Accepted Manuscript

Full-length Article

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PII: S0889-1591(16)30459-7
DOI: http://dx.doi.org/10.1016/j.bbi.2016.10.003
Reference: YBRBI 2982

To appear in: *Brain, Behavior, and Immunity*

Received Date: 26 July 2016
Revised Date: 28 September 2016
Accepted Date: 5 October 2016

Please cite this article as: Klyne, D.M., Barbe, M.F., Hodges, P.W., Systemic inflammatory profiles and their relationships with demographic, behavioural and clinical features in acute low back pain, *Brain, Behavior, and Immunity* (2016), doi: http://dx.doi.org/10.1016/j.bbi.2016.10.003

Title: Systemic inflammatory profiles and their relationships with demographic, behavioural and clinical features in acute low back pain

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Disclosures: This research was funded by the National Health and Medical Research Council (NHMRC) of Australia (Program Grant: ID631717; Project Grant ID631369). PWH supported by NHMRC Fellowship ID1002190. There are no conflicts of interest related to this work.

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Abstract

Systemic inflammation is linked with development and persistence of many pathological pain states. Although chronic phase inflammatory responses are well reported, the acute phase has received limited attention. Here we investigated circulating pro-inflammatory cytokines and C-reactive protein (CRP), and explored their relationships with symptom severity and other factors in acute low back pain (LBP). Ninety-nine individuals within two weeks of onset of acute LBP and 55 pain-free controls completed questionnaires related to their pain (visual analogue scale, VAS) and disability, behaviour, sleep quality and psychological status. CRP, interleukin-6 (IL-6), tumor necrosis factor (TNF) and interleukin-1β (IL-1β) were measured from serum samples. Biomarkers were compared between LBP and control participants, and in a separate analysis, for those with "high-pain" (VAS≥4) and "low-pain" (VAS<4). The relationships between biomarkers and all other variables, including other cytokines/CRP were assessed. CRP was higher in LBP than controls and in those with high- than low-pain ($p<0.01$). IL-6 was higher in those with high- than low-pain ($p<0.05$), but not controls. Various pain and non-pain factors were associated with each biomarker differently. These findings suggest systemic CRP and IL-6 are important contributors to inflammation in the early post-onset phase of LBP and that various factors can shape these responses.

1. Introduction

Development and persistence of many pain states has been linked with a systemic inflammatory response [18,117]. Attention has focused on inflammatory cytokines and mediators that play key roles in the immune response following injury or harmful stimuli [17]. Cytokines are proteins produced by numerous cells that have specific effects on the interactions and communications between immune-related cells, and play an integral role in the initiation, perpetuation and downregulation of the immune response [125]. In addition to their role in the immune response, pro-inflammatory cytokines sensitise nociceptors either directly or via stimulation of the release of agents that modulate nociception both peripherally and centrally [1,118]. Pro-inflammatory cytokines have also been implicated in the development of "sickness" symptoms such as depression, anxiety, sleep disturbances and cognitive deficits [70,104]. These relationships are bidirectional; peripheral pro-inflammatory cytokines can induce sickness behaviours and sickness behaviours can upregulate peripheral pro-inflammatory cytokines [69].

Many chronic pain conditions are linked with elevated circulating pro-inflammatory cytokines. Elevated tumor necrosis factor (TNF) has been reported in chronic low back pain (LBP) [117], interleukin-6 (IL-6) in fibromyalgia [50] and interleukin-1β (IL-1β) in chronic tension-type headache [29]. In some cases cytokines are related to symptom severity (e.g. upper-extremity overuse disorders [18]; mixed chronic musculoskeletal pain conditions [57]). C-reactive protein (CRP) is a reactant in the acute phases of inflammation primarily synthesised in the liver in response to IL-6 [48], an effect that is enhanced by IL-1β [61,64] and TNF [73]. Elevated CRP has been reported in pain disorders [18,87,92,121] and is also associated with symptom severity [18,105]. Although the role is unclear, CRP is an important clinical marker of inflammation [96], predictor of some inflammatory diseases [73,93], and marker of treatment response [65].

As the relationship between pro-inflammatory cytokines and pain-related behavioural/psychosocial symptoms is considered bidirectional, whether they develop simultaneously or sequential cannot be determined from cross-sectional studies of chronic pain. For instance, poor sleep quality and elevated IL-6 have both been reported in people with chronic LBP [47,66], increased IL-6 before sleep onset correlates with poorer sleep quality [51], and conversely, poorer sleep quality correlates with higher IL-6 during the day [52,113]. Further, increased IL-6 and TNF are commonly observed in both depression [30] and chronic pain [37,47], and depression is linked with elevated pro-inflammatory cytokines and reduced sleep quality [71]. Further complicating the interpretation is the influence of age, gender and body mass index (BMI) on cytokines [8,17,116].

Most studies report inflammatory profiles in individuals already in chronic pain. Although the role of local inflammation immediately after an injury is clear, understanding of the systemic inflammatory response in the acute stage of LBP is limited. Here we aimed to: i) evaluate systemic pro-inflammatory markers (CRP, TNF, IL-6 and IL-1β) in people within two weeks of onset of an episode of acute LBP and controls with no history of major LBP; ii) compare these markers between participants with mild and moderate-to-severe LBP; and iii) examine the relationships between each cytokine/CRP and demographic, behavioural and clinical features plus the other cytokines/CRP.

2. Methods

2.1 Participants

Ninety-nine people in an acute episode of LBP (53 M, 46 F) aged 29±8 (mean±SD) years and fifty-five healthy controls (23 M, 32 F) aged 27±6 years participated in the study. Participants were recruited through advertisements around the university and local community, social media, and via a professional recruitment agency. Ethical clearance was

obtained from the university Medical Research Ethics Committee. All participants provided informed consent and procedures were conducted in accordance with the Declaration of Helsinki.

LBP participants were recruited within two weeks of onset of an acute episode of LBP following at least one month without pain. A LBP episode was defined as pain that had lasted for longer than 24 hours, caused functional limitation, and caused them to seek or seriously consider medical or allied health intervention. Participants were excluded if they had known or suspected serious spinal pathology (e.g. fracture, inflammatory/infective spinal disease, cauda equine syndrome, metastasis and neurological disorders). Participants were also excluded if they were less than 18 years old, had major pain or injury to other body regions in the previous 12 months, or had other major diseases or disorders (e.g. chronic renal/endocrine disorders). To control factors that might influence inflammation, participants were excluded if they were using corticosteroids, anti-cytokine therapy [18], or were greater than 50 years old to exclude undiagnosed cardiovascular disease. Participants were allowed to use pain medications that do not affect cytokines (e.g. paracetamol) and if required could use non-steroidal anti-inflammatory pain medication (e.g. Ibuprofen) provided it ceased five days prior to blood collection. After data collection, five participants were excluded from the dataset because they were identified to have a comorbidity known to influence inflammation (hypothyroidism [N=2], endometriosis [N=2] and reactive arthritis [N=1]).

On arrival at the assessment session participants were asked to rate their “average” level of LBP over the last week using a visual analogue scale (VAS) anchored with “no pain” at 0 and “worst pain imaginable” at 10. Pain-related disability was also assessed using the Roland Morris Disability Questionnaire (RMDQ) [95], which is a self-administered questionnaire consisting of 24 items associated with physical functions likely to be affected by LBP. An item receives a score of 1 if it is applicable to the respondent or a score of 0 if it

is not, with a total score range of 0 (no disability) to 24 (severe disability). Potential control participants who reported a score >0 on the VAS and/or RMDQ were excluded from the study. Potential LBP participants who reported pain of <1 on the VAS and/or a score of <1 on the RMDQ in the past week were excluded from the study.

2.2 Categorization of participants

Participants were categorised in two ways: 1) those with (LBP) and without LBP (controls), and 2) those with moderate-to-severe LBP (VAS≥4, "high-pain"), those with mild LBP (VAS<4, "low-pain"), and controls. The VAS cut-off used to distinguish between moderate-to-severe and mild LBP is based on those reported by Boonstra et al. [13].

2.3 Questionnaires

Demographic and health variables: Age, gender, co-morbidities, and body mass index (BMI: weight [kg] divided by the squared height) were collected. Participants reported smoking history (current/previous smoker), alcohol habits (frequency and amount consumed), and whether they had experienced previous LBP (yes/no).

Centre for Epidemiological Studies of Depression Scale (CES-D): The CES-D is a 20-item measure of depressive symptoms in the past week. Respondents' rate how often over the past week they experienced symptoms associated with depression using a four-point Likert scale ranging from 0 ("rarely or none of the time") to 3 ("most or all of the time"), for an overall score out of 60. Scores greater than 15 identify individuals at high risk for clinical depression with high sensitivity and specificity [62]. Both total CES-D scores and whether participants were experiencing clinically significant depressive symptoms, defined as a total score greater than 15 [86], were considered in the analysis.

Pain Catastrophizing Scale (PCS): The 13-item PCS is a valid and reliable measure of thoughts and feelings related to pain, which suggest catastrophic cognitions [76]. Responses to questions are quantified on a five-point Likert scale ranging from 0 ("not at

all") to 4 ("all the time") with respect to how often the respondent experiences certain thoughts and feelings when in pain. Components of the PCS include scores of magnification ("I become afraid that the pain will get worse"), rumination ("I worry all the time whether the pain will end") and helplessness ("I feel I can't go on"). Together, these components provide an overall score of catastrophizing, ranging from 0 to 52. Individual component and total PCS scores were used for analyses.

Fear Avoidance Beliefs Questionnaire (FABQ): The FABQ assesses fearful and avoidant behaviours with 16 items that measure the agreement of statements related to physical activity (FABQ-PA: 5 items) and work (FABQ-W: 11 items) that affect the participant's LBP [115]. Responses to each item are quantified on a 0 ("completely disagree") to 7 ("completely agree") scale for a maximum score of 30 and 66 for the FABQ-PA and FABQ-W, respectively. Higher scores indicate higher levels of fear-avoidance beliefs, which has been validated in chronic LBP patients [43]. Unemployed participants were removed from the final FABQ-W dataset.

Pain Self-Efficacy Questionnaire (PSEQ): The PSEQ is a 10-item questionnaire that quantifies the confidence people with pain have in performing activities while in pain [75]. Respondents rate how confidently they can perform a range of activities (e.g. work, household chores and socialising) on a seven-point Likert scale ranging from 0 ("not at all confident") to 6 ("completely confident"), for an overall score ranging from 0 to 60. Higher scores reflect stronger self-efficacy beliefs and correlate with measures of pain-related disability, different coping strategies, and activity-specific self-efficacy beliefs (Self-Efficacy Scale) [54,109].

Pittsburgh Sleep Quality Index (PSQI): Sleep duration and quality were assessed with the PSQI. The 19-item questionnaire evaluates global sleep complaints along seven dimensions, including subjective sleep quality, sleep duration and latency (time it takes to fall

asleep), and the frequency and severity of specific sleep-related complaints in the previous month [16]. Scores from each dimension (ranging from 0 to 3) are individually reported as component scores and summed to derive a sleep quality maximum score of 21; higher scores reflect greater sleep complaints. A PSQI score greater than 5 indicates poor sleep [16]. The PSQI and its psychometric properties have been validated in various populations including those with insomnia [7,16,19]. For analyses we used self-reported hours of actual sleep, individual component and global PSQI scores.

2.4 Cytokine and CRP measurements in serum

Approximately 8 ml of venous blood was drawn from each participant and collected into a Serum Separator Tube (Becton, Dickinson and Company), inverted four to five times, and allowed to clot for 30 min at room temperature. Samples were then centrifuged at 2,500 rpm for 15 min and the supernatant serum was stored at -80 degrees in approximately 450 uL aliquots. Concentrations of CRP, TNF, IL-6 and IL-1β were determined using separate "high sensitive" (assay sensitivity: CRP=0.022 ng/ml, IL-6=0.110 pg/ml, IL-1β=0.14 pg/ml, TNF=0.191 pg/ml) enzyme-linked immunosorbent assays (ELISA, R&D Systems, Minneapolis, Minnesota). Absorbance was measured at 490/650 nm for IL-6, TNF and IL-1β, and 450/540 for CRP, using the Spark 10M (Tecan, Männedorf, Switzerland) or Paradigm (Beckman Coulter, Brea, California) microplate reader. Values below the sensitivity of the test were allocated a zero score.

2.5 Data collection

Participants completed all questionnaires online within 24 hours of providing a blood sample. Participants who were considered eligible following the evaluation phase provided informed consent and a blood sample. All samples were collected between 8:00 am and 6:00 pm by research staff trained in venepuncture. Participants were instructed to refrain from moderate to high-level exercise for at least 24 hours before blood collection [18].

2.6 Statistical analysis

Demographic features and questionnaire data were compared between control and LBP groups, and between the control, low-pain (VAS<4) and high-pain (VAS≥4) groups using either chi-squared tests (categorical variables) or independent t-tests (continuous variables). As data for serum concentrations of CRP, TNF and IL-6 were skewed (Kolmogorov-Smirnov test: $p<0.05$), values were log-transformed before further analysis. Because over 50% of IL-1β values were allocated a zero score (concentrations below the accuracy of the test), no suitable transformation method achieved a normal distribution, and nonparametric tests (i.e. Mann-Whitney U test, Kruskal-Wallis ANOVA and quantile regression) were used with raw IL-1β values. Cytokine/CRP concentrations were first compared between LBP and controls using independent t-tests (log-transformed CRP, TNF and IL-6) or the Mann-Whitney U test (raw IL-1β). Univariate ANOVAs (group: 3 levels x cytokine/CRP) were then used to explore differences between the high-pain, low-pain and control groups for each cytokine or CRP. Blood collection times (24 h) were compared between control and LBP participants, and between high-pain and low-pain participants using t-tests to consider the effect of diurnal variation in biomarker levels between groups. Duncan's multiple range test was used for post-hoc analysis.

Stepwise (forward and backward selection) linear regression models were undertaken to investigate the relationships between each cytokine (except IL-1β) or CRP separately with demographic, behavioural and clinical features and the other cytokines/CRP. In the same manner, quantile regression was performed to calculate the association of these variables by quantiles (50^{th}, 60^{th}, 70^{th}, $80^{th}$and 85th) of IL-1β. Quantile regression is similar to linear regression, but instead of estimating the mean of the dependant variable, estimates the median (or other specified quantile), conditional on the values of the independent variable. Quantile regression does not require distributional assumption and is more robust in response

to extreme outliers [58]. Regression analyses were also undertaken without inclusion of the control participants to determine the association of variables only assessed in the LBP participants (FABQ-PA, FABQ-W and PSEQ). Analyses were performed using Statistica v12 (StatSoft) and Stata v14 (Stata Corp). P-values <0.05 were considered statistically significant.

3. Results

3.1 Group characteristics

Participants with and without LBP did not differ significantly in terms of age, gender, BMI and current smoking status. A greater proportion of LBP participants were previous smokers (p=0.005) and had a greater incidence of at least one previous episode of LBP (p<0.001) than controls. LBP participants reported higher CES-D scores (p<0.001), higher total and component (magnification, rumination and helplessness) PCS scores (p<0.014), and less sleep hours per night (p=0.002). A greater proportion of participants with LBP were experiencing clinical depressive symptoms (CES-D>15, p=0.002) than controls (Table 1).

Participants with LBP categorised as having either low-pain (VAS<4) or high-pain (VAS≥4) shared similar characteristics except that reported pain intensity (VAS, p<0.001), disability (RMDQ, p=0.036) and feelings of pain-related helplessness (PCS, p=0.048) were greater in the high-pain group (Table 2).

3.2 Group comparison of serum cytokine/CRP concentrations

Serum cytokine/CRP concentrations for each group are shown in Fig. 1 and 2. CRP was higher in participants with LBP than controls (p=0.003), but there were no significant differences between groups for TNF (p=0.174), IL-6 (p=0.141) or IL-1β (p=0.197). As shown in Fig. 2, the three-group comparison of high-pain, low-pain and control participants revealed a main effect for *group* with respect to both CRP ($F [2, 146] = 7.9$, p<0.001) and IL-6 ($F [2, 150] = 3.2$, p=0.045). CRP was higher in those with high-pain than low-pain (post-

hoc: p=0.005) and controls (post-hoc: p=0.005), and IL-6 was higher in those with high-pain than low-pain (post-hoc: p=0.034), but not controls (post-hoc: p=0.114). There were no differences in TNF and IL-1β when the three groups were compared. Time of blood collection was not different between control and LBP participants (p=0.510), or between high-pain and low-pain participants (p=0.301).

3.3 Relationship between each cytokine/CRP, demographic, behavioural and clinical features and other cytokines/CRP

Linear and quantile regression analyses revealed significant associations between each biomarker and various non-pain factors (Table 3). CRP was positively associated with IL-6 (β=0.42, p<0.001), pain intensity (VAS: β=0.17, p=0.002) and BMI (β=0.16, p=0.02). TNF was positively associated with previous LBP (β=0.26, p=0.006) and BMI (β=0.19, p=0.037), and negatively associated with a lower frequency (less than monthly) of consumption of five or more alcoholic drinks (β=-0.36, p=0.006) and higher pain rumination scores (β=-0.20, p=0.031). Although not statistically significant, there was a tendency for lower TNF to be associated with more sleep hours per night (β=-0.32, p=0.066). Higher IL-6 was associated with higher CRP (β=0.43, p<0.001) and less sleep (β=0.56, p=0.01). Interestingly, lower IL-6 was associated with the second highest category (7–9 drinks) relating to the number of alcoholic drinks typically consumed when drinking (β=-0.48, p=0.02). Quantile regression revealed a significant negative affect of IL-6 on the 70^{th} (-0.16, p=0.010), 80^{th} (-0.23, p<0.001) and 85^{th} (-0.22, p<0.001) quantiles of IL-1β and a significant positive affect of CRP on the corresponding 80^{th} (0.13, p=0.010) and 85^{th} (0.19, p<0.001) quantiles of IL-1β. Higher levels of IL-1β (80^{th} and 85^{th} quantile) were also associated with lower pain intensity ratings (VAS: -0.07/-0.05, p<0.05) and higher pain magnification scores of the PCS (0.11, p<0.01). Further, the 85^{th} quantile of IL-1β was higher in individuals who consumed alcohol more frequently (0.61/0.44, p<0.01) and who had a lower BMI (-0.03,

p=0.043). No associations were found in other quantiles of IL-1β. Separate regression analyses without controls did not identify associations between cytokines/CRP and variables specific to LBP, including the FABQ-W, FABQ-PA and PSEQ.

4. Discussion

This study has three major findings: i) CRP was elevated in acute LBP, particularly in those with greater pain, ii) IL-6 was elevated in participants with high-pain, and iii) factors other than pain explained some of the variance in cytokine/CRP levels. These data provide the first evidence for a relationship between systemic inflammatory markers, symptom severity and other features in the acute-phase of LBP. Early CRP and IL-6 responses may be key mediators of immune events that determine LBP outcome.

Elevation of CRP, but not primary pro-inflammatory cytokines in acute LBP

CRP provides a generic marker of active inflammation through its interplay with IL-6 [48], and to a lesser extent TNF and IL-1β, all of which stimulate CRP synthesis [12,73]. There are several possible reasons why CRP was elevated in acute LBP without concurrent increases in individual cytokines. First, injury severity may explain the results. Pro-inflammatory cytokine concentrations relate to the degree of tissue injury [44,100]. Although the injury may have been insufficient to stimulate specific cytokines detectably, the cumulative effect of cytokines may underlie detectable CRP production. Consistent with *in-vitro* evidence of enhanced CRP production in response to IL-6 when combined with IL-1β [41,61,124], our data show CRP is positively correlated with both IL-6 and IL-1β.

Second, the results may be explained by the time since injury. IL-6 at the injury site clears rapidly from circulation [20], but can accumulate in the liver where it continues to stimulate CRP synthesis [68]. Other work shows low systemic IL-6 levels accompanied by high systemic CRP levels in arthritic populations with high synovial fluid IL-6 levels

[9,79,106]. Further, cytokines exhibit distinct time-dependant response patterns with differences in times of peak expression and rates of development/recovery [80]. Failure to identify significant elevation of individual cytokines may be partly explained by variation in time since LBP onset between participants (within the 2-week window). However, the majority of participants presented between 10-14 days after onset limiting such time-dependent variation in cytokine expression.

Third, variation in individual responses to tissue injury requires consideration. Genetic differences (i.e. polymorphisms) can affect cytokine production in response to comparable triggers [10]. Some polymorphisms are cytokine specific (see [10]) whereas others have more global effects (i.e. enhanced/suppressed production of multiple cytokines) [120]. Demographic[99], physical [94,102] and psychosocial [25,28,31,70] factors can also shape cytokine/CRP profiles, independent of tissue injury (see below). Together these variations could limit identification of changes in specific cytokines, but still be detectable using robust generic markers of inflammation such as CRP [82].

CRP as a contributor to inflammation

Evidence is accumulating that CRP is not only a marker of inflammation and disease but also a director contributor [32-34]. CRP is proposed to exert pro-inflammatory effects via activation of the complement pathway [45,81], stimulating various inflammatory products including cytokines that sensitise peripheral nociceptors [91] and can exacerbate tissue damage [81]. As shown here in LBP, systemic CRP concentrations relate to pain/disease severity [18,85,105]. However, involvement of CRP in underlying pathological processes has been debated [5,123] because it has both pro- and anti-inflammatory effects [107]. These have been reported separately [6,82] and explained by two distinct CRP structural forms associated with opposite inflammatory properties [34]. Under inflammatory conditions

circulating (native) CRP can dissociate into a pro-inflammatory and predominantly tissue-bound form of CRP [34,107,119]. Prevention of this dissociation has therapeutic benefit (inhibition of pro-inflammatory effects) in a rat model [83]. Taken together, CRP is a likely contributor to increased pain and the many symptoms attributable to inflammation.

IL-6 elevated only in high-pain

Elevated IL-6 only in the high-pain group, and no correlation with pain intensity suggests a non-linear relationship between these variables. For instance, physiological reactivity (such as pain) to IL-6 may require a threshold concentration. There are other examples: patients undergoing total knee replacement have a two-fold increase in serum IL-6 levels post-surgery without linear relationship to knee pain [39]; and patients undergoing total hip replacement increase cerebrospinal fluid IL-6 levels post-surgery without linear correlation to postoperative pain [15]. Some studies of trauma or chronic pain do report correlation between IL-6 and symptom severity [26,42,57,103], but circulating IL-6 levels are likely to be much higher in these populations (e.g. chronic LBP [60,117]) than the acute LBP group reported here. The few studies that have examined IL-6 in acute conditions report similar findings to the present study. Carpe et al. [18] reported higher serum IL-6 levels in people with more severe upper extremity musculoskeletal disorders of <12 weeks, though levels were not correlated with symptoms. Rechardt et al. [88] showed that serum IL-6 (also IL-1β and TNF) levels were not different between controls and patients with musculoskeletal injuries of varying severity with symptom duration of <4 weeks.

Possible anti-inflammatory role of IL-6

Several aspects of our data imply that IL-6 might have an anti-inflammatory role in the acute phase of LBP. IL-6 is an integral pro-inflammatory driver in response to injury, stimulating the release of acute phase proteins, cell growth and proliferation [11,49,63,72].

However, it also possesses anti-inflammatory properties, which include direct and indirect inhibition of IL-1β and TNF [3,11,98,108,111]. Such effects could attenuate IL-6 levels given TNF and IL-1β both stimulate IL-6 [67,74]. Carpe et al. [18], hypothesised that individuals less severely affected by musculoskeletal injuries would benefit most from the anti-inflammatory properties of IL-6 to prevent hyper-inflammation in the early post-injury period. This could suppress IL-1β and TNF [11]; we found a non-significant tendency for reduced IL-1β in our LBP participants. On the other hand, inflammation augmented by IL-6 may be less controlled or favour those more severely affected.

Further evidence of an immunosuppressant function of IL-6 is provided by the observation that lower concentrations of IL-1β related to higher IL-6 and pain. An inverse relationship between systemic IL-1β/TNF and IL-6 levels has been reported in inflammatory diseases such as fibromyalgia and rheumatoid arthritis [59]. In addition, systemic TNF levels are markedly elevated in IL-6 knockout mice [4,122]. These findings concur with those reported during and post-exercise. Systemic elevation of muscle-derived IL-6 during exercise, thought to facilitate glucose metabolism [77], are accompanied by low TNF and IL-1β levels [55,89,90,102]. As with injury, the mechanisms by which IL-6 attenuates TNF and IL-1β is through stimulation of IL-1-receptor antagonist and TNF receptors, and the potent anti-inflammatory cytokine IL-10 [11,101,108]. Taken together, we speculate that IL-6 operates to control the extent of inflammation and that impaired regulation could have detrimental short- and long-term effects (e.g. hyper-inflammation or immunosuppression). Subtle or premature reductions in IL-6 anti-inflammatory activities could unmask other pro-inflammatory mediators such as TNF, which are elevated in chronic LBP [116,117] and was associated with previous LBP in the current study. Longitudinal studies are needed.

Relationship between cytokine/CRP levels and non-pain factors

Several factors other than pain were associated with concentrations of cytokines/CRP. Consistent with previous reports, CRP, IL-6 and TNF were higher in those with a greater BMI [8,24,56,78]. TNF and IL-6 are considered responsible for the dyslipidemia common in obesity [24]. This adds to accumulating evidences that inflammation is reciprocally linked with obesity [36,94]. Age was unrelated to cytokine/CRP levels when accounting for all biomarkers in our regression model. Although this differs from the general view [21,35,40,99], we only included participants below 50 years, which is younger than the threshold age at which inflammation is considered to be affected by the ageing process [22]. Alcohol is a well-known immune-modulator. Numerous studies show correlation between low intake and low pro-inflammatory cytokines [2,25,114]. Our results reflect these findings for TNF and IL-1β but not IL-6, which had the opposite effect. The later result should be interpreted with caution because of the low number of participants (7/154) who reported this drinking category.

Associations between inflammation, sleep loss and psychological symptoms are well documented in a variety of conditions [14,27,28,31,38,46,70]. Although the interrelationships are not fully understood, abnormal hypothalamic-pituitary adrenal-axis (HPA-axis) function has emerged as a possible explanation [27]. Disturbance to any one of these domains can profoundly impact the others. Here, LBP participants reported less/poorer sleep, higher pain catastrophizing, more depressive symptoms and a greater proportion were considered significantly depressed than controls. Less sleep was associated with increased IL-6 and a non-significant tendency for greater TNF, consistent with sleep restriction studies. . β, respectively.

Methodological considerations

An important limitation in this study was that blood samples were not collected at the same time of day for each participant. Although blood collection times were not different between groups, cytokine diurnal rhythms (i.e. natural ebb and flow of cytokine levels throughout the day) [84,97,112] may explain some variation in our data. Unlike cytokines however, CRP is generally reported to have minimal daytime variation and is unlikely to be influenced by our collection methods.

Conclusions

This study provides insight into the early response profiles of systemic cytokines/CRP in acute LBP. CRP was elevated in LBP and correlated with pain, adding to evidence of its role in inflammation. IL-6 was only elevated in high-pain participants but not linearly related with pain, and the data point to it having a possible anti-inflammatory role. Involvement of these immune mediators systemically in the acute-phase of LBP seem both important in modulating inflammation but potentially harmful if not controlled appropriately. These responses are likely predisposed by various demographic, behavioural and psychosocial factors, some of which are bi-directionally linked. Future longitudinal studies should consider whether early inflammatory profiles and associated factors are predictive of persistent inflammation and pain.

Acknowledgements

This research was funded by the National Health and Medical Research Council (NHMRC) of Australia (Program Grant: ID631717; Project Grant ID631369). PWH supported by NHMRC Fellowship ID1002190. There are no conflicts of interest related to this work.

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Table captions

Table 1. LBP (N = 99) and control (N = 55) participant characteristics.

Table 2. High-pain (N = 75) and low-pain (N = 23) participant characteristics.

Figure captions

Fig. 1. Serum concentrations of biomarkers in participants with acute low back pain (LBP) and pain-free controls. Box-plots represent median (*horizontal line*), 25th and 75th percentiles (*box*), and 10th and 90th percentiles (*lines outside the box*). **p<0.01. IL-6 – interleukin-6; TNF – tumor necrosis factor; IL-1β – interleukin-1β; CRP – C-reactive protein.

Fig. 2. Serum concentrations of biomarkers in acute low back pain participants divided into those with high-pain (VAS≥4) and low-pain (VAS<4) and control participants. Descriptions of the box-plots are given in Fig. 1. *p<0.05; **p<0.01. IL-6 – interleukin-6; TNF – tumor necrosis factor; IL-1β – interleukin-1β; CRP – C-reactive protein.

Table 1. LBP (N = 99) and control (N = 55) participant characteristics.

	Control		**LBP**		
Characteristic	**Mean (SD)**	**Range**	**Mean (SD)**	**Range**	**P-value**
Age (yrs)	26 (6)	18–46	29 (8)	18–50	0.052
Gender male (%)	42	-	54	-	0.164
BMI (kg/m^2)	23.0 (4.1)	17.4–43.4	24.1 (3.8)	16.6–39.2	0.100
Current smoker (%)	1.9	-	5.7	-	0.271
Previous smoker (%)	16.7	-	39.0	-	**0.005****
Previous LBP (%)	5.8	-	92.6	-	**<0.001*****
Sleep hours per night (h)	7.5 (1.1)	4–10	6.9 (1.2)	4–10	**0.002****
Sleep quality[a]	3.4 (2.2)	0–9	6.3 (3.2)	1–15	**<0.001*****

Depressive symptoms[b]	7.0 (5.4)	1–27	12.4 (8.0)	0–36	**<0.001*****
Significant depression (%)[c]	7.4	-	28.9	-	**0.002****
Pain catastrophizing[d]:	6.6 (9.3)	0–41	13.0 (10.4)	0–49	**<0.001*****
Rumination	2.8 (3.7)	0–12	4.4 (3.9)	0–16	**0.014***
Magnification	1.4 (2.1)	0–9	3.4 (2.6)	0–12	**<0.001*****
Helplessness	2.4 (4.3)	0–23	5.2 (4.9)	0–21	**<0.001*****

[a]Scored using the 19-item Pittsburgh Sleep Quality Index. Higher scores reflect poorer sleep.

[b]Scored using the 20-item Epidemiological Studies of Depression Scale. Higher scores reflect greater depressive symptoms.

[c]Interpreted from the Epidemiological Studies of Depression Scale. Scores greater than 15 indicate clinically significant depressive symptoms.

[d]Scored using the 13-item Pain Catastrophizing Scale. Higher scores reflect greater pain-related catastrophizing. Components of the questionnaire include rumination, magnification and helplessness.

Significant values are in bold font (*p<0.05; **p<0.01; ***p<0.001).

Table 2. High-pain (N = 75) and low-pain (N = 23) participant characteristics.

	Low-Pain		**High-Pain**		
Characteristic	**Mean (SD)**	**Range**	**Mean (SD)**	**Range**	**P-value**
Age (yrs)	30 (9)	18–49	29 (8)	18–50	0.754
Gender male (%)	57	-	53	-	0.743
BMI (kg/m^2)	24.1 (3.6)	16.9–31.5	24.0 (3.9)	16.6–39.2	0.901
Current smoker (%)	4.8	-	6.0	-	0.835
Previous smoker (%)	39.1	-	39.0	-	0.984
Previous LBP (%)	95.5	-	91.7	-	0.554
Pain intensity[a]	2.4 (0.7)	1–3	5.8 (1.3)	4–9	**<0.001*****
Disability severity[b]	4.7 (3.3)	1–14	6.9 (4.4)	1–22	**0.036***
Sleep hours per night (h)	7.1 (1.1)	5–8.5	6.8 (1.2)	4–10	0.368
Sleep quality[c]	5.7 (2.8)	1–12	6.4 (3.4)	1–15	0.385

Depressive symptoms[d]	13.2 (9.7)	0–36	12.2 (7.5)	0–33	0.614
Significant depression (%)[e]	30.4	-	28.4	-	0.849
Fear avoidance (work)[f]	11.5 (8.7)	0–27	11.7 (9.7)	0–36	0.926
Fear avoidance (activity)[g]	13.3 (5.8)	0–24	15.0 (5.3)	0–24	0.191
Pain self-efficacy[h]	47.7 (10.7)	14–60	44.3 (11.0)	18–60	0.275
Pain catastrophizing[i]:	9.9 (8.0)	2–34	14.0 (10.9)	0–49	0.102
Rumination	3.3 (3.2)	0–12	4.8 (4.1)	0–16	0.123
Magnification	3.2 (2.1)	0–9	3.5 (2.8)	0–12	0.636
Helplessness	3.4 (3.2)	0–13	5.7 (5.2)	0–21	**0.048***

[a]Scored on a 0 to 10 visual analogue scale (0 = no pain, 10 = worst pain imaginable)

[b]Scored using the 24-item Roland Morris Disability Questionnaire. Higher scores reflect greater disability.

[c]Scored using the 19-item Pittsburgh Sleep Quality Index. Higher scores reflect poorer sleep.

[d]Scored using the 20-item Epidemiological Studies of Depression Scale. Higher scores reflect greater depressive symptoms.

[e]Interpreted from the Epidemiological Studies of Depression Scale. Scores greater than 15 indicate clinically significant depressive symptoms.

[f]Scored using the 5-item Fear Avoidance Beliefs Questionnaire-Work. Higher scores indicate higher levels of fear-avoidance beliefs related to work.

[g]Scored using the 11-item Fear Avoidance Beliefs Questionnaire-Physical Activity. Higher scores indicate higher levels of fear-avoidance beliefs related to physical activity.

[h]Scored using the 10-item Pain Self-Efficacy Questionnaire. Higher scores reflect stronger self-efficacy beliefs with respect to performing activities while in pain.

[i]Scored using the 13-item Pain Catastrophizing Scale. Higher scores reflect greater pain-related catastrophizing. Components of the questionnaire include rumination, magnification and helplessness.

Significant values are in bold font (*p<0.05; **p<0.01; ***p<0.001).

Table 3. Association of all factors including other biomarkers on serum cytokine and CRP levels.

Variable	CRP	TNF	IL-6	IL-1β: 70th quantile	IL-1β: 80th quantile	IL-1β: 85th quantile
CRP	-	-	**0.43 (0.27–0.59)*** **	0.10 (0.00–0.19)	**0.13 (0.03–0.22)***	**0.19 (0.11–0.28)*** **
IL-6	**0.42 (0.28–0.57)*** **	-	-	**-0.16 (-0.28–-0.04)***	**-0.23 (-0.34– -0.11)*** **	**-0.22 (-0.33– -0.12)*** **
Pain intensity	**0.17 (0.03–0.31)***	-	-	-0.03 (-0.09–0.20)	**-0.07 (-0.12– -0.02)***	**-0.05 (-0.10–0.00)***
Age	-	-	-	0.01 (-0.01–0.02)	0.00 (-0.01–0.02)	0.00 (-0.01–0.01)
BMI	**0.16 (0.02–0.31)***	**0.19 (0.01–0.36)***	-	0.01 (-0.02–0.04)	0.00 (-0.03–0.03)	**-0.03 (-0.05–0.00)***
Previous LBP	-	**0.26 (0.08–0.45)** **	-	-0.14 (-0.45–0.16)	-0.04 (-0.33–0.26)	0.08 (-0.18–0.34)
≥7 drinks[a]	-	-	**-0.48 (-0.91– -0.06)***	0.04 (-0.53–0.62)	-0.03 (-0.58–0.52)	-0.07 (-0.56–0.42)
<monthly 5+ drinks[b]	-	**-0.36 (-0.62– -0.11)** **	-	0.01 (-0.20–0.22)	-0.08 (-0.28–0.12)	-0.11 (-0.29–0.07)
Drink freq ≥4 times/w[c]	-	-	-	0.07 (-0.46–0.59)	0.26 (-0.25–0.76)	**0.61 (0.16–1.10)** **
Drink freq 2-3 times/w[c]	-	-	-	0.06 (-0.27–0.39)	0.18 (-0.14–0.50)	**0.44 (0.15–0.72)** **
<5 h sleep[d]	-	-	**0.56 (0.14–0.99)***	-0.39 (-1.85–1.07)	-0.24 (-1.64–1.16)	-0.50 (-1.75–0.75)
≥6 h sleep[d]	-	-0.32 (-0.65–0.02)	-	-0.19 (-0.59–0.22)	0.01 (-0.38–0.39)	0.04 (-0.30–0.39)
Pain rumination	-	**-0.20 (-0.37– -0.02)***	-	0.01 (-0.05–0.06)	0.04 (-0.01–0.10)	-0.02 (-0.07–0.03)
Pain magnification	-	-	-	0.06 (-0.02–0.15)	**0.11 (0.03–0.19)** **	**0.11 (0.03–0.18)** **
Overall fit (R^2)	**0.30**	**0.21**	**0.31**	**-**	**-**	**-**

Data represent regression coefficients (95% confidence intervals) of various factors demonstrating an association with: i) CRP, TNF and IL-6 using step-wise linear regression models, and ii) different quantiles (those with significant factors only shown) of IL-1β using quantile

regression. CRP, TNF and IL-6 levels are log transformed. IL-6 – interleukin-6; TNF – tumor necrosis factor; IL-1β – interleukin-1β; CRP – C-reactive protein. Significant values are in bold font (*p<0.05; **p<0.01; ***p<0.001).

[a]Refers to the number of alcoholic drinks typically consumed when drinking.

[b]Refers to the frequency at which 5 or more alcoholic drinks are consumed in a single drinking session.

[c]Refers to the frequency at which alcohol is consumed

[d]Refers to the average number of hours slept per night over the past week.

- Findings point to circulating IL-6 and CRP as mediators of inflammation following an acute episode of low back pain
- Physiological reactivity (such as pain) to IL-6 may require a threshold concentration
- Demographic, behavioural and psychosocial factors have a likely role in shaping these acute inflammatory responses
- Data differ from those in chronic LBP and provide a basis to study changes in inflammatory profiles in the transition from acute to persistent back pain

Fig. 1.

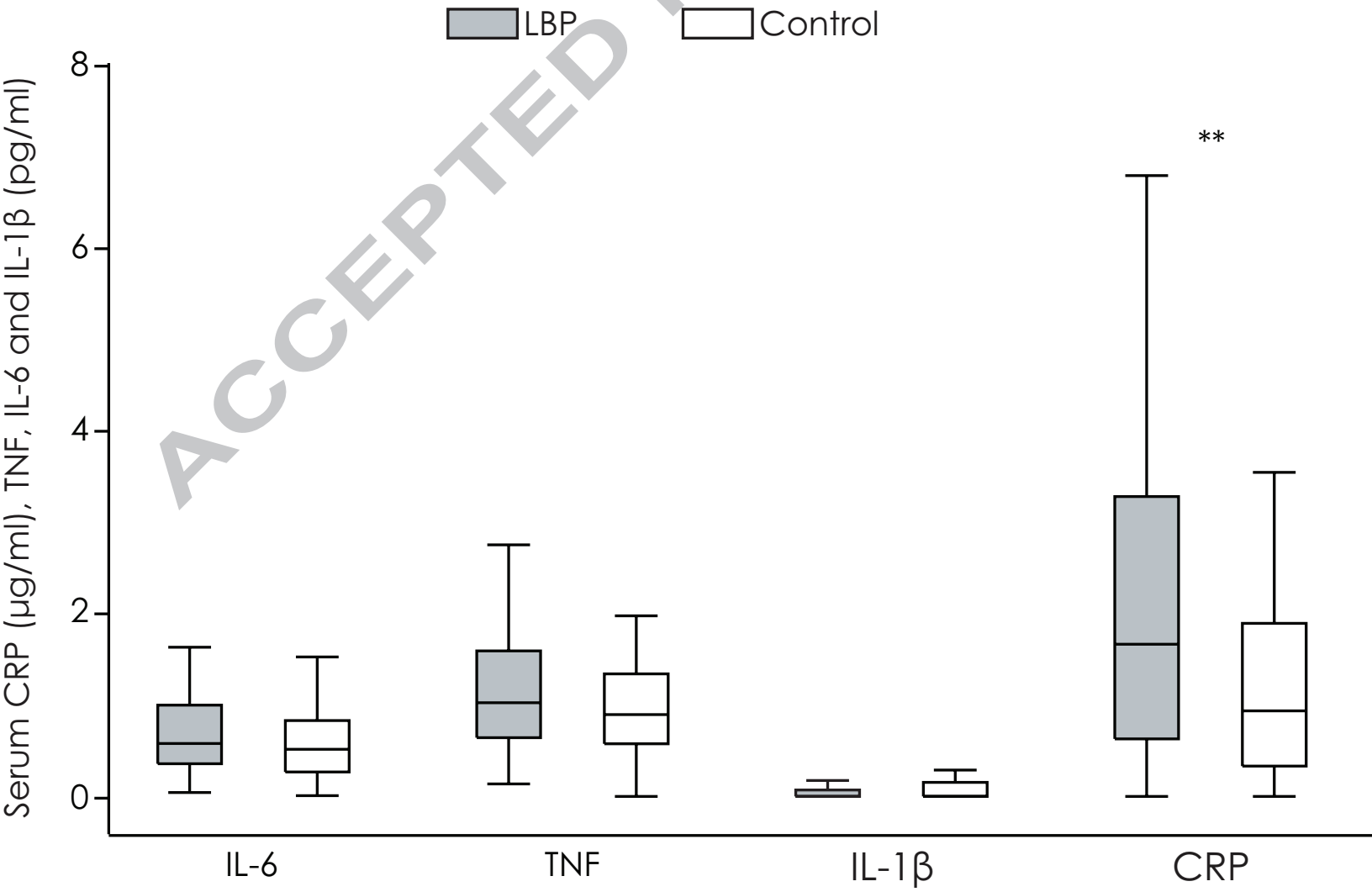

Fig. 2.