
<tr>
<td>Q9</td>
<td>-0.58</td>
<td>-0.47</td>
</tr>
<tr>
<td>Q10</td>
<td>0.61</td>
<td>0.65</td>
</tr>
</tbody>
</table>

Table 3 legend: R - reverse-scored; Note: Factor loadings < 0.25 are not shown in pattern matrix.

In the three-factor model, Factor 1 loaded four items (6, 10, 8 and 2). The highest item loaded onto the first factor was Q6, which asks how often ‘you found you could not cope with all the things that you had to do’. The other items loading onto the first factor were similarly concerned with inability to overcome difficulties, such that this factor was labelled *Overwhelmed*. Factor 2 grouped items concerned with emotional behaviour. The highest loading item Q1 asks how often the respondent has ‘been upset’. Because they were negatively loaded, this suggested that the factor represented the opposite of emotional, thus labelled *Emotional Control*.

The highest item loaded on Factor 3 was Q5 (‘things going your way’) and the other two items were similarly concerned with positive coping ability. However, they had been reverse-scored prior to the EFA. This factor was thus labelled *Not*. 
Coping. The correlations between the factors were moderate (between .52 - .6), suggesting that the factors are clearly distinct constructs.

In summary, EFA was used to explore whether the ten items of the PSS could be adequately represented by a smaller number of factors. One, two and three model solutions were tested. No one factorial solution was found to possess all the criteria for a good representation of the data. Nevertheless, the three-factor solution was found to best represent the structures inherent within the data of Sample 1, evidenced by a non-significant chi-square and lower proportion of large residuals in the reproduced correlation matrix.

**Confirmatory Factor Analysis (CFA)**

The three-factor exploratory model as determined by the EFA, complete with its four cross-loadings, was then modelled using CFA in MPlus. CFA found this model a very good fit to the data ($\chi^2 (28) = 36.01, p = .14$, RMSEA = 0.04, TLI = 0.98). In the interests of parsimony, the model was modified with the aim of finding the closest fitting model without the presence of any items loading onto more than one factor (cross-loading). Competing models were subsequently tested and model fit indices compared. The final three-factor confirmatory model had a significant chi-square ($\chi^2 (32) = 49.76, p = .02$), nonetheless the overall model fit statistics indicated a reasonable fit (RMSEA = .54, CI: 90% = .02 - .08, TLI = .97).

**Table 4** Comparison of model fit statistics for confirmatory models of the PSS-10 using Sample 1 data

<table>
<thead>
<tr>
<th>CFA Model</th>
<th>Goodness-of-fit Tests</th>
<th>Chi-Sq</th>
<th>df</th>
<th>p</th>
<th>RMSEA</th>
<th>TLI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Factor</td>
<td></td>
<td>91.05</td>
<td>35</td>
<td>$&lt;.0005^*$</td>
<td>.09</td>
<td>.90</td>
</tr>
<tr>
<td>2 Factor (EFA)</td>
<td></td>
<td>68.00</td>
<td>34</td>
<td>.0005*</td>
<td>.07</td>
<td>.94</td>
</tr>
<tr>
<td>2 Factor (literature)</td>
<td></td>
<td>79.40</td>
<td>34</td>
<td>$&lt;.0005^*$</td>
<td>.08</td>
<td>.92</td>
</tr>
<tr>
<td>3 Factor</td>
<td></td>
<td>49.76</td>
<td>32</td>
<td>.02*</td>
<td>.05</td>
<td>.97</td>
</tr>
</tbody>
</table>

Table 4 legend: *denotes statistical significance at $p < 0.05$ level; $p$ - significance level; RMSEA - Root Mean Square Error of Approximation; TLI - Tucker-Lewis Index

A comparison of the model fit statistics, using the chi-square difference test, provided in Table 4, demonstrated that the three-factor model had the lowest and least significant chi-square statistic. Therefore the three factor model, as
illustrated in Figure 2, best represented the structural relationships within the data for Sample 1.

**Figure 2** Three factor confirmatory model (Sample 1 data; \( n = 191 \))

![Diagram of the three-factor confirmatory model](image)

*Figure 2 legend: Fully standardised model; Model \( \chi^2(32) = 49.76, p = .02, \) RMSEA = .54, CI: 90% = .02 - .08, TLI = .97*

**Model cross-validation**

The three-factor model was tested in the new data (\( n = 117 \)), the chi-square was once again significant but once again the other fit indices indicated an adequate fit to the data (\( \chi^2(32) = 51.42, p = .02, \) RMSEA = .07 CI: 90% = .03 - .11, TLI = .92). However, when the two-factor model from the literature was tested in Sample 2, it was a much better fit to this data than was the three-factor model (\( \chi^2(34) = 39.54, p = .24, \) RMSEA = .04, CI: 90% = .000 - .08, TLI = .98). Comparisons of model fit statistics for Sample 2 data are provided in Table 5. The two-factor model from the literature as applied to Sample 2 data is illustrated in Figure 3.
Table 5 Comparison of model fit for confirmatory models of the PSS-10 using Sample 2 data

<table>
<thead>
<tr>
<th>CFA Model</th>
<th>Goodness-of-fit Tests</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Factor</td>
<td>Chi-Sq: 78.36</td>
<td>df: 35</td>
<td>p: &lt;.0005*</td>
<td>RMSEA: .1</td>
<td>TLI: .83</td>
</tr>
<tr>
<td>2 Factor (EFA)</td>
<td>Chi-Sq: 77.12</td>
<td>df: 34</td>
<td>p: &lt;.0005*</td>
<td>RMSEA: .10</td>
<td>TLI: .83</td>
</tr>
<tr>
<td>2 Factor (literature)</td>
<td>Chi-Sq: 39.54</td>
<td>df: 34</td>
<td>p: .24</td>
<td>RMSEA: .04</td>
<td>TLI: .98</td>
</tr>
<tr>
<td>3 Factor</td>
<td>Chi-Sq: 51.42</td>
<td>df: 32</td>
<td>p: .02*</td>
<td>RMSEA: .07</td>
<td>TLI: .92</td>
</tr>
</tbody>
</table>

Table 5 legend: *denotes statistical significance at \( p < 0.05 \) level; \( p \) - significance level; RMSEA - Root Mean Square Error of Approximation; TLI - Tucker-Lewis Index

**Figure 3** Two factor confirmatory model (Sample 2 data; \( n = 117 \))

![Figure 3](image)

Figure 3 legend: Fully standardised model; Model \( \chi^2_{(34)} = 39.54, p = .24, \) RMSEA = .04, CI: 90% = .000 - .08, TLI = .98

**Summary of CFA**

The three-factor model was the best fit for the first sample (\( n = 191 \)), while the two-factor model was the best fit for the second sample (\( n = 117 \)).
Structural equation modelling

When the three cytokines were regressed on the three-factor measurement model, as shown in Figure 4, the model was an adequate fit to the data, given by the relative chi-square < 2 ($\chi^2_{(53)} = 89.34, p = .001$, RMSEA = .06, CI: 90% = .04 - .08, TLI = .93). The standardised regression coefficients, provided in Figure 4, indicate that the different factors impact the proinflammatory cytokines in different ways; some were negative while others were positive, some were weak and not significant while others were strong and significant.

Figure 4. The differential effects of the three factors of the PSS-10 on the production of proinflammatory cytokines in Sample 1

The factor labelled Overwhelmed as with the factor labelled Coping were inversely related to the cytokines, such that as scores on these factors increased, the production of all three proinflammatory cytokines decreased. This relationship was most significant for the impact of Overwhelmed on IL-6, such that for every one standard deviation increase in Overwhelmed, there is a corresponding reduction of 77% of one standard deviation in the secretion of IL-6.
For every one standard deviation increase in positive *Coping* scores, there was a corresponding reduction in the production of IL-6 (61% of one standard deviation) and an increase in TNF secretion (63% of one standard deviation). For each standard deviation increase in the factor labelled *Emotionally Reactive* there was a direct increase of 47% of a standard deviation increase in the production of IL-1. Clearly, these differential effects, a mixture of direct and inverse and significant and nonsignificant, were diluted in the one factor model, but were evident in the three-factor model.

The three cytokines were regressed, as an exercise of interest, on the two factors from the two-factor model (literature). Not surprisingly, the model was not as well fitting as the SEM using the three-factor measurement model for the items on the PSS-10 but better fitting than the SEM using the one-factor measurement model ($\chi^2_{(58)} = 110.32, p = .000$, RMSEA = .07, CI: 90% = .05 - .09, TLI = .94). There were no significant regressions among the cytokines on the stress factors, although TNF was quite strongly related to both factors, labelled for convenience *Strain* as it loaded the six stress-related items (-.59, $p = .07$) and *Not Coping* as it loaded the coping-related items that were reverse-scored (-.60, $p = .06$) and IL-6 with strain (-.44, $p = .15$) and *Coping* (-.53, $p = .09$). Interestingly, the direct impact that the *Emotional* factor had on IL-1 was lost in this two-factor model.

The one-factor model for the PSS-10 with the three cytokines regressed on *Perceived Stress* was not a close fitting model, given by the relative ratio > 2 and the TLI < .9 ($\chi^2_{(62)} = 132.49, p \leq .00005$, RMSEA = .08, CI: 90% = .06 - .10, TLI = .88). The standardised regressions of the cytokines on the one factor *Perceived Stress* were not significant (.05, $p = .56$ for IL-1; .07, $p = .35$ for IL-6; and .01, $p = .93$ for TNF). The cytokines were not significantly regressed on *Perceived Stress*, as measured by a one-dimensional measurement model, which is assumed by use of the total score of the PSS.

**Conclusion**

This analysis demonstrates the importance of assessing the measurement model used to represent stress, particularly in research seeking to model stress on cytokine profiles. Perceived stress is a complex construct, made up of several inherent factors. When the appropriate factor structure is taken into account by including it in the model, more of the error variance is explained, which facilitates a clearer delineation of the more subtle effects of stress on functional biomarkers, such as the production of proinflammatory cytokines. Moreover, these subtle effects risk being diluted in models that do not adequately represent the relationships within measurement models of a given data set.
The two factor model from the literature (which loaded the four positively worded items on one factor and the remaining six items on the other) was first tested in Sample 1 and found not to adequately represent the relationships inherent in the data. Hence, the three-factor model was arrived at de novo in the first sample through first identifying the factor structure using EFA and then confirming it using CFA. Interestingly, the three-factor model was not cross-validated in the second data set (Sample 2). Indeed, the two-factor model from the literature was found to be the best representation of the relationships within the data for the second sample. Notably, neither the two- nor the three-factor measurement models were invariant across samples, indicating that the relationships amongst the items had different structures in the different samples. This may have been influenced by the different stress levels between the samples. Sample 1 included a wide range of stress levels, where the participants in Sample 2 had chronic work stress, therefore higher than average stress levels. It may be that different facets of stress become more or less significant as stress levels vary.

In this analysis, there was a clear distinction between the Emotional and Overwhelm factors in Sample 1, the sample from a wide cross-section of stress levels in the community. In contrast, the distinction between the Emotional and Overwhelm factors was much less important in Sample 2, the sample of people with high chronic work stress. In Sample 2, the two factors were blended to form one general factor, called Strain. According to the theory, long term exposure to stress precipitates an adaptation process, which involves physiological and behavioural components. Perhaps these findings signal a psychological adaptation, a blending of the two extremes of the psychological stress reaction; a ‘getting on with it’ approach to dealing with ongoing stress, as distinct from a more motivational related ‘emotional’ and perhaps withdrawal related ‘overwhelmed’ initial responses to acute stress.

These differences suggest that different models of stress may better represent different types of stress (for example acute versus chronic). A much larger study is required to firmly establish whether these findings hold in similar samples or whether there is indeed a ‘one size fit all’ model for stress in the population norms.

When the one-factor and three-factor measurement models for perceived stress were modelled to assess their respective impact on cytokines, the three-factor model explained more variance in the cytokines. Critically, the three-factor model allowed subtle, differential effects of the stress factors on the cytokines. Thus the three-factor model provided much more information than the one-factor model, which may have diluted its effects by the grouping of the stress outcomes from three outcomes to a single construct. It should be noted that these findings are specific to Sample 1. Unfortunately Sample 2 used in this analysis did not have corresponding cytokine data. Therefore, this exploratory SEM (Figure 4) should
be cross-validated in an independent sample before any meaningful interpretations can be generalised to the wider population.

Statistical modelling provides a sophisticated and powerful methodology for assessing the impact of stress perception on a range of biomarkers. In order to detect subtle differences in the immunological correlates for a complex, multidimensional construct such as stress, the most representative measurement model of the construct itself should first be established. The construct known as perceived stress is made up of several inherent factors. Therefore, modelling the inherent factors provides closer fitting models, accounting for more of the variance (noise) in the measurement model and facilitating stronger structural relationships (signals) between stress factors and biomarker outcomes.

References


Appendix – The Perceived Stress Scale (PSS-10)

Instructions: The questions in this section ask you about your feelings and thoughts during the last month. In each case, please indicate with a check how often you felt or thought a certain way.

<table>
<thead>
<tr>
<th>Item</th>
<th>Never</th>
<th>Almost never</th>
<th>Sometimes</th>
<th>Fairly often</th>
<th>Very often</th>
</tr>
</thead>
<tbody>
<tr>
<td>In the last 30 days, how often have you been upset because of something that happened unexpectedly?</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>In the last 30 days, how often have you felt that you were unable to control the important things in your life?</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>In the last 30 days, how often have you felt nervous and &quot;stressed&quot;?</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>In the last 30 days, how often have you felt confident about your ability to handle your personal problems?</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>In the last 30 days, how often have you felt that things were going your way?</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>In the last 30 days, how often have you found that you could not cope with all the things that you had to do?</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>In the last 30 days, how often have you been able to control irritations in your life?</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>In the last 30 days, how often have you felt that you were on top of things?</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>In the last 30 days, how often have you been angered because of things that were outside of your control?</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>In the last 30 days, how often have you felt difficulties were piling up so high that you could not overcome them?</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

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