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Biological Stress Systems, Adverse Life Events, and the Improvement of Chronic Multisite Musculoskeletal Pain Across a 6-Year Follow-Up

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Abstract: Dysfunction of biological stress systems and adverse life events, independently and in interaction, have been hypothesized to predict chronic pain persistence. Conversely, these factors may hamper the improvement of chronic pain. Longitudinal evidence is currently lacking. We examined whether: 1) function of biological stress systems, 2) adverse life events, and 3) their combination predict the improvement of chronic multisite musculoskeletal pain. Subjects of the Netherlands Study of Depression and Anxiety (NESDA) with chronic multisite musculoskeletal pain at baseline (N = 665) were followed-up 2, 4, and 6 years later. The Chronic Pain Grade Questionnaire was used to determine improvement (not meeting the criteria) of chronic multisite musculoskeletal pain at follow-up. Baseline assessment of biological stress systems included function of hypothalamic-pituitary-adrenal axis (1-hour cortisol awakening response, evening level, and post dexamethasone level), the immune system (basal and lipopolysaccharide-stimulated inflammatory markers), the autonomic nervous system (heart rate, pre-ejection period, SD of the normal-to-normal interval, and respiratory sinus arrhythmia). The number of adverse life events were assessed at baseline and 2-year follow-up using the List of Threatening Events Questionnaire. We showed that hypothalamic-pituitary-adrenal axis, immune system, and autonomic nervous system functioning and adverse life events were not associated with the improvement of chronic multisite musculoskeletal pain, either as a main effect or in interaction. This longitudinal study could not confirm that biological stress system dysfunction and adverse life events affect the course of chronic multisite musculoskeletal pain.

***Perspective:** Biological stress systems and adverse life events are not associated with the improvement of chronic multisite musculoskeletal pain over 6 years of follow-up. Other determinants should thus be considered in future research to identify in which persons pain symptoms will improve.*

***Key words:** Chronic pain, cortisol, inflammation, autonomic nervous system, life change events.*

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Chronic pain is a common and persistent problem in the community.[16] However, some persons show improvement in pain symptoms[13] and it is useful to examine which factors identify these persons. Biological and psychosocial stress factors may play a role in the deterioration, persistence, or the improvement of chronic pain.[13]

Considering biological factors, subjects with chronic pain have previously shown alterations in biological stress systems.[1,23] Previous cross-sectional studies indicate that dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis, the immune system (IMS), and the autonomic nervous system (ANS) promote neural alterations that have been associated with central sensitization processes in pain disorders.[29,33] Furthermore, psychosocial trigger incidents, such as adverse life events, may among others exacerbate chronic pain through their effect on biological stress systems.[33,36] Support for this model (see Fig 1) comes mainly from cross-sectional findings hence causal directions remain uncertain.

To be more precise, previous cross-sectional studies showed lower levels of HPA axis cortisol[20,34,44] and increased sensitivity to feedback inhibition of the HPA axis[50] in chronic pain. In other studies, individuals with chronic pain showed an increased innate immune response[18,26] and altered parasympathetic and sympathetic activity of the ANS.[12,41] Longitudinal studies on the role of biological stress systems in chronic pain are scarce and mainly focused on the onset of chronic pain.[36] One prospective study reported that altered function of the HPA axis predicted the onset of chronic widespread pain.[36] Thus, although function of biological stress systems might be altered in chronic pain, its association with the persistence or improvement of chronic pain remains unclear.

With regard to psychosocial stress factors, cross-sectional studies suggest an association between recent adverse life events and chronic pain.[3,27] Longitudinal studies showed that adverse life events were associated with the future onset of chronic pain.[24,25] One prospective study reported that experiencing 2 or more negative events (related to relationships, unemployment, illness, or financial problems) predicted new-onset chronic widespread pain 15 months later.[24] Nevertheless, the role of life events in relation to the future course of chronic pain remains to be elucidated.

Most previous studies on biological stress systems and life events in relation to chronic pain did not take relevant confounders into account, although previous work has indicated that lifestyle,[13,37,47] chronic diseases,[13,37] and psychopathology[21,47] may influence biological stress systems and chronic pain. For example, older age and chronic diseases (such as cardiovascular disease) have been associated with increased cortisol levels,[48] and an increased risk of persisting chronic pain.[32,37] In addition, previous research has shown that psychopathology[20] and pain status at baseline[16] might not only confound, but also moderate the association between biological function and chronic pain. We showed in a previous study that hypocortisolemia was only present in subjects with chronic pain without depressive and anxiety disorders, because these disorders seem to conversely increase cortisol levels.[20] To test this, interaction of biological variables with psychopathology have to be examined in the association between biological stress systems and chronic pain. There is a lack of large-scale longitudinal studies that take these relevant confounders and moderators into account.

This 6-year longitudinal study followed 665 subjects who had chronic multisite musculoskeletal pain at baseline to determine the association of baseline: 1) dysfunction of the HPA axis, IMS and ANS, 2) adverse life events, and 3) the interaction of biological dysfunction and life events, with the improvement of chronic multisite musculoskeletal pain across a 6-year follow-up, while adjusting for sociodemographic characteristics, lifestyle, chronic diseases, depression, and anxiety. We hypothesize that biological stress systems and life events contribute to the maintenance of chronic pain.

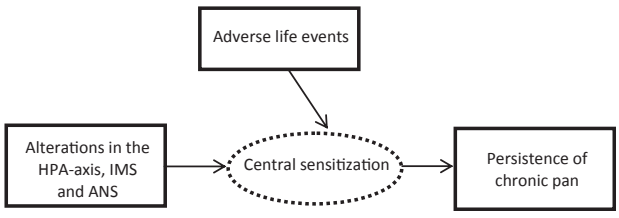

Figure 1. Hypothetical framework suggesting that alterations in biological stress systems induce central sensitization, ultimately resulting in the persistence of chronic pain, an effect that is aggravated by adverse life events.[33]

Methods

Study Design, Data Collection Procedures, and Study Sample

The present study used longitudinal data from the Netherlands Study of Depression and Anxiety (NESDA): an ongoing cohort study in which 2981 participants (aged 18–65 years at baseline), from which 94.8% were of North European ancestry, were monitored biannually for 6 years to investigate the long-term course and consequences of depressive and anxiety disorders. Participants were recruited from the community (19%), primary care (54%), and specialized mental health care (27%). The community sample builds on 2 Dutch cohorts that were available through previous studies.[8,28] Primary care patients were recruited from 65 general practitioners through a 3-stage screening procedure including screening questionnaires and phone interviews. People in different developmental stages of psychopathology as well as control participants with no psychiatric diagnosis participated. The disorders included dysthymia, major depressive disorder, general anxiety disorder, panic disorder, social phobia, and agoraphobia. Because of the design of the NESDA study, exclusion criteria were not being fluent in Dutch or a diagnosis of a primary psychiatric diagnosis other than depression and anxiety (eg, psychotic disorder, obsessive compulsive disorder, or severe addiction disorder assessed using Diagnostic and

Statistical Manual of Mental Disorders criteria using the Composite International Diagnostic Interview[42]). Baseline data collection took place between 2004 and 2007, with follow-up assessments 2, 4, and 6 years later. Data collection was performed by specially trained clinical research staff at the 3 research sites in the Netherlands (Amsterdam, Groningen, and Leiden) and included assessment of written questionnaires, interviews, a medical examination, and collection of blood and saliva samples. The NESDA study contains a high proportion of subjects with chronic multisite musculoskeletal pain and provides a unique opportunity to control for relevant variables such as depressive and anxiety disorders. The research protocol was approved by the ethical committee of participating universities and written informed consent was obtained from all participants. Penninx et al[39] provided a detailed description of the NESDA study design and sampling procedures.

Participants with chronic multisite musculoskeletal pain at baseline were included in the present study (n = 767) and followed up for the improvement of chronic multisite musculoskeletal pain over 6 years. Of these eligible subjects, 665 subjects had data on pain at follow-up (2-, 4-, or 6-year) available (therefore, n = 102 were lost to follow-up). Baseline data were assessed on biological stress systems function and adverse life events. All subjects had baseline data available for adverse life events or for at least 1 marker of the biological stress systems.

Improvement of Chronic Multisite Musculoskeletal Pain

Chronic multisite musculoskeletal pain was defined using an updated version of the Chronic Pain Grade Questionnaire (CPG[45]). The CPG first inquires about the presence of pain in the previous 6 months in the extremities (joints of the arms, hands, legs, or feet), back, neck, head, abdomen, chest, and the orofacial area (mouth and face).[45] The subsequent questions in the CPG refer to the most painful location and inquire: 1) days in pain in the previous 6 months (scale of 0–180), 2) pain at this moment (scale of 0–10), 3) worst pain in the previous 6 months (scale of 0–10), 4) average pain in the previous 6 months (scale of 0–10), 5) disability days in the previous 6 months (scale of 0–180), 6) disability in daily activities (scale of 0–10), 7) disability in spare time, social life, and family activities (scale of 0–10), and 8) disability in work (scale of 0–10). For the assessment of pain severity, a total pain intensity score was calculated using questions 2, 3, and 4 of the CPG, and a total pain disability score was calculated using questions 6, 7, and 8 of the CPG (average of the 0–10 ratings of the 3 questions multiplied by 10 resulting in a 0–100 score). Disability points (scale of 0–6) were calculated adding the total disability score (0–100) and the indicated points for disability days (question 5 of the CPG; see Von Korff et al[46] and Supplementary Table 1). Five grades were categorized: grade 0 (pain-free, no pain in the previous 6 months); grade I (low disability [less than 3 disability points], low intensity [score below 50]); grade II (low disability, high intensity [score 50 or higher]); grade III (high disability, moderately limiting [3–4 disability points regardless of pain intensity score]); and grade IV (high disability, severely limiting [5–6 disability points regardless of pain intensity score]; see Von Korff et al[46] and Supplementary Table 2). Following our previous studies,[18,20] we defined chronic multisite musculoskeletal as grade I, II, III, or IV on the CPG and pain present in the previous 6 months in the extremities, the back, and the neck. Improvement was defined as being free of chronic multisite musculoskeletal pain (in the previous 6 months) at 1 of the follow-up time points (2, 4, or 6 years). Time to improvement described the first time point at which improvement occurred. Subjects whose chronic multisite musculoskeletal pain did not improve were classified as 'persistent' and still met the criteria of chronic multisite musculoskeletal pain at all follow-up time points. All subjects were censored in the analysis at the occurrence of improvement or at the last recorded follow-up.

Biological Stress Systems

Function of the HPA axis was assessed by collection of saliva samples at home using Salivettes (Sarstedt AG and Co, Numbrecht, Germany) at 7 time points within a median of 9 days (interquartile range [IQR] = 5–22) after the baseline interview. Cortisol analysis was performed using competitive electrochemiluminescence (E170; Roche, Basel, Switzerland). The cortisol awakening response included sampling points at awakening, and 30, 45, and 60 minutes later. Using formulas described by Pruessner et al,[40] the area under the curve with respect to the ground (AUCg) and with respect to the increase (AUCi) were calculated on the basis of the 4 morning cortisol measures.[48] The AUCg is an estimate of the total cortisol secretion over the first hour of awakening, whereas the AUCi is a measure of the dynamic of the cortisol awakening response related to the sensitivity of the system and emphasizing the rate of change of the cortisol levels after awakening. In line with our previous work,[47] we only used AUCg and AUCi as markers of the cortisol awakening curve in our analyses. Evening cortisol was averaged over 2 evening values (10 PM and 11 PM). To provide a measure of the negative feedback system of the HPA axis, the cortisol suppression ratio was calculated as the cortisol value at awakening on the first day divided by the cortisol value at awakening on the next day after ingestion of .5 mg dexamethasone the evening before (directly after the saliva sample at 11 PM). Cortisol values were assigned as missing if samples were collected outside of a margin of 5 minutes before or after the time protocol. Also, a pilot test has been performed to rule out noncompliance with dexamethasone ingestion (see Vreeburg et al[47,48] for details on cortisol assessment and data cleaning procedures).

For function of the IMS, basal inflammation and the innate immune response were assessed because they have previously been related to chronic pain.[18] Fasting morning blood samples were kept frozen at −80°C and basal levels of inflammatory markers C-reactive protein

(CRP), interleukin (IL)-6, and tumor necrosis factor (TNF)-α were assayed. CRP was assayed using a high-sensitivity in-house enzyme-linked immunosorbent assay (ELISA) on the basis of purified protein and polyclonal anti-CRP antibodies (Dako, Glostrup, Denmark). IL-6 was assayed using a high-sensitivity ELISA (PeliKine-Compact ELISA, Sanquin, Amsterdam, The Netherlands). TNF-α was assayed using a high-sensitivity solid-phase ELISA (Quantikine HS Human TNF-α Immunoassay, R&D Systems Inc, Minneapolis, MN). Following our previous studies,[18,19] a basal summary index was calculated as the standardized sum of all 3 standardized ln-transformed basal inflammatory markers. The innate immune response of 13 cytokines was determined in whole blood that was ex vivo stimulated with lipopolysaccharide (LPS; 10 ng/mL blood). LPS-stimulated samples were laid flat and incubated at a slow rotation for 5 to 6 hours at 37°C. Plasma was kept frozen at −80°C. Using a multianalyte profile (Human CytokineMAP A version 1.0; Myriad RBM, Austin, TX), interferon-γ, IL-2, IL-4, IL-6, IL-8, IL-10, IL-18, C-C motif chemokine (CCL) 2, CCL3, CCL4, matrix metalloproteinase-2, TNF-α, and TNF ligand superfamily member 1 were determined. Because all cytokines were highly intercorrelated (mean Pearson r ± SD = .60 ± .18), an LPS summary index was calculated as the standardized sum of all 13 standardized ln-transformed markers. To avoid multiple testing, only the basal summary index and LPS summary index were used in the analyses.

ANS assessment was performed using the VU University Ambulatory Monitoring System device, recording the electrocardiogram and changes in thorax impedance from 6 electrodes placed on the chest and back for a period of approximately 2 hours.[14,49] ANS markers included heart rate and pre-ejection period as markers of sympathetic activity; and SD of the normal-to-normal interval and respiratory sinus arrhythmia (RSA) as markers of parasympathetic activity of the ANS (see Barakat et al[5] and Licht et al[31] for details).

Adverse Life Events

Adverse life events were assessed at baseline using the List of Threatening Events Questionnaire.[9,10] Twelve recent life stressors, such as death of close friend or relative or serious financial problems, were assessed at baseline (events occurring in the year before baseline assessment) and at 2-year follow-up (events occurring in the 2 years in between the baseline and this follow-up assessment). We additionally inquired whether subjects experienced any other adverse life event in the past year.[43] The total number of life events (0–13) were calculated and used in the analyses.

Covariates

Baseline covariates were selected a priori on the basis of previous findings of important determinants of biological stress systems function and chronic pain. A first set of covariates included sociodemographic characteristics (age, sex, and years of education) and sampling factors. Older age,[16,32] female sex, and less education[32] have been associated with persistence of chronic pain. For HPA axis analyses, cortisol sampling factors were considered including awakening time, working on day of sampling (yes/no), season (light vs dark month), and < 6 hours of sleep (yes/no) because they have previously been reported to determine HPA axis cortisol levels.[48] For the LPS summary index analyses, laboratory site (Amsterdam, Groningen, Leiden, Heerenveen) was considered to account for potentially small differences in the assessment of LPS-stimulated inflammatory levels across sites. For RSA analyses, respiration rate (breaths per minute) was considered as a covariate because it influences RSA and vagal tone.[22] A second set of covariates comprised several lifestyle and disease factors, which were selected on the basis of previous associations with either biological stress systems[13,47] or pain.[37] Higher body mass index,[37] alcohol consumption,[6] former smoking,[37] lower physical fitness level,[17] and chronic diseases[37] were previously associated with chronic pain. Body mass index was calculated as body weight in kilograms divided by height in meters squared. Smoking was categorized as never, former, or current smoker. Alcohol intake was categorized as nondrinker, mild/moderate drinker, or heavy drinker.[53] Physical activity was assessed using the International Physical Activity Questionnaire and expressed as one's resting metabolic rate multiplied by minutes of physical activity per week.[2] Total number of chronic diseases (including cardiovascular disease, epilepsy, diabetes mellitus, osteoarthritis, stroke, cancer, chronic lung disease, thyroid disease, liver disease, intestinal disorders, and ulcers) were assessed according to self-report. Medication use was on the basis of drug container inspection of all drugs used in the past month and classified according to the World Health Organization Anatomical Therapeutic Chemical (ATC) classification.[52] In line with our previous work,[5,19] we considered frequent use (daily or > 50% of the time) of medication that affects either biological stress systems or pain.[15,52] For IMS analyses, anti-inflammatory medication (ATC codes: M01A, M01B, A07EB, A07EC) was considered. For ANS analyses, heart medication (β-blocking agents [ATC code: C07] and other heart medication [ATC codes: C01, C02, C03, C04, C05, C08, C09]) were considered. A third set of covariates included lifetime (current and previous) diagnoses of depressive and/or anxiety disorders and use of antidepressant medication. Depression and anxiety have previously been associated with altered sympathetic and parasympathetic balance, HPA axis dysregulation, and altered immune function.[33] Pain increases the onset of these disorders.[21] In addition, depression and anxiety might moderate the association between biological stress systems, such as the HPA axis, and chronic pain.[20] Use of antidepressants might supress depression, anxiety, and pain.[33] Lifetime diagnoses of depressive disorders (major depressive disorder, dysthymia) and anxiety disorders (panic disorder, agoraphobia, generalized anxiety disorder, social phobia) were established with the Composite International Diagnostic Interview (World Health Organization version 2.1) according to the Diagnostic and Statistical Manual of Mental Disorders-Fourth Edition criteria.[42] Use of tricyclic

antidepressants (ATC code: N06AA), selective serotonin reuptake inhibitors (ATC code: N06AB), and other antidepressant medications (ATC codes: N06AF/AG/AX) was assessed.

Statistical Analyses

Baseline characteristics were compared between subjects with and without improvement of chronic multisite musculoskeletal pain using independent-samples t-tests for continuous variables, χ^2 tests for dichotomous and categorical variables, and Mann-Whitney U tests for non-normally distributed variables. Cox regression analyses were performed to determine the associations between biological stress systems and adverse life events at baseline and improvement of chronic multisite musculoskeletal pain over 6 years of follow-up, while adjusting for confounders in 3 steps: 1) sociodemographic variables (age, sex, and years of education), cortisol sampling factors (in HPA axis analyses), laboratory site (in LPS analyses), and respiration rate (breaths per minute, in RSA analyses); 2) 'lifestyle and disease': body mass index, smoking, alcohol intake, physical activity, chronic diseases, anti-inflammatory medication (IMS analyses) and β-blocking agents and heart medication (ANS analyses); and 3) lifetime depression and/or anxiety and antidepressant use. Unique aspects of the Cox approach are studying time at risk for an event and the censoring of data at the last recorded follow-up measure if subsequent data are missing or if no event occurs. Proportional hazards were verified. Values for evening cortisol, cortisol suppression ratio, and basal inflammatory markers were ln-transformed before analyses to normalize distributions. Missing data of biological stress systems were at random (Little Missing Completely At Random test: P = .45). Life events at 2-year follow-up were used for sensitivity analyses to test whether they were further predictive of improvements in chronic multisite musculoskeletal pain at later follow-up measures.

A potential moderating effect of life events in the association between function of biological stress systems and improvement of chronic multisite musculoskeletal pain was examined by adding an interaction term (Biological marker × Number of life events) to each analysis. Also, because psychopathology[20] and pain intensity at baseline[16] might moderate the association between biological function and chronic multisite musculoskeletal pain, interaction terms ([Biological marker × Lifetime depression and/or anxiety]; [Biological marker × Pain intensity at baseline]) were separately tested and added to the analyses.

For all statistical tests, a probability level of ≤ 5% was regarded as significant. To test interactions, this level was set at ≤ 10%. The statistical calculations were performed using SPSS 20 for Windows (IBM, Armonk, NY).

Results

Included subjects (n = 665) had lower baseline values for cortisol suppression ratio (median [IQR] = 2.3 [1.7–3.0] vs 2.9 [2.1–3.6], P = .02), CRP (median [IQR] = 1.3 [.6–2.9] vs 1.5 [.9–4.7], P = .05), RSA (mean [SD] = 40.5 [21.4] vs 48.0 [33.1], P = .003) and higher total number of adverse life events (mean [SD] = 1.0 [1.4] vs .7 [1.0], P = .02), but did not differ from excluded subjects who were lost to follow-up (n = 102) in the other variables of biological stress systems (P > .05).

Of 665 subjects with chronic multisite musculoskeletal pain at baseline, 28% (n = 185) still met the criteria for chronic multisite musculoskeletal pain at all available follow-up time points. Of the subjects who improved (n = 480, 72%), average time to improvement was 3 years (46% improved after 2 years, an additional 17% after 4 years, and an additional 9% after 6 years). Subjects with improvements in chronic multisite musculoskeletal pain had lower baseline pain scores, were significantly younger, had more years of education, worked more often on day of cortisol sampling, and slept more than subjects with persistence of chronic multisite musculoskeletal pain. Moreover, the group with pain improvement had lower body mass index, were more often moderate alcohol drinkers, were more often physically active, had less often chronic diseases, used less often other heart medication, had less often a lifetime depressive or anxiety disorder, had lower values of IL-6, and higher values of SD of the normal-to-normal interval and RSA than the persistent group (see Table 1).

Table 2 shows associations between function of the biological stress systems and improvement of chronic multisite musculoskeletal pain. None of the HPA axis, IMS, and ANS variables were associated with improvement of chronic multisite musculoskeletal pain after adjustment for covariates (all P > .05). Table 3 reports the association between the total number of adverse life events and improvement of chronic multisite musculoskeletal pain. Similar to the unadjusted model, this association did not appear to be significant after adjusting for sociodemographic or more extensive sets of covariates (all P > .05).

Additional analyses showed that the number of life events assessed at 2-year follow-up were not further predictive of improvement of chronic multisite musculoskeletal pain at later follow-up measures (n = 628; fully adjusted hazard ratio = .96, 95% confidence interval, .90–1.02, P = .18). Also, no moderating effects of life events in the association between any of the markers of the biological stress systems and improvement of chronic multisite pain were found (all P interaction > .10). Moreover, no evidence was found for moderating effects of psychopathology and pain intensity at baseline in the association between biological stress systems and improvement of chronic multisite musculoskeletal pain (all P interaction > .10). Additional analyses examining a short follow-up period restricted to 2 years also showed no associations between biological stress systems and life events with improvement of chronic multisite musculoskeletal pain (n = 628; similar hazard ratios and all P > .30). To test whether our findings were not influenced by chronic pain severity at baseline, we performed analyses while adjusting for all covariates including baseline pain intensity scores and also found similar

Table 1. Baseline Characteristics* Comparing Subjects With Persistence and With Improvement of Chronic Multisite Musculoskeletal Pain

Characteristic	Persistence of Chronic Multisite Pain (n = 185)	Improvement of Chronic Multisite Pain (n = 480)	P†
Pain scores			
Pain days	134.8 (61.4)	100.0 (70.2)	<.001
Pain intensity	58.3 (15.6)	50.6 (18.0)	<.001
Pain disability	48.3 (23.1)	35.9 (26.0)	<.001
Sociodemographic factors			
Age, y	48.4 (10.6)	43.8 (12.2)	<.001
Women, %	78.4	71.2	.06
Education, y	10.8 (3.2)	11.6 (3.2)	.002
Cortisol sampling factors			
Time of awakening	7:30 (1:00)	7:30 (0:58)	.66
Working on day of sampling, %	57.8	68.5	.009
Sampling in month with more daylight, %	68.1	70.8	.49
≤6 h of sleep, %	43.8	31.0	.003
Lifestyle and disease factors			
Median body mass index [IQR]	26.0 [23.3–29.4]	24.9 [22.4–29.0]	.03
Smoking, %			.31
Never smoker	27.0	26.5	
Former smoker	36.2	30.8	
Current smoker	36.8	42.7	
Alcohol use, %			.001
Nondrinker	48.6	35.4	
Mild/moderate alcohol drinker	38.4	54.0	
Heavy alcohol drinker	13.0	10.6	
Median physical activity, 1000 MET min/wk [IQR]	3.3 [1.9–5.0]	3.7 [1.5–5.7]	.02
Number of chronic diseases	1.4 (1.2)	.9 (.9)	<.001
Anti-inflammatory medication, %	29.7	25.6	.28
β-Blocking agents, %	12.4	8.8	.15
Other heart medication, %	20.0	13.3	.03
Depression and anxiety factors			
Lifetime depressive or anxiety disorder, %	92.4	86.7	.04
Antidepressant medication, %	30.8	30.4	.92
Biological stress systems function			
HPA axis			
AUCg, nmol/L/h	18.9 (6.9)	18.6 (6.5)	.70
AUCi, nmol/L/h	2.8 (6.4)	2.5 (6.1)	.69
Evening level, nmol/L, median [IQR]	5.4 [3.4–6.9]	4.7 [3.3–6.6]	.25
Median cortisol suppression ratio [IQR]	2.2 [1.7–2.9]	2.4 [1.7–3.1]	.39
IMS			
Median basal CRP, mg/L [IQR]	1.4 [.68–3.5]	1.3 [.57–2.8]	.29
Median basal IL-6, pg/mL [IQR]	.95 [.60–1.5]	.78 [.51–1.2]	.03
Median basal TNF-α, pg/mL [IQR]	.80 [.60–1.10]	.80 [.60–1.0]	.14
Basal summary index	.11 (.98)	−.04 (1.0)	.10
LPS summary index	.15 (.74)	−.06 (1.1)	.11
ANS			
Heart rate, beats per minute	71.6 (9.7)	71.9 (9.2)	.73
Pre-ejection period, msec	120.6 (20.0)	120.8 (17.5)	.89
SDNN, msec	60.5 (22.4)	66.7 (24.3)	.003
RSA, msec	37.3 (19.4)	41.7 (22.0)	.02
Respiration rate, breaths per minute	17.0 (1.2)	17.0 (1.1)	.81
Total number of adverse life events	.69 (.96)	.77 (1.06)	.37

Abbreviations: MET, resting metabolic rate multiplied by minutes of physical activity per week; SDNN, SD of the normal-to-normal interval.
*Values are mean ± SD unless otherwise indicated.
†Based on independent samples t-tests for continuous variables, χ^2 tests for dichotomous and categorical variables, and Mann-Whitney U tests for non-normally distributed variables.

nonsignificant hazard ratios for each predicting variable (all $P > .10$). Similarly, adjusting for pain disability or number of pain days at baseline did not affect our findings. Following a previous study,[13] we additionally tested whether pain severity at follow-up affected our findings. However, the inclusion of pain intensity or pain disability at 2-year follow-up separately as confounders in our fully adjusted analyses showed similar hazard ratios (analyses restricted to 2-year follow-up, n = 628; all $P > .40$). Approximately 12% of our sample regularly used

Table 2. Associations* Between Biological Stress Systems Function at Baseline and Improvement of Chronic Multisite Musculoskeletal Pain Over 6-Year Follow-Up

Characteristic	n	Improvement of Chronic Multisite Pain, Hazard Ratio Per 1 SD (95% Confidence Interval)	P
HPA axis			
AUCg†	403		
Sociodemographic adjusted‡		.99 (.88–1.11)	.82
Lifestyle and disease adjusted§		1.00 (.88–1.13)	.99
Depression and anxiety adjusted¶		1.01 (.89–1.14)	.90
AUCi†	403		
Sociodemographic adjusted‡		1.00 (.89–1.12)	.96
Lifestyle and disease adjusted§		1.01 (.89–1.14)	.89
Depression and anxiety adjusted¶		1.01 (.90–1.14)	.85
Mean evening level†	439		
Sociodemographic adjusted‡		.99 (.89–1.11)	.91
Lifestyle and disease adjusted§		1.01 (.89–1.14)	.91
Depression and anxiety adjusted¶		1.01 (.89–1.15)	.87
Cortisol suppression ratio†	415		
Sociodemographic adjusted‡		1.00 (.89–1.13)	.97
Lifestyle and disease adjusted§		.99 (.89–1.12)	.92
Depression and anxiety adjusted¶		1.00 (.89–1.12)	.97
IMS			
CRP‖	650		
Sociodemographic adjusted‡		.99 (.90–1.08)	.80
Lifestyle and disease adjusted§		.99 (.89–1.10)	.87
Depression and anxiety adjusted¶		.99 (.89–1.10)	.99
IL-6‖	649		
Sociodemographic adjusted‡		.99 (.90–1.09)	.84
Lifestyle and disease adjusted§		1.00 (.91–1.10)	.99
Depression and anxiety adjusted¶		1.00 (.91–1.10)	.96
TNF-α‖	647		
Sociodemographic adjusted‡		.98 (.89–1.07)	.61
Lifestyle and disease adjusted§		.98 (.89–1.08)	.69
Depression and anxiety adjusted¶		.98 (.89–1.08)	.69
Basal summary index‖	643		
Sociodemographic adjusted‡		.97 (.89–1.07)	.57
Lifestyle and disease adjusted§		.98 (.88–1.09)	.71
Depression and anxiety adjusted¶		.98 (.89–1.09)	.73
LPS summary index‖,**	266		
Sociodemographic adjusted‡		.88 (.73–1.05)	.16
Lifestyle and disease adjusted§		.88 (.72–1.07)	.19
Depression and anxiety adjusted¶		.86 (.70–1.05)	.14
ANS			
Heart rate††	641		
Sociodemographic adjusted‡		.99 (.90–1.09)	.86
Lifestyle and disease adjusted§		1.00 (.91–1.10)	.96
Depression and anxiety adjusted¶		.99 (.90–1.10)	.88
PEP††	632		
Sociodemographic adjusted‡		1.03 (.94–1.13)	.56
Lifestyle and disease adjusted§		1.03 (.94–1.13)	.61
Depression and anxiety adjusted¶		1.03 (.93–1.14)	.57
SDNN††	641		
Sociodemographic adjusted‡		1.03 (.94–1.14)	.48
Lifestyle and disease adjusted§		1.03 (.94–1.13)	.55
Depression and anxiety adjusted¶		1.03 (.94–1.14)	.52

Table 2. Continued

Characteristic	n	Improvement of Chronic Multisite Pain, Hazard Ratio Per 1 SD (95% Confidence Interval)	P
RSA††,‡‡	641		
Sociodemographic adjusted‡		.99 (.89–1.11)	.91
Lifestyle and disease adjusted§		.98 (.88–1.09)	.66
Depression and anxiety adjusted¶		.97 (.87–1.08)	.61

Abbreviations: PEP, pre-ejection period; SDNN, SD of the normal-to-normal interval.
*Using Cox regression analyses; hazard ratio per 1 SD increase; ln-transformed variables were used for evening cortisol, post-dexamethasone cortisol, and basal inflammatory markers.
‡Adjusted for sex, age, years of education.
†Adjusted for sex, age, years of education and cortisol sampling factors
**Adjusted for sex, age, years of education and cortisol sampling factors; laboratory site
‡‡Adjusted for sex, age, years of education and cortisol sampling factors; laboratory site; and respiration rate.
§Additionally adjusted for alcohol intake, smoking, body mass index, number of chronic diseases and physical activity.
‖Additionally adjusted for alcohol intake, smoking, body mass index, number of chronic diseases and physical activity and use of anti-inflammatory medication.
††Additionally adjusted for alcohol intake, smoking, body mass index, number of chronic diseases and physical activity and use of anti-inflammatory medication; and use of β-blocking agents or other heart medication.
¶Additionally adjusted for lifetime diagnoses of depressive and anxiety disorders and use of antidepressants.

benzodiazepines (ATC codes: N03Ae, N05BA, N05CD, N05CF). We therefore performed additional analyses adding this as a confounder to our model. This did not affect our findings: similar nonsignificant hazard ratios were found for biological variables and life events with chronic pain improvement.

Discussion

This longitudinal study showed that improvement of chronic multisite musculoskeletal pain was neither predicted by HPA axis, IMS, and ANS functioning, nor by adverse life events or the interaction between biological stress system function and life events. Therefore, our study does not confirm the hypothesis that biological or psychosocial stress contribute to the maintenance of chronic pain.[33]

Previous cross-sectional studies, including NESDA studies, did indicate modest associations between biological stress systems and chronic multisite musculoskeletal pain.[18,20,34] However, our previous prospective NESDA study did also show no associations between biological stress systems and the onset of chronic multisite musculoskeletal pain.[19] Because concurrent but no prospective associations are found between biological stress systems and the onset or persistence of chronic pain, it appears that biological alterations do not precede chronic pain. Instead, biological alterations might be a consequence of chronic pain (eg, by pain acting as a chronic stressor, or as a result of associated symptoms of chronic pain such as fatigue, sleep disturbance, depressed mood, and lower physical activity levels).[17] Another explanation is that a third factor, such as a genetic factor, induces pain and biological changes.

Table 3. Association* Between Adverse Life Events at Baseline and Improvement of Chronic Multisite Musculoskeletal Pain Over 6-Year Follow-Up (N = 665)

Number of Adverse Life Events	Improvement of Chronic Multisite Pain, Hazard Ratio (95% Confidence Interval)	P
Sociodemographic adjusted†	1.00 (.93–1.09)	.98
Lifestyle and disease adjusted‡	1.02 (.93–1.11)	.71
Depression and anxiety adjusted§	1.02 (.93–1.12)	.65

*Using Cox regression analyses; hazard ratio per 1 additional life event.
†Adjusted for sex, age, and years of education.
‡Additionally adjusted for alcohol intake, smoking, body mass index, number of chronic diseases, and physical activity.
§Additionally adjusted for lifetime diagnoses of depressive and anxiety disorders and use of antidepressants.

This study indicated that also adverse life events did not predict the course of chronic multisite musculoskeletal pain over 6 years. Perhaps life events play a role in the etiology rather than the persistence of chronic pain. In agreement with this, our previous study showed that adverse life events, but not biological stress systems, were associated with a higher risk of developing chronic multisite musculoskeletal pain over 6 years.[19] From a transactional point of view, life events alone will not affect persistence of chronic multisite musculoskeletal pain but rather life events in interaction with psychosocial factors such as stress and distress reactions[35] that are also determined by resilience factors. Moreover, attitudes and concerns about illness and health,[35] avoidance behavior, and catastrophizing cognitions[30] may have an effect on persistence of chronic pain. These factors were not examined in our study.

Some other determinants were indicated to be of importance for improvement of chronic pain. Our finding that older age and a higher number of chronic diseases were associated with persistence of chronic pain concurs with previous research.[7,16,37] Other relevant determinants of persisting chronic pain might be sociodemographic variables such as female sex and less education, as indicated by previous research.[32] Also, health characteristics such as obesity,[37] physical fitness,[17] and additional physical or psychological symptoms[32] have been shown to influence the course of chronic pain. Whereas poor sleep[37] and high levels of

fatigue[32] were previously reported to predict persisting chronic pain, restorative sleep was reported to predict the resolution of chronic pain in another study.[13] Moreover, high psychological distress and illness behavior such as medical care-seeking behavior has been shown to predict chronic pain persistence.[35] It is possible that our findings for biological stress systems were masked by other variables not assessed in our study (eg, by associated symptoms of pain such as fatigue or by genetic variables). Nevertheless, this longitudinal study controlled for the effects of a multitude of relevant sociodemographic, pain-related, health-related, and psychopathological variables.

The number of subjects with improved chronic multisite musculoskeletal pain in our study (46% within 2 years, 63% within 4 years, and 72% within 6 years) appears relatively low compared with other previous studies (44% within 1 year[35]; 65% within 2 years[32]). This lower rate of improvement may be explained by differences in measurement of pain or by a rather high rate of psychological distress[35] in our study because of the NESDA design. Nevertheless, we tested whether psychopathology influenced our findings, as a potential confounder and moderator, and this was not the case.

There are several methodological considerations in our study related to the assessment of biological variables and pain. First, we assessed biological variables in 1 day, whereas HPA axis, ANS, and IMS functioning fluctuates from day to day. Second, data were partially missing for LPS-stimulated inflammation and for HPA axis markers. Persons with missing data on LPS-stimulated inflammation did not differ in basal inflammatory levels and chronic pain from those with LPS data.[18] However, missing data may be slightly differential for HPA axis markers because our subjects collected salivary samples at home.[20] A pilot test has been performed to rule out noncompliance with dexamethasone ingestion (see Vreeburg et al[47,48]). Also, additional missing value analyses showed that all biological data in our sample were indicated to be missing at random. Third, our study did not assess biological function at follow-up. Future studies could elucidate whether changes in biological function over time rather than baseline function determine improvements in chronic pain. Fourth, pain may fluctuate and we assessed pain in the 6 months before the follow-up visits, suggesting that some persons might have been misclassified. Fifth, our study did not measure type of pain, and therefore cannot entirely exclude that some of our findings may not hold for specific types of pain. For example, distinct brain alterations have previously been reported for neuropathic pain, compared with inflammatory pain conditions.[4,38] A final consideration concerns the selection of our primary outcome. We examined improvement of chronic multisite musculoskeletal pain in this study to best profit from the survival data and the large sample size. We were able to study time at risk for an event (ie, improvement) and to censor data at the last recorded follow-up measure if subsequent data were missing or if no event occurred. Also, the Cox regression approach allows inclusion of multiple follow-up time points rather than 1 follow-up time point, as was done in previous studies.[13,37] Our approach may thus decrease the chance of misclassification of subjects. Nevertheless, reduction of symptoms rather than passing a cutoff criterion of chronic multi-site pain may yield somewhat different results. Our classification of chronic multisite musculoskeletal pain was pain in the extremities, the back, and the neck, whereas most previous studies assessed chronic widespread pain (pain in all 4 body quadrants).[51] However, setting broader parameters in studying mechanisms underlying chronic pain might be useful because multisite musculoskeletal pain without meeting the widespread criteria is also associated with severe disability, high pain intensity, and significant psychological distress.[11] Nonetheless, this study has many strong aspects such as the longitudinal design, the large sample size, and the assessment of a wide range of biological markers in relation to chronic pain improvements while adjusting for a large number of covariates. Thus, if dysfunction of biological stress systems would play an important role in the persistence of chronic pain, we should have been able to show this, especially because our cross-sectional findings did indicate biological alterations in chronic multisite musculoskeletal pain.[18,20]

Conclusions

This longitudinal study showed that neither HPA axis, immune, and autonomic function nor adverse life events affect the course of chronic multisite musculoskeletal pain.

Supplementary Data

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.jpain.2016.10.010.

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