Inflammatory biomarkers increase with severity of upper-extremity overuse disorders

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ABSTRACT

MSDs (musculoskeletal disorders) from overuse are common occupational health problems that cause pain, functional loss and loss of work time. The aim of the present study was to determine whether a relationship exists between the severity of early-onset overuse-related MSDs of the upper extremity and serum levels of IL-1β (interleukin-1β), TNF-α (tumour necrosis factor-α), IL-6 (interleukin-6) and CRP (C-reactive protein). Twenty-two subjects with upper-extremity MSDs due to overuse for no longer that 12 weeks were stratified according to the severity of upper-extremity signs and symptoms as determined by a UBMA (upper-body musculoskeletal assessment). Nine asymptomatic subjects also participated. Serum cytokines were analysed using ELISA, and CRP was analysed using a laser nephelometry technique. CRP was strongly correlated, and TNF-α, IL-1β and IL-6 were moderately correlated, with UBMA scores. Only CRP and TNFα were significantly associated with UBMA scores in an ordinal logistic regression analysis in which age and BMI (body mass index) were covariates. These results are of clinical importance as they suggest that early-onset overuse-related MSDs may have an inflammatory component. The possibility of using a combination of serum biomarkers to follow the progression of overuse-related MSDs or their response to therapeutic intervention may be of interest to clinical practitioners and should be the focus of future research.

INTRODUCTION

According to the most recent U.S. Department of Labor survey of occupational injuries and illnesses, which reports on U.S. private industry injury and illness records from 2004, MSDs (musculoskeletal disorders) accounted for 402700 (32%) of the injuries and illnesses associated with days away from work [1]. Service industries accounted for 69% of all lost-work-day MSDs, and goods-producing industries accounted for 31% of all MSD cases. Upper-extremity MSDs have a relatively high impact on the number of days away from work. For example, carpal tunnel syndrome is associated with the highest number of lost-work days (median of 28 days), and injuries to the shoulder and wrist accounted for median lost-work days of 17 and 14 respectively. In addition, exposure to repetitive motion, such as grasping tools, scanning groceries and typing, resulted in the longest absences from work by exposure type (median of 20 days).

Despite epidemiological evidence linking the onset and severity of MSDs to repetitive and forceful tasks

Key words: C-reactive protein (CRP), cytokine, interleukin, musculoskeletal disorder, overuse injury, tumour necrosis factor (TNF), upper-body musculoskeletal assessment.

Abbreviations: BMI, body mass index; CRP, C-reactive protein; IL, interleukin; MSD, musculoskeletal disorder; TNF-α, tumour necrosis factor-α; UBMA, upper-body musculoskeletal assessment.

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[2,3], there is still much to learn about the underlying pathophysiology of these disorders. Previously, we conducted a series of studies using a rat model to explore the early onset of overuse MSDs in which we found motor behavioural and physiological tissue changes similar to that seen in humans with overuse MSDs [4–7]. We have shown a dose-dependent relationship between reach rate (i.e. level of overuse exposure) and the concentrations of the pro-inflammatory cytokines IL (interleukin)-1α and IL-1β in serum [4,5]. This body of work leads us to hypothesize that there is a relationship between severity of early-onset upper-extremity MSDs due to overuse and the serum concentration of inflammatory markers, such as pro-inflammatory cytokines.

The cytokines IL-1, TNF-α (tumour necrosis factor-α) and IL-6 are intercellular signalling polypeptides produced by most injured cells as well as activated immune cells, including activated monocytes and macrophages [6,8]. Cytokines are the chief stimulators of acute-phase-response proteins and contribute to the development and remediation of signs and symptoms of acute and chronic inflammation, such as the recruitment of immune cells. IL-1 and TNF-α can potently stimulate immune and stromal cell production of other cytokines and chemokines as well as most mechanisms of inflammation, including phagocyte proliferation and activation, adhesion and angiogenesis [8]. IL-6, a pleiotropic cytokine, has many pro-inflammatory effects that overlap with those of IL-1 and TNF-α. For example, IL-6 induces all positive acute-phase-response proteins [9]. IL-6 also increases in response to exercise, independent of muscle damage [10]. Interstitial muscle and peritendon IL-6 production, and subsequent release into the bloodstream, is relative to the intensity and duration of the exercise and occurs as a result of muscle glycogen depletion [10]. Circulating IL-6 then acts in a hormone-like fashion to regulate lipolysis and fat oxidation [11]. IL-1β and TNF-α are not influenced by the glycogen content of muscle, and thus are not elevated in serum during exercise unless tissue damage has occurred [11].

The acute-phase response is a general response designed to aid tissue repair and facilitate a return to physiological homoeostasis. CRP (C-reactive protein) is a sensitive acute-phase reactant produced by hepatocytes in the liver [12,13]. CRP is a prototypic inflammatory marker of underlying low-grade inflammation [12,14] that is beneficial in identifying individuals with unstable angina pectoris underlying coronary artery disease [15] or risk of future stroke [13,16]. CRP is also elevated in smokers, elderly at risk of mortality and patients with metabolic disease and diabetes [17]. Although controversial, there is emerging evidence that CRP is not only a marker of the acute-phase response, but may also be causal in the pathogenesis of inflammatory disease [17,18].

Kramer et al. [19] developed a tool to quantify the severity of work-related MSDs known as the UBMA (upper-body musculoskeletal assessment) instrument. UBMA takes a regional approach to diagnosis using clinically common tests, resulting in a single composite outcome score that quantifies severity of work-related MSDs, irrespective of specific diagnosis, and may be useful in clinical diagnosis and evaluation of progress toward remediation. Test–retest reliability of UBMA has been shown to be excellent (intra-class correlation coefficient = 0.88 for side of workplace equipment use; and intra-class correlation coefficient = 0.94 for side opposite equipment use) among patients with upper-extremity MSDs due to overuse [19]. In addition, UBMA has been shown [19] to distinguish between a group of healthy controls and a group of workers with MSDs for both the side of equipment use and the side opposite equipment use ($P < 0.001$).

In the present study, we have utilized UBMA in combination with biochemical assays of human serum to determine if a relationship exists between the severity of early-onset overuse MSDs of the upper extremity and circulating inflammatory mediators and markers. Twenty-two subjects with upper-extremity MSDs due to overuse for no longer than 12 weeks and nine healthy asymptomatic subjects participated. Serum was collected and examined for the presence of IL-1β, IL-6, TNF-α and CRP. We found that each of these proteins was progressively elevated with increasing UBMA score; however, only CRP and TNF-α were significantly associated with UBMA score in an ordinal logistic regression model.

MATERIALS AND METHODS

Subjects

Subjects were recruited from an outpatient physical therapy clinic, Chestnut Hill HealthCare facilities in Philadelphia, PA, U.S.A. for participation in the study. Both the Temple University Institutional Review Board and Chestnut Hill Hospital Institutional Review Board approved all procedures. The research was carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association. All subjects signed an informed consent form after full explanation of the purpose, nature and risk of all procedures. Individuals with a history of upper-extremity MSDs due to overuse of no longer than 12 weeks duration were included in the study. A standardized demographic questionnaire covering medical history and medication use was administered. Exclusion criteria included a history of inflammatory disease (e.g. lupus, rheumatoid arthritis, diabetes and non-medically controlled hypertension), cancer, known coronary artery disease, disease processes that required ongoing treatment with steroids or NSAIDs (non-steroidal anti-inflammatory drugs) and cigarette smoking. Subjects were also excluded if they were medically unable or unwilling to refrain from taking NSAIDs

or full-dose aspirin for a period of 7 days. Individuals with a history of hypertension were included if the hypertension was currently controlled with medication. The demographic questionnaire used in the study included characteristics such as age, gender and race. Information was obtained on the subject's referring diagnosis, occupation and/or primary activity related to the development of MSD. BMI (body mass index) was calculated as weight (in kg) divided by the square of height (in m).

Subjects were examined by the same physical therapist using UBMA, as described by Kramer et al. [19]. A total of 22 symptomatic subjects were recruited using a stratified non-random sampling procedure, where the strata were predefined UBMA score ranges (50–74, 75–99 and $\geqslant$100; $n = 6$–9 subjects per stratum). This sampling procedure was undertaken to obtain a full range of UBMA scores. Nine healthy asymptomatic subjects were also included, and their UBMA scores were < 50.

Biochemical analysis of serum

Subjects were asked to refrain from performing strenuous exercise and taking NSAIDs or full-dose aspirin (325 mg/day) for 7 days prior to the drawing of blood samples. Subjects taking low-dose aspirin (81 mg/day) were included in the study. Venous blood samples were drawn from all subjects between 07.00 and 12.00 hours, and were collected into sterile ECTA (enzyme-catalysed therapeutic activation) tubes (5×10^{-3} mol/l; Labco) containing aprotinin (bovine lung 15–30 units/ml; Sigma) at 0.67 unit/ml. Serum was immediately separated from cells and platelets by centrifugation at 400 *g* for 7 min, and then at 10 000 *g* for 7 min at 4 °C. Following removal of appropriate quantities of serum for CRP analysis, the aqueous phase was aspirated and stored in 150 μl aliquots at − 80 °C until assayed as described below. Aliquots were assayed in batches to avoid any thawing and refreezing.

CRP was measured using a modification of the laser nephelometry technique (Berhing Diagnostics) by the Chestnut Hill Hospital Pathology laboratory, according to standard protocols. The CRP assay was standardized according to the World Health Organization First International Reference Standard. Data are expressed in mg/dl to be consistent with conventional units of reporting in the clinical literature.

For analysis of cytokines, serum samples were allowed to thaw on ice. Commercially available ELISA kits (OptEIA ELISA kits for human serum; BD Biosciences) with high-sensitivity for serum cytokine levels were used to determine the circulating levels of IL-1β, IL-6 and TNF-α. Each sample was assayed in duplicate to control pipetting error. A mean was calculated from the duplicate values for further analysis. Data are expressed in pg/ml of serum to be consistent with conventional units of reporting in the clinical literature.

Statistical analysis

A comparison between UBMA scores for the asymptomatic and symptomatic subjects was performed using a Mann–Whitney *U* test. In addition, the number of single- and multiple-site impairments in symptomatic subjects was compared with that in asymptomatic subjects using a χ^2 test. Comparisons of BMI and age between symptomatic and asymptomatic subjects were performed using Student's *t* tests for independent samples. Spearman's rank correlation tests were used to determine correlations between each inflammatory biomarker and UBMA score. Ordinal logistic regression analysis with backward elimination was used to determine which combination of the inflammatory biomarkers, age and BMI were associated with UBMA score. SAS (SAS Institute) was used for the statistical analyses. *P* values < 0.05 were considered significant for all analyses.

RESULTS

General characteristics of the subjects, existence of medically controlled hypertension, occupation and/or primary activity related to MSD are given in Table 1, as are the UBMA scores. Table 1 also summarizes the referring diagnoses, symptom duration and medication used during the time of the study for each subject. All of the referring diagnoses involved disorders of the upper extremity. The average duration of symptoms was 51.7 $\pm$ 18.9 days (value is mean $\pm$ S.D.) with a range of 17–76 days. Student's *t* tests for independent samples revealed no significant differences between the asymptomatic and symptomatic subjects with respect to age ($P = 0.23$) and BMI ($P = 0.65$). A Mann–Whitney *U* test demonstrated that the UBMA score was significantly higher in the symptomatic group compared with the asymptomatic group ($P < 0.0001$). Table 2 shows the UBMA results describing the number and type of impairment. The frequency of single-site impairments (i.e. those related to a single anatomical region) was greater in symptomatic than in asymptomatic subjects (7 and 53 respectively), as was the frequency of multiple-site impairments (i.e. those related to multiple anatomical regions; 1 and 28 respectively). χ^2 analysis confirmed this difference between symptomatic and asymptomatic groups in single- and multiple-site impairments ($\chi^2 = 348$; critical value = 10.83, $P = 0.001$).

Spearman's rank correlation tests revealed that all of the biomarkers were positively correlated with the UBMA score. As shown in Figure 1, CRP was the most highly correlated, with an r_s (Spearman's rank correlation coefficient) of 0.81 ($P < 0.0001$). IL-1β was a moderately correlated biomarker, with an r_s of 0.70 ($P < 0.0001$), as were TNF-α ($r_s = 0.66$, $P < 0.0001$) and IL-6 ($r_s = 0.52$, $P = 0.003$).

Table 1 Demographic characteristics of subjects ranked by UBMA score

Values are means (S.D.) for age, BMI and symptom duration; values are means (mean rank) for the UBMA score, as determined by Mann–Whitney *U* test. *Post-menopausal subject; †subject menstruating at the time of serum collection; ‡history of hypertension that is currently controlled medically; §statin medication. ||Significantly different from asymptomatic subjects as determined by Mann–Whitney *U* test ($P < 0.0001$). HCTZ, hydrochlorothiazide; Mvi, multivitamins; N/A, not applicable.

UBMA score	Gender	Age (years)	Race	BMI (kg/m^2)	Occupation/primary activity related to MSD	Referring diagnosis	Symptom duration (days)	Medication
Asymptomatic subjects								
10	Female*	47	Caucasian	19.2	Nurse	N/A	N/A	
11	Female*‡	49	Caucasian	23.0	Biomedical engineer	N/A	N/A	HCTZ
12	Male	46	Caucasian	22.0	Maintenance engineer	N/A	N/A	Flonase
16	Male	29	Caucasian	24.6	Physical therapist	N/A	N/A	
22	Female	37	Caucasian	20.3	Clerk	N/A	N/A	Flonase
30	Female	19	Caucasian	16.8	Administrative assistant	N/A	N/A	Mvi
31	Male	30	Caucasian	25.2	Clerk	N/A	N/A	
31	Female*	46	Caucasian	24.0	Biomedical engineer	N/A	N/A	Tylenol
41	Female*	48	African–American	29.3	Hospital administrator	N/A	N/A	Mvi
39.0 (5.0)		39.0 (10.8)		22.7 (3.7)				
Symptomatic subjects								
50	Male	29	Caucasian	24.4	Nurse	Median neuropathy	61	Atenelol
51	Female	28	Caucasian	20.6	Engineer	Lateral epicondylitis	52	
52	Female*	69	Caucasian	20.1	Gardener/potter	Wrist tendonitis	18	Tylenol/Pravachol§
56	Female†	26	Caucasian	16.9	Recreation therapist	Wrist sprain	30	
56	Male	33	Caucasian	26.2	Clerk	Wrist strain	45	Motrin
59	Female	26	Caucasian	25.0	Nurse	Myalgia	33	
66	Male	42	Caucasian	23.5	Stockroom clerk	Overuse syndrome	66	
66	Female	34	Hispanic	20.4	Nurse (retired for 1 month)	Wrist extensor strain	61	Birth control pills
78	Male	25	Caucasian	21.0	Engineer	Rotator cuff tendonitis, wrist strain	66	
78	Female	31	Caucasian	27.0	Cardiologist	Lateral epicondylitis	72	Birth control pills
83	Female	39	Caucasian	23.4	Engineer	Carpal tunnel syndrome	29	Birth control pills
84	Male	73	Caucasian	27.3	Gardener/house painter	Early compartmental syndrome	17	Low-dose aspirin/Mvi/Plavix
86	Female	39	Caucasian	22.1	Teacher	Overuse syndrome forearm	32	Tylenol/Neurontin
88	Male	29	Caucasian	24.9	Cable TV installer	Tendonitis	72	
90	Male	49	Caucasian	24.0	Laboratory technician	Tendonitis	48	Tylenol/Neurontin
91	Female*	62	Caucasian	21.1	Computer programmer	Carpal tunnel syndrome	55	Prilosec
102	Male	66	Caucasian	23.5	Woodworker	Carpal tunnel syndrome	64	
105	Male	54	Caucasian	19.7	Manager	Cervical strain/pronator teres syndrome	71	Low-dose aspirin/Pravachol§
106	Male‡	74	African–American	26.9	Artist (painter)	Bicipital tendonitis/hand oedema	68	Mvi/Lopressor/Dilantin
109	Male	57	Caucasian	19.1	Consultant	Bicipital tendonitis	34	
119	Female*	56	Caucasian	32.6	Manager	Wrist tendonitis	67	Zocor§
124	Female*	54	Caucasian	24.0	Cook	Carpal tunnel syndrome	76	Tylenol/Pravachol§
81.6 (20.5)||		45.2 (16.7)		23.4 (3.5)			51.7 (18.9)	

Table 2 Impairments identified by UBMA in asymptomatic and symptomatic subjects with MSDs

*Significantly higher observed frequency than expected from the frequency in asymptomatic subjects as determined by χ^2 analysis ($P < 0.001$). N/A, not applicable because none of these tests are administered to multiple sites; MMT, manual muscle test; PROM, passive range of motion.

Impairment	Single-site impairments (*n*)	Multiple-site impairments (*n*)
Asymptomatic subjects		
Resisted MMT	0	0
PROM	1	0
Trigger point	5	1
Median tinel	1	N/A
Finkelstein	0	N/A
Phalen	0	N/A
Adson	0	N/A
Grasp/pinch	0	N/A
Total	7	1
Symptomatic subjects		
Resisted MMT	10	9
PROM	7	10
Trigger point	10	17
Median tinel	6	N/A
Finkelstein	4	N/A
Phalen	7	N/A
Adson	3	N/A
Grasp/pinch	15	N/A
Total	53*	28*

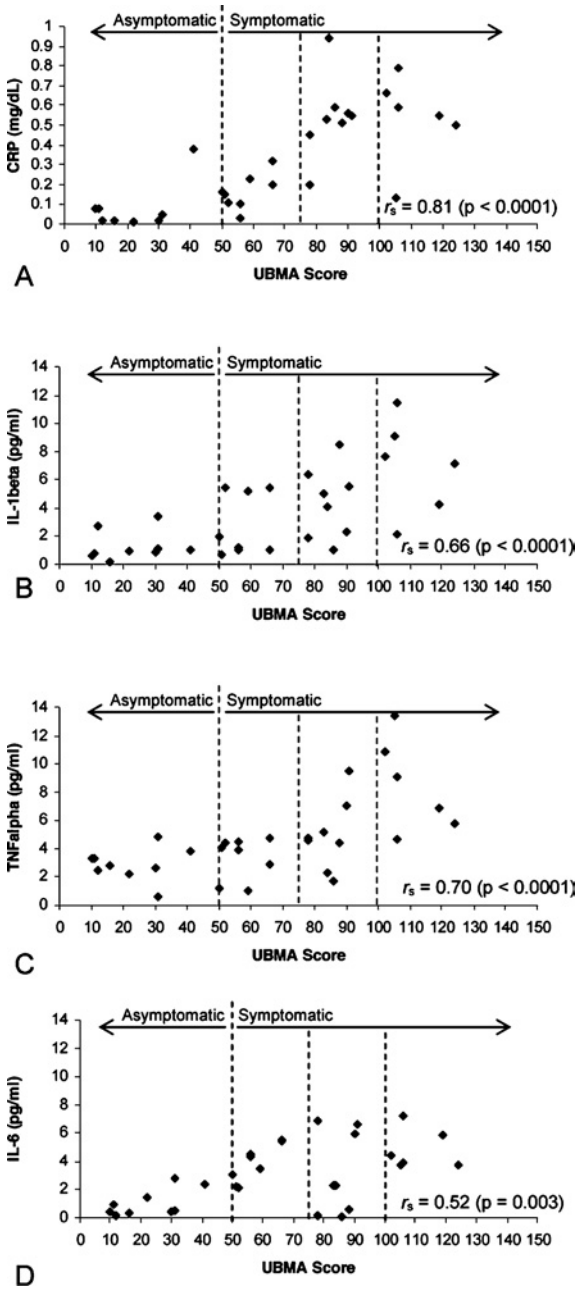

Figure 1 Concentrations of inflammatory biomarkers compared with the UBMA scores for all symptomatic ($n = 22$) and asymptomatic ($n = 9$) subjects

(A) CRP; (B) IL-1β; (C) TNF-α; and (D) IL-6. Vertical broken lines show cut-off points used in ordinal logistic regression. Horizontal arrows indicate the ranges of scores for asymptomatic (left arrow) and symptomatic (right arrow) subjects. Spearman's rank correlation coefficients (r_s) are shown.

Mean values for the serum levels of each biomarker are shown in Table 3 and reflect the correlational findings of increasing biomarker level with increasing UBMA score. Ordinal logistic regression analysis using the cut-off points for UBMA score indicated in Table 3 showed that CRP [odds ratio, > 999.9 (95 % confidence interval, 111.8 to > 999.9); $P = 0.0003$] and TNF-α [odds ratio, 2.2 (95 % confidence interval, 1.4–3.4); $P = 0.0007$] were significantly associated with an increased UBMA score. IL-1β and IL-6 were removed from the model with P values of 0.09 and 0.48 respectively. The covariates age ($P = 0.16$) and BMI ($P = 0.62$) were also removed from the model.

DISCUSSION

Overall, there was a positive relationship between MSD severity, as reflected by the UBMA score, and cytokine and CRP concentrations. All of the markers were positively correlated with UBMA: CRP was strongly correlated, and IL-1β, TNF-α and IL-6 were moderately correlated. However, only CRP and TNF-α were significantly associated with the UBMA score in an ordinal logistic regression model. These results are consistent with the progressive increase in the levels of these markers with increasing severity of the UBMA score.

The results from the UBMA showed the presence of local signs of pain, tenderness, peripheral nerve irritation, weakness and limited motion that progressively increased

Table 3 Inflammatory marker concentrations in serum from the asymptomatic and symptomatic groups calculated according the cut-off points used in the ordinal logistic regression analysis

Values are means (S.D.).

	Inflammatory marker concentration			
UBMA score range	CRP (mg/dl)	TNF-α (pg/ml)	IL-1β (pg/ml)	IL-6 (pg/ml)
0–49 (asymptomatic) ($n = 9$)	0.08 (0.12)	2.87 (1.18)	1.29 (1.04)	1.05 (0.94)
50–74 ($n = 8$)	0.16 (0.09)	3.35 (1.49)	2.73 (2.18)	3.82 (1.32)
75–99 ($n = 8$)	0.54 (0.20)	4.93 (2.49)	4.33 (2.50)	3.11 (2.93)
100–150 ($n = 6$)	0.54 (0.22)	8.45 (3.32)	6.95 (3.35)	4.80 (1.42)

in number with increasing serum CRP, IL-1β and TNF-α levels. The number of cases with involvement of multiple anatomical sites also increased with increased MSD severity and increased serum CRP, IL-1β and TNF-α levels. These results are similar to those of Ravaglia et al. [20], showing that at least CRP has a strong association with functional impairment. These results are also similar to those from an earlier study of IL-1β using a rat model of MSD developed in our laboratory [4,5]. It seems intuitive that the more severe the MSD, the more sites are injured, the larger the acute-phase response and thus the larger the concentration of serum inflammatory mediators and markers. Therefore we suggest that the systemic inflammatory response is associated with local signs and symptoms of inflammation in humans as in our rat model. It is likely that the systemic response is initiated by a local response and is proportionally amplified in the presence of greater tissue injury and inflammation (i.e. greater number of physical signs, symptoms and impairment). The increase in the number of affected anatomical sites may either be a cause or an effect of the systemic inflammatory response.

In contrast with IL-1 and TNF-α, circulating IL-6 has a more complex relationship with MSD severity in the present study. The pleiotropic effects of IL-6 may help explain these results. IL-6 is a tightly regulated cytokine that is not normally detected in serum unless there is trauma, infection, cellular/tissue stress, or during or following muscle glycogen-depleting exercise intensities [10,11,21]. In the case of infection or inflammation, IL-6 is an early cytokine responder [21,22]. Pro-inflammatory effects of IL-6 include induction of cell growth and proliferation, inflammation and acute-phase responses [22,23]. It also appears to have anti-inflammatory actions, such as inducing increases in circulating levels of IL-1 receptor antagonist and soluble TNF receptor [22,24]. These activities would have a potent anti-inflammatory effect by suppressing the actions of circulating IL-1 and TNF-α. We would expect the IL-6 concentrations in the serum of our subjects to fluctuate depending upon whether IL-6 is inducing pro- or anti-inflammatory effects, and would hypothesize further that symptomatic subjects less severely affected (i.e. those with UBMA scores in the 50–75 range) would benefit most from the anti-inflammatory functions of IL-6, such as attenuating IL-1β and the highly cytotoxic TNF-α. Indeed, in our subjects, serum levels of TNF-α are within the range of control values at UBMA scores between 50 and 75 (Figure 1C), as are IL-1β levels in three of the eight subjects (Figure 1B). Among the more severely affected subjects (i.e. those with UBMA scores > 100), IL-6 may augment the inflammatory response or it may be unable to down-regulate the production of other proinflammatory cytokines.

Our serum results differ from those obtained in a study by Freeland et al. [25] in which serum and tenosynovial tissues were examined in 41 patients with idiopathic carpal tunnel syndrome (most with abnormal nerve conduction velocity changes). Serum was collected 1 week prior to carpal tunnel release surgery, and tendosynovial tissue was collected at the time of surgery. Their findings of increased serum malondialdehyde, a marker of cell injury resulting from reperfusion, an absence of serum IL-1 and IL-6 as well as increased malondialdehyde, prostaglandin E2 and IL-6 in the tendosynovial tissues, led them to hypothesize that idiopathic carpal tunnel syndrome has a non-inflammatory ischaemia/reperfusion aetiology that results in fibrosis of carpal tunnel tissues. We also found fibrosis of carpal tunnel tissues in our rat model between 9 and 12 weeks, a few weeks after the onset of the inflammatory response [7]. Fibrotic carpal tunnel changes were accompanied by a significant decline in median nerve conduction velocity. Recent results from our rat model (M. F. Barbe and A. E. Barr, unpublished work) indicate that the serum cytokine levels return to normal after the changeover to a fibrotic tissue state. We hypothesize that our observed serum inflammatory responses in both the present human study and in our previous rat studies [4,5] are signs of early-onset of MSDs and are not indicative of later stages of MSDs. We would also like to highlight that the present study is not entirely comparable with the study by Freeland et al. [25]. In the present study, we only had four patients diagnosed with carpal tunnel syndrome. The remainder of our symptomatic subjects had diagnoses of musculoskeletal overuse. Further experimentation is needed to determine whether the involvement of musculoskeletal tissues instead of, or in addition to, nerve tissue is necessary for an increase in serum cytokines.

There are many patient characteristics in addition to the presence of MSD that may affect serum concentrations of these inflammatory biomarkers. Although intense and prolonged exercise elevates IL-6 due to exercise-induced glycogen depletion [10,11], repetitive low-intensity exercise does not appear to contribute to elevated serum IL-6 levels [26]. In a study using microdialysis catheters to measure IL-6 levels in the trapezius following 20 min of repetitive low-force exercise, interstitial muscle levels of IL-6 were elevated, but not serum levels [26]. Conversely, in another study by Chan et al. [11], serum levels of IL-6 increased significantly after 2.5 h of high-intensity glycogen-depleting exercise. Circulating levels of IL-6 have been shown to remain increased for 48 h after prolonged exercise [27]. Therefore our subjects were asked to refrain from vigorous exercise for 7 days before having their blood taken. Although we did not ask them to refrain from light-intensity exercise, it is unlikely that our measurements were affected by low-intensity physical activity, as suggested by Rosendal et al. [26].

The more severely affected symptomatic subjects had similar CRP levels to those of elderly subjects [28] and patients with non-medically controlled angina [15], but greater than smokers [16]. Hypertension, obesity and high coffee intake contribute to levels of serum CRP and TNF-α that are similar to those seen in our more severely affected subjects and to serum levels of IL-6 seen in all of our symptomatic subjects [29–31]. The IL-6 levels in our subjects with MSD were also similar to the very elderly, aged smokers and aged patients with significantly reduced functional status [32], although these were considerably higher than levels found in elderly subjects by Hager et al. [9]. All of our subjects had CRP and serum cytokine levels that were considerably lower than those seen in studies examining patients with severe trauma with risk for mortality, cardiogenic shock and sepsis [33–36].

Obesity is associated with an increase in serum CRP, TNF-α and IL-6 [30,37]. In the present study, the mean BMIs for the asymptomatic and MSD groups were below the threshold for obesity of 30 kg/m^2 defined by the American Heart Association (Table 1; http://www.americanheart.org). Only one of our symptomatic subjects would be considered obese. Therefore BMI was not a significant covariate in our regression model, although a sample with a larger number of obese individuals might give a different result.

Medication may affect serum levels of inflammatory mediators. Statins have been shown to reduce serum levels of IL-1β [38] and CRP [38,39], but not TNF-α [40]. Low-dose aspirin has been shown to reduce IL-1β but not CRP [38]. We did not exclude subjects who were prescribed and taking low-dose aspirin (81 mg/day) and statins. Five of our symptomatic subjects were prescribed statin therapy or low-dose aspirin (Table 1). As statins and aspirin tend to lower CRP and pro-inflammatory cytokine levels, their ingestion by any of our subjects would lower the concentration of the biomarkers, thereby diluting our main effects and, therefore, at least not falsely supporting our hypothesis.

Hormonal factors also affect the production of inflammatory mediators and may help explain the increased susceptibility of women to overuse MSDs. Oestrogens exacerbate inflammation, making women more vulnerable to chronic inflammatory disorders [41]. Lack of oestrogen, through naturally occurring or medically induced menopause, is associated with systemic increases in IL-6 and localized tissue increases in IL-1, IL-6 and TNF-α [42–44]. In addition, the use of hormone-replacement therapy might be associated with an enhanced thrombotic tendency [45,46]. The evidence is equivocal about the relationship between pro-inflammatory cytokines and oestrogen. We are uncertain as to the effect of not stratifying our female subjects by their hormonal status other than recording whether the subject was post-menopausal or menstruating at the time of serum collection. Of the nine asymptomatic subjects four were postmenopausal, whereas five of the 22 symptomatic subjects were postmenopausal or menstruating at the time of serum collection. The effect of this small number of subjects would tend to dilute our main effects, thus not falsely supporting our hypothesis.

The effect of aging on CRP and cytokine production has also been studied extensively. Roubenoff et al. [47] found that serum concentrations of IL-6, CRP and IL-1 receptor antagonist, but not IL-1β or TNF-α, were increased in elderly subjects (mean age, 79 years) compared with younger control subjects (mean age, 39 years). In addition, the increased concentration of IL-6 was correlated with an increased serum concentration of CRP. The authors [47] postulated a dysregulation of IL-6, CRP and IL-1 receptor antagonist with aging; however, since the findings by Macy et al. [12] and Chrysohoou et al. [29] indicate a weak or absent association of CRP with age, perhaps the elevation of CRP in the elderly population studied by Roubenoff and co-workers [47] was due to inflammatory co-morbidities, which were not reported. On the other hand, in a large study of community-dwelling elderly subjects (aged 65 years and older) by Cohen et al. [32], elevation of IL-6 was correlated strongly with age, independent of several selected disease states and disorders of aging. Similar results of increased pro-inflammatory cytokine activity after menopause have been found in numerous studies [32,48,49]. In the present study, even though the mean age of our symptomatic subjects was higher than that of our asymptomatic subjects, the difference was not statistically significant. Despite there being four individuals among our symptomatic subjects >65 years of age (Table 1), age was not found to be a significant covariate in the ordinal logistic regression model. Although inclusion of a

larger number of aged subjects might change this finding, the age group included in the present study is certainly representative of working-age adults, which is a group greatly affected by overuse MSDs [1–3].

Cardiovascular problems, such as severe unstable angina [15], underlying coronary artery disease, hypertension and risk of future stroke [13,16], are well known to result in the elevation of CRP. CRP and pro-inflammatory cytokines have been shown to be among the first responders in many inflammatory and/or infectious conditions. For example, Fida et al. [36] evaluated the utility of using serum levels of IL-1α, IL-6, TNF-α and CRP in differentiating sepsis from meningitis in children. They found that optimal cut-off points (i.e. the points that allow detection of as many true positive and as few false-positive results as possible) were 0.70 mg/l for CRP, 12.07 pg/ml for IL-1α, 5.43 pg/ml for IL-6 and 27.35 pg/ml for TNF-α. Liuzzo et al. [50] evaluated the prognostic value of CRP in adults with unstable angina. Using a cut-off point for CRP of 0.3 mg/dl as a marker for subsequent cardiac events or the need for urgent angioplasty, they found a sensitivity of 90 % and a specificity of 82 %. Our findings also suggest a relationship between the severity of MSD and the plasma concentrations of CRP and pro-inflammatory cytokines. Although serum concentrations among our subjects were below the cut-off points for severe conditions, such as sepsis, they are comparable with those for cardiac disease risk. If CRP is a mediator of future cardiac events, as some recent hypotheses in the literature suggest, then elevated CRP levels induced by overuse leading to MSDs could contribute to future cardiac problems if left unchecked. Clearly, the clinical use of these measurements in determining the specific cause of an inflammatory disease process has limitations and it is necessary to control for potentially confounding factors when using CRP and pro-inflammatory cytokines as disease markers. However, their usefulness in identifying an underlying inflammatory component may add significantly to the understanding of a disease process, and these biomarkers may prove useful in determining the exacerbation or remission of the inflammatory response in MSDs such as might occur with chronic risk factor exposure or clinical intervention.

Conclusions

Several key points are supported by these findings. First, early-onset MSDs due to overuse are associated with a systemic inflammatory response, as demonstrated by a direct positive relationship between serum concentrations of key markers of inflammation and symptom severity in subjects with early-onset MSDs. Secondly, increases in serum biomarkers are associated with an increase in the number of local signs of pain, tenderness, peripheral nerve irritation, weakness and limited motion as well as the involvement of multiple anatomical sites among these subjects. Recent work in our animal model of repetitive motion injury [4,5] has shown that local tissue injury and inflammatory responses are linked to a systemic release of pro-inflammatory cytokines. Therefore the results of the present study indicate that inflammatory markers reflect an underlying low-grade inflammatory process induced by overuse. Thirdly, the magnitude of the systemic serum response in the patients with more severe MSD signs and symptoms is of the same order as several low-grade chronic inflammatory conditions known to produce elevated serum pro-inflammatory biomarkers, although not as high as major organ trauma, serious infections or inflammatory diseases. Therefore, in any study that monitors the serum levels of pro-inflammatory cytokines and CRP, care must be taken to control for other confounding characteristics, diseases and injuries. On the basis of our results, MSDs should be counted among these confounding variables. Fourthly, although the non-specificity of the serum cytokine and CRP response presents limitations to the use of these markers in the diagnosis of specific overuse MSD conditions, the potential use of multiple serum inflammatory marker levels as indicators of the exacerbation and resolution of MSDs in combination with the UBMA or a similar assessment tool is compelling, and may be of interest to clinical practitioners and should be the focus of future research.

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REFERENCES

1 Bureau of Labor Statistics (2005) Lost-worktime injuries and illnesses: characteristics and resulting days away from work, 2004, United States Department of Labor News USDL 05-2312, Washington, D.C.
2 National Institute for Occupational Safety and Health (1997) Musculoskeletal Disorders and Workplace Factors: a Critical Review of Epidemiologic Evidence for Work-Related Musculoskeletal Disorders of the Neck, Upper Extremity, and Low Back (Bernard, B., ed.), U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, Publication no. 97–141
3 National Research Council and Institute of Medicine (2001) Musculoskeletal Disorders and the Workplace, National Academy Press, Washington, DC
4 Barbe, M. F., Barr, A. E., Gorzelany, I., Amin, M., Gaughan, J. P. and Safadi, F. F. (2003) Chronic repetitive reaching and grasping results in decreased motor performance and widespread tissue responses in a rat model of MSD. J. Orthop. Res. **21**, 167–176
5 Barr, A. E., Amin, M. and Barbe, M. F. (2002) Dose–response relationship between reach repetition and indicators of inflammation and movement dysfunction in a rat model of work-related musculoskeletal disorder. Proceedings of the Human Factors and Ergonomics Society 46th Annual Meeting, 1486–1490

6 Al-Shatti, T., Barr, A. E., Safadi, F., Amin, M. and Barbe, M. F. (2005) Increase in pro- and anti-inflammatory cytokines in median nerves in a rat model of repetitive motion injury. J. Neuroimmunol. **167**, 13–22
7 Clark, B. D., Al-Shatti, T. A., Barr, A. E., Amin, M. and Barbe, M. F. (2004) Performance of a high-repetition, high-force task induces carpal tunnel syndrome in rats. J. Orthop. Sports Phys. Ther. **34**, 244–253
8 Ruminy, P., Gangneux, C., Claeyssens, S., Scotte, M., Daveau, M. and Salier, J. P. (2001) Gene transcription in hepatocytes during the acute phase of a systemic inflammation: from transcription factors to target organ. Inflamm. Res. **50**, 383–390
9 Hager, K., Machein, U., Krieger, S., Platt, D., Seefried, G. and Bauer, J. (1994) Interleukin-6 and selected plasma proteins in healthy persons of different ages. Neurobiol. Aging **15**, 771–772
10 Pedersen, B. K., Steensberg, A. and Schjerling, P. (2001) Muscle-derived interleukin-6: possible biological effects. J. Physiol. **526**, 329–337
11 Chan, M. H., Carey, A. L., Watt, M. J. and Febbraio, M. A. (2004) Cytokine gene expression in human skeletal muscle during concentric contraction: evidence that IL-8, like IL-6, is influenced by glycogen availability. Am. J. Physiol. Regulatory Integrative Comp. Physiol. **287**, R322–R327
12 Macy, E. M., Hayes, T. E. and Tracy, R. P. (1997) Variability in the measurement of C-reactive protein in healthy subjects: implications for reference intervals and epidemiological applications. Clin. Chem. **43**, 52–58
13 Beckman, J. A., Preis, O., Ridker, P. M. and Gerhard-Herman, M. (2005) Comparison of usefulness of inflammatory markers in patients with versus without peripheral arterial disease in predicting adverse cardiovascular outcomes (myocardial infarction, stroke, and death). Am. J. Cardiol. **96**, 1374–1378
14 Jialal, I. and Devaraj, S. (2001) Inflammation and atherosclerosis: the value of the high-sensitivity C-reactive protein assay as a risk marker. Am. J. Clin. Pathol. **116** (Suppl.), S108–S115
15 Liuzzo, G., Biasucci, L. M., Gallimore, J. R. et al. (1994) The prognostic value of C-reactive protein and serum amyloid a protein in severe unstable angina. N. Engl. J. Med. **331**, 417–424
16 Ridker, P. M., Cushman, M., Stampfer, M. J., Tracy, R. P. and Hennekens, C. H. (1997) Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. N. Engl. J. Med. **336**, 973–979
17 Nijmeijer, R., Lagrand, W. K., Visser, C. A., Meijer, C. J., Niessen, H. W. and Hack, C. E. (2001) CRP, a major culprit in complement-mediated tissue damage in acute myocardial infarction? Int. Immunopharmacol. **1**, 403–414
18 Lagrand, W. K., Visser, C. A., Hermens, W. T. et al. (1999) C-reactive protein as a cardiovascular risk factor: more than an epiphenomenon? Circulation **100**, 96–102
19 Kramer, J. F., Potter, P., Harburn, K. L., Speechley, M. and Rollman, G. B. (2001) An upper body musculoskeletal assessment instrument for patients with work-related musculoskeletal disorders: a pilot study. J. Hand Ther. **14**, 115–121
20 Ravaglia, G., Forti, P., Maioli, F. et al. (2004) Peripheral blood markers of inflammation and functional impairment in elderly community-dwellers. Exp. Gerontol. **39**, 1415–1422
21 Ershler, W. B. and Keller, E. T. (2000) Age-associated increased interleukin-6 gene expression, late-life diseases, and frailty. Annu. Rev. Med. **51**, 245–270
22 Biffl, W. L., Moore, E. E., Moore, F. A. and Peterson, V. M. (1996) Interleukin-6 in the injured patient. Marker of injury or mediator of inflammation?Ann. Surg. **224**, 647–664
23 Akira, S., Taga, T. and Kishimoto, T. (1993) Interleukin-6 in biology and medicine. Adv. Immunol. **54**, 1–78
24 Tilg, H., Trehu, E., Atkins, M. B., Dinarello, C. A. and Mier, J. W. (1994) Interleukin-6 as an anti-inflammatory cytokine: induction of circulating IL-1 receptor antagonist and soluble tumor necrosis factor receptor p55. Blood **83**, 113–118
25 Freeland, A. E., Tucci, M. A., Barbieri, R. A., Angel, M. F. and Nick, T. G. (2002) Biochemical evaluation of serum and flexor tenosynovium in carpal tunnel syndrome. Microsurgery **22**, 378–385
26 Rosendal, L., Sogaard, K., Kjaer, M., Sjogaard, G., Langberg, H. and Kristiansen, J. (2005) Increase in interstitial interleukin-6 of human skeletal muscle with repetitive low-force exercise. J. Appl. Physiol. **98**, 477–481
27 Langberg, H., Olesen, J. L., Gemmer, C. and Kjaer, M. (2002) Substantial elevation of interleukin-6 concentration in peritendinous tissue, in contrast to muscle, following prolonged exercise in humans. J. Physiol. **542**, 985–990
28 Ballou, S. P., Lozanski, F. B., Hodder, S. et al. (1996) Quantitative and qualitative alterations of acute-phase proteins in healthy elderly persons. Age Ageing **25**, 224–230
29 Chrysohoou, C., Pitsavos, C., Panagiotakos, D. B., Skoumas, J. and Stefanadis, C. (2004) Association between prehypertension status and inflammatory markers related to atherosclerotic disease: the ATTICA Study. Am. J. Hypertens. **17**, 568–573
30 Panagiotakos, D. B., Pitsavos, C., Yannakoulia, M., Chrysohoou, C. and Stefanadis, C. (2005) The implication of obesity and central fat on markers of chronic inflammation: the ATTICA study. Atherosclerosis **183**, 308–315
31 Zampelas, A., Panagiotakos, D. B., Pitsavos, C., Chrysohoou, C. and Stefanadis, C. (2004) Associations between coffee consumption and inflammatory markers in healthy persons: the ATTICA study. Am. J. Clin. Nutr. **80**, 862–867
32 Cohen, H. J., Pieper, C. F., Harris, T., Rao, K. M. and Currie, M. S. (1997) The association of plasma IL-6 levels with functional disability in community-dwelling elderly. J. Gerontol. A. Biol. Sci. Med. Sci. **52**, M201–M208
33 de Werra, I., Jaccard, C., Corradin, S. B., Chiolero, R. et al. (1997) Cytokines, nitrite/nitrate, soluble tumor necrosis factor receptors, and procalcitonin concentrations: comparisons in patients with septic shock, cardiogenic shock, and bacterial pneumonia. Crit. Care Med. **25**, 607–613
34 Strecker, W., Gebhard, F., Perl, M. et al. (2003) Biochemical characterization of individual injury pattern and injury severity. Injury **34**, 879–887
35 Plank, L. D. and Hill, G. L. (2000) Sequential metabolic changes following induction of systemic inflammatory response in patients with severe sepsis or major blunt trauma. World J. Surg. **24**, 630–638
36 Fida, N. M., Al-Mughales, J. and Farouq, M. (2006) Interleukin-1α, interleukin-6 and tumor necrosis factor-α levels in children with sepsis and meningitis. Pediatr. Int. **48**, 118–12436
37 Ziccardi, P., Nappo, F., Giugliano, G. et al. (2002) Reduction of inflammatory cytokine concentrations and improvement of endothelial function in obese women after weight loss over one year. Circulation **105**, 9075–9076
38 Ferroni, P., Martini, F., Cardarello, C. M., Gazzaniga, P. P., Davi, G. and Basili, S. (2003) Enhanced interleukin-1β in hypercholesterolemia: effects of simvastatin and low-dose aspirin. Circulation **108**, 1673–1675
39 Ansell, B. J., Watson, K. E., Weiss, R. E. and Fonarow, G. C. (2003) HsCRP and HDL effects of statins trial (CHEST): rapid effect of statin therapy on C-reactive protein and high-density lipoprotein levels: a clinical investigation. Heart Dis. **5**, 2–7
40 Zubelewicz-Szkodzinska, B., Szkodzinski, J. et al. (2003) Effects of simvastatin on pro-inflammatory cytokines in patients with hypercholesteremia. Kardiol. Pol. **59**, 465–474
41 Flake, N. M., Bonebreak, D. B. and Gold, M. S. (2005) Estrogen and inflammation increase the excitability of rat temporomandibular joint afferent neurons. J. Neurophysiol. **93**, 1585–1597
42 Pacifici, R., Rifas, L., Teitelbaum, S. et al. (1987) Spontaneous release of interleukin 1 from human blood monocytes reflects bone formation in idiopathic osteoporosis. Proc. Natl. Acad. Sci. U.S.A. **84**, 4616–4620

43 Ralston, S. H. (1994) Analysis of gene expression in human bone biopsies by polymerase chain reaction: evidence for enhanced cytokine expression in postmenopausal osteoporosis. J. Bone Miner. Res. **9**, 883–890
44 Seck, T., Diel, I., Bismar, H., Ziegler, R. and Pfeilschifter, J. (2002) Expression of interleukin-6 (IL-6) and IL-6 receptor mRNA in human bone samples from pre- and postmenopausal women. Bone **30**, 217–222
45 Leng, X. H., Zhang, W., Nieswandt, B. and Bray, P. F. (2005) Effects of estrogen replacement therapies on mouse platelet function and glycoprotein VI levels. Circ. Res. **97**, 415–417
46 Yildirir, A., Aybar, F., Tokgozoglu, L. et al. (2002) Effects of hormone replacement therapy on plasma homocysteine and C-reactive protein levels. Gynecol. Obstet. Invest. **53**, 54–58
47 Roubenoff, R., Harris, T. B., Abad, L. W., Wilson, P. W., Dallal, G. E. and Dinarello, C. A. (1998) Monocyte cytokine production in an elderly population: effect of age and inflammation. J. Gerontol. A. Biol. Sci. Med. Sci. **53**, M20–M26
48 Giuliani, N., Sansoni, P., Girasole, G. et al. (2001) Serum interleukin-6, soluble interleukin-6 receptor and soluble gp130 exhibit different patterns of age- and menopause-related changes. Exp. Gerontol. **36**, 547–557
49 Pfeilschifter, J., Koditz, R., Pfohl, M. and Schatz, H. (2002) Changes in proinflammatory cytokine activity after menopause. Endocr. Rev. **23**, 90–119
50 Liuzzo, G., Biasucci, L. M., Gallimore, J. R. et al. (1994) The prognostic value of C-reactive protein and serum amyloid A protein in severe unstable angina. N. Engl. J. Med. **331**, 417–424

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