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EXTENDED REPORT

Biological stress systems, adverse life events and the onset of chronic multisite musculoskeletal pain: a 6-year cohort study

Ellen Generaal,[1] Nicole Vogelzangs,[1] Gary J Macfarlane,[2] Rinie Geenen,[3] Johannes H Smit,[1] Eco J C N de Geus,[4] Brenda W J H Penninx,[1] Joost Dekker[1,5]

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For numbered affiliations see end of article.

Correspondence to
Ellen Generaal, Department of Psychiatry and EMGO Institute for Health and Care Research, VU University Medical Center, A.J. Ernststraat 1187, room M1.14, Amsterdam 1081 HL, The Netherlands; e.generaal@ggzingeest.nl

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ABSTRACT

Objectives Dysregulated biological stress systems and adverse life events, independently and in interaction, have been hypothesised to initiate chronic pain. We examine whether (1) function of biological stress systems, (2) adverse life events, and (3) their combination predict the onset of chronic multisite musculoskeletal pain.

Methods Subjects (n=2039) of the Netherlands Study of Depression and Anxiety, free from chronic multisite musculoskeletal pain at baseline, were identified using the Chronic Pain Grade Questionnaire and followed up for the onset of chronic multisite musculoskeletal pain over 6 years. Baseline assessment of biological stress systems comprised function of the hypothalamic-pituitary-adrenal axis (1-h cortisol awakening response, evening levels, postdexamethasone levels), the immune system (basal and lipopolysaccharide-stimulated inflammation) and the autonomic nervous system (heart rate, pre-ejection period, SD of the normal-to-normal interval, respiratory sinus arrhythmia). The number of recent adverse life events was assessed at baseline using the List of Threatening Events Questionnaire.

Results Hypothalamic-pituitary-adrenal axis, immune system and autonomic nervous system functioning was not associated with onset of chronic multisite musculoskeletal pain, either by itself or in interaction with adverse life events. Adverse life events did predict onset of chronic multisite musculoskeletal pain (HR per event=1.14, 95% CI 1.04 to 1.24, p=0.005).

Conclusions This longitudinal study could not confirm that dysregulated biological stress systems increase the risk of developing chronic multisite musculoskeletal pain. Adverse life events were a risk factor for the onset of chronic multisite musculoskeletal pain, suggesting that psychosocial factors play a role in triggering the development of this condition.

INTRODUCTION

Chronic pain without a known pathological source may be the consequence of alterations in biological stress systems function resulting in abnormal pain perception.[1 2] A dominant hypothesis suggests that dysregulations of the hypothalamic-pituitary-adrenal (HPA) axis, the immune system (IMS) and the autonomic nervous system (ANS) contribute to initiating chronic pain.[3] This may occur through central sensitisation, a process of hypersensitivity of neural nociceptive pathways.[4] Adverse life events may trigger central sensitisation and aggravate the impact of dysfunction of biological stress systems on chronic pain.[3 5]

Previous studies found impaired HPA axis function, mostly indicated by hypocortisolemia, in chronic pain.[6–10] One prospective study found that a blunted diurnal cortisol rhythm and non-suppression of the HPA axis were associated with new-onset chronic widespread pain among psychologically at-risk subjects.[5] Also, IMS dysfunction has been demonstrated in chronic pain.[11] We recently found unaltered levels of basal inflammatory markers but elevated levels of lipopolysaccharide (LPS)-stimulated inflammatory markers in chronic multisite musculoskeletal pain, possibly indicative of an enhanced innate immune response.[12] Moreover, impaired sympathetic activity and parasympathetic activity of the ANS have been shown in chronic pain,[3 13–15] although we recently showed parasympathetic dysregulations to be associated with the severity, but not the presence of chronic pain.[16] Some cross-sectional studies suggest an association between recent adverse life events and chronic pain.[17 18] One longitudinal study found that experiencing two or more adverse life events (related to relationships, unemployment, illness and financial problems) predicted chronic widespread pain onset.[19] Although longitudinal evidence is limited, trigger incidents, such as adverse life events, in combination with dysregulated biological stress systems have been hypothesised as a risk factor for developing chronic pain.[3 5]

Most previous studies that examined biological stress systems and life events in relation to chronic pain were cross-sectional and did not take relevant confounders such as lifestyle, chronic diseases, depression and anxiety into account. This 6-year longitudinal study examines whether (1) function of biological stress systems (HPA-axis, IMS and ANS), (2) adverse life events, and (3) the co-occurrence of biological function and adverse life events predict the onset of chronic multisite musculoskeletal pain, while adjusting for the aforementioned confounders.

METHODS

Sample

Longitudinal data are from the Netherlands Study of Depression and Anxiety, an ongoing cohort study conducted among 2981 adults (18–65 years at baseline) with and without depression or anxiety[20] (see online supplementary text). Baseline

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data collection took place between 2004 and 2007, with follow-up assessments 2 years, 4 years and 6 years later. Subjects who were free of chronic multisite musculoskeletal pain at baseline (n=2213; see below) were selected. Of these eligible subjects, 2039 subjects had follow-up data on pain available. Included subjects (n=2039) had lower pain intensity and disability scores at baseline (p<0.001), had fewer years of education (p<0.001), had less often a lifetime depressive and/or anxiety disorder (p<0.001) and less often experienced adverse life events (p=0.02), but did not differ in sex, age and biological stress systems function, compared with persons who were lost to follow-up (n=174). From these 2039 subjects, baseline data for HPA axis were available of 1464 subjects (persons using corticosteroids were excluded: n=72), for basal inflammation of 2022 subjects, for LPS-stimulated inflammation of 862 subjects, for ANS of 1951 subjects and for life events of all 2039 subjects.

Onset of chronic multisite musculoskeletal pain

Chronic multisite musculoskeletal pain was defined using the Chronic Pain Grade (CPG).[21] The CPG first inquires about the presence of pain in the prior 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).[22] The subsequent questions in the CPG refer to the most painful location and include: (1) days in pain in the prior 6 months (scale 0–180); (2) pain at this moment (scale 0–10); (3) worst pain in the prior 6 months (scale 0–10); (4) average pain in the prior 6 months (scale 0–10); (5) disability days in the prior 6 months (scale 0–180); (6) disability in daily activities (scale 0–10); (7) disability in spare time, social life and family activities(scale 0–10); and (8) disability in work (scale 0–10). A total pain intensity score was calculated using questions 2, 3 and 4 of the CPG; a total pain disability score was calculated using questions 6, 7 and 8 of the CPG (average of the 0–10 ratings of the three questions multiplied by 10 resulting in a 0–100 score).[21] According to the CPG protocol, five grades were categorised from these measures: grade 0 (pain-free, no pain in the prior 6 months); grade I (low disability, low intensity); grade II (low disability, high intensity); grade III (high disability, moderately limiting); and grade IV (high disability, severely limiting).[21] We defined chronic multisite musculoskeletal pain as grade I, II, III or IV on the CPG and pain present in the extremities, the back, and the neck.[12] Subjects with chronic multisite musculoskeletal pain at baseline were excluded from analyses. Participants were classified as having onset of chronic multisite musculoskeletal pain if they met these criteria at one of the follow-up assessments (2 years, 4 years or 6 years). We further refer to chronic multisite musculoskeletal pain as 'chronic pain'.

Biological stress systems

HPA axis

Saliva samples were collected at home using Salivettes (Sarstedt AG and Co, Numbrecht, Germany) at seven time points within a median of 9 days (IQR=5–22) after the baseline interview.[23] Cortisol analysis was performed by competitive electrochemiluminescence (E170 Roche, Switzerland). The cortisol awakening response included sampling points at awakening, and 30 min, 45 min and 60 min later. Using formulas described by Pruessner *et al*,[24] the area under the curve with respect to the ground (AUCg) and with respect to the increase (AUCi) were calculated based on the four morning cortisol measures. Evening cortisol was averaged over two evening values (22:00 and 23:00). 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 0.5 mg dexamethasone the evening before (directly after the saliva sample at 23:00).

Immune system

Basal inflammatory markers included C reactive protein (CRP), interleukin (IL)-6 and tumour necrosis factor (TNF)-α. Fasting morning blood samples were kept frozen at −80°C. CRP was assayed using a high-sensitivity inhouse ELISA based on purified protein and polyclonal anti-CRP antibodies (Dako, Glostrup, Denmark). IL-6 was assayed using a high-sensitivity ELISA (PeliKine-Compact ELISA, Sanquin, Amsterdam). TNF-α was assayed using a high-sensitivity solid-phase ELISA (Quantikine HS Human TNF-α Immunoassay, R&D systems, Minneapolis, USA). A basal summary index was calculated as the standardised sum of all three standardised ln-transformed basal inflammatory markers.

The innate immune response of 13 cytokines was determined in whole blood that was ex vivo stimulated with LPS (10 ng/mL blood). LPS-stimulated samples were laid flat and incubated at a slow rotation for 5–6 h at 37°C. Plasma was kept frozen at −80°C. Using a multianalyte profile (Human CytokineMAP A V.1.0; Myriad RBM, Austin, Texas, USA), interferon γ, IL-2, IL-4, IL-6, IL-8, IL-10, IL-18, C-C motif chemokine 2 (CCL2), CCL3, CCL4, matrix metalloproteinase 2, TNF-α and TNF ligand superfamily member 1 were determined. An LPS summary index was calculated as the standardised sum of all 13 standardised ln-transformed markers and used in the analyses.[12]

Autonomic nervous system

ANS functioning was assessed unobtrusively during the interview using the VU University Ambulatory Monitoring System (VU-AMS), recording the ECG and changes in thorax impedance from six electrodes placed on the chest and back for a period of ~2 h.[25 26] Breaks and non-stationary moments were removed from the registration. Mean heart rate, pre-ejection period, SD of the normal-to-normal interval (SDNN) and respiratory sinus arrhythmia (RSA) were determined.[16 27]

Adverse life events

The number of adverse life events (0–13) was assessed at baseline using the List of Threatening Events Questionnaire.[28 29] The list assesses 12 recent life stressors in the year preceding the baseline assessment such as death of close friend or relative.[30] We additionally inquired whether subjects experienced any other adverse life event in the past year.

Covariates

Baseline covariates were selected a priori based on previous studies on biological stress systems function and chronic pain. A first set of covariates included sociodemographic variables, cortisol sampling factors (HPA axis analyses),[31] laboratory site (LPS analyses) and respiration rate (RSA analyses).[32] A second set of covariates included several lifestyle and disease factors (body mass index, smoking, alcohol intake,[33] physical activity,[34] chronic somatic diseases, use of anti-inflammatory medication (IMS analyses), and use of β-blocking agents and other heart medication (ANS analyses)). Since depression and anxiety may confound the association between biological variables and pain, the third set of covariates comprised lifetime diagnoses of depressive and anxiety disorders,[35] and use of antidepressants (see online supplementary text for details).

Statistical analyses

Baseline characteristics were compared between subjects with and without onset of chronic 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. Adjusted Cox's regression analyses were used to examine the associations of each biological variable and adverse life events with the onset of chronic pain. Values for evening cortisol, cortisol suppression ratio and basal inflammatory markers were ln-transformed to normalise distributions. Proportional hazards were verified. A potential moderating effect of life events in the association between biological stress systems and chronic pain onset was examined by adding an interaction term (biological variable×number of life events) to each analysis. Since the HPA axis could be hypoactive or hyperactive,[9] non-linear associations between cortisol measures and chronic pain onset were examined by entering quadratic terms of cortisol measures to the model also including the linear terms. Since our previous study found hypocortisolemia particularly among chronic pain subjects without depressive or anxiety disorders,[9] we tested whether lifetime depression and/or anxiety moderated the association between biological stress systems and chronic pain onset by including biological variable×psychopathology (yes/no) interaction terms in the analyses. Follow-up time was included as 2 years, 4 years or 6 years. Subjects were censored in the analysis at the last recorded follow-up.

Three models were tested: (1) adjusted for sociodemographic variables (and cortisol sampling factors, laboratory site and respiration rate); (2) additionally adjusted for lifestyle and disease factors (and use of anti-inflammatory medication, β-blocking agents and other heart medication), and; (3) additionally adjusted for lifetime depression and/or anxiety and use of antidepressants (see covariates).

For all statistical tests, a probability level of ≤5% was regarded as significant. For testing interactions, this level was set at ≤10%. The statistical calculations were performed using SPSS V.20 for Windows (SPSS, Chicago, USA).

RESULTS

Sample characteristics

Of 2039 subjects, 11% developed chronic multisite musculoskeletal pain over 2 years, 17% over 4 years and 21% over 6 years.

Persons who developed chronic pain had higher pain scores at baseline, were significantly older, were more often female, had less years of education, had a higher body mass index, had more chronic diseases, used more often heart medication, had more often a lifetime depressive and/or anxiety disorder, had higher evening cortisol, lower RSA and SDNN, and had more adverse life events at baseline (see table 1 for baseline characteristics).

Biological stress systems

Table 2 reports the association between biological stress systems function and the onset of chronic multisite musculoskeletal pain over 6 years. None of the HPA axis, IMS and ANS variables were associated with the onset of chronic pain after adjustment for covariates. Controlling for age eliminated the significant univariate associations as shown in table 1 (evening cortisol: Δβ=16%; RSA: Δβ=46%; SDNN: Δβ=38%).

No evidence was found for non-linear associations between cortisol measures and chronic pain (all quadratic terms: p>0.10). No moderating effect of depression and/or anxiety was found in the association between any biological variable with the onset of chronic pain (all p-interaction>0.10). We additionally checked whether associations existed for the first cortisol value at awakening and the diurnal cortisol slope (calculated by subtracting the value at 23:00 from the cortisol value at awakening and dividing it by the number of hours in-between the two samples), as these were cross-sectionally associated with chronic pain before,[9] but no such associations were found either. Analyses considering only subjects with chronic pain for ≥90 days (n=215) showed similar HR for all biological variables (p>0.05) but evening cortisol (fully adjusted HR (95% CI) 1.30(1.09 to 1.55); p=0.003). Analyses adjusting for all covariates including pain intensity at baseline showed no significant associations for all biological variables (p>0.05). Additional analyses only including subjects who were largely pain-free at baseline (grade 0 or grade I on CPG and pain in at most two locations; n=831) showed similar non-significant HR for all biological variables (p≥0.10), but AUCg (HR (95% CI) 1.33(1.04 to 1.73); p=0.04).

Adverse life events

Table 3 shows the association between adverse life events and the onset of chronic multisite musculoskeletal pain over 6 years.

An increased number of recent adverse life events was significantly associated with a higher hazard of chronic pain onset before and after adjustment for confounders (fully adjusted: HR per life event=1.14 (95% CI 1.04 to 1.24, p=0.005). Additional analyses considering life events as a categorical variable (0 events (reference, n=1172), 1 event (n=515), 2 events (n=239) and 3 or more events (n=113)) showed that this association with chronic pain onset followed a linear trend (fully adjusted HR=1.13, 1.41 and 1.60 subsequently). When adjusting for all covariates including pain intensity at baseline, we also showed significant associations with chronic pain onset (fully adjusted HR (95% CI) 1.12(1.02 to 1.22), p=0.02). Additional analyses only including largely pain-free subjects at baseline (n=831) showed similar HR, however non-significant (fully adjusted: OR (95% CI) 1.14(0.90 to 1.44), p=0.27).

Figure 1 shows the association between individual adverse life events and the onset of chronic pain. Nearly each life event showed a positive association with chronic pain onset, which were significant for financial problems (5.8%; HR (95% CI) 1.54(1.05 to 2.25); p=0.03), separation from partner (6.1%; HR (95% CI) 1.49(1.02 to 2.18); p=0.04) and other adverse life events (9.3%; HR (95% CI) 1.59(1.18 to 2.14), p=0.003; which included mainly death of others than friend or family (9.9%), problems with relationships (6.0%) and physical/sexual abuse (3.3%)).

Analyses showed no moderating effect of adverse life events in the association between biological stress systems function and chronic pain onset (all interaction terms: p>0.10, with the exception of evening cortisol: p=0.06).

DISCUSSION

This longitudinal study showed that HPA axis, IMS and ANS functioning did not predict the onset of chronic multisite musculoskeletal pain, either as a main effect or in interaction with adverse life events. However, adverse life events by themselves did predict the onset of chronic multisite musculoskeletal pain independently of biological stress systems function, sociodemographics, lifestyle, chronic diseases, depression and anxiety.

In contrast with a dominant hypothesis in the field,[3] our large-scale study including a multitude of biological variables could not confirm that altered function of three major biological

Table 1 Baseline characteristics* comparing subjects with and without onset of chronic multisite musculoskeletal pain over 6 years follow-up

	No onset of chronic pain (n=1608)	Onset of chronic pain (n=431)	p Value†
Pain scores			
Pain intensity	33.0 (19.3)	44.3 (18.4)	<0.001
Pain disability	19.7 (22.7)	29.0 (25.8)	<0.001
Sociodemographic factors			
Age, years	40.4 (13.4)	42.8 (12.4)	0.001
Women, %	62.6	69.6	0.007
Education, years	12.7 (3.2)	12.1 (3.2)	0.001
Sampling factors			
Time of awakening	7:25 (1:01)	7:28 (1:16)	0.33
Working on day of sampling, %	73.9	68.2	0.02
Sampling in month with more daylight, %	68.1	65.9	0.39
≤6 h sleep, %	19.8	29.9	<0.001
Respiration rate, breaths/min	17.1 (1.2)	17.1 (1.1)	0.45
Lifestyle and disease factors			
Body mass index, kg/m^2	25.1 (4.7)	25.9 (5.5)	0.001
Former smoker, %	34.6	34.6	0.99
Current smoker, %	35.6	38.5	0.27
Mild/moderate alcohol drinker, %	60.2	57.1	0.24
Heavy alcohol drinker, %	12.0	10.7	0.45
Physical activity, 1000 MET min/week, median (IQR)	2.9 (1.5–4.8)	3.0 (1.5–4.7)	0.29
Number of chronic diseases	0.43 (0.72)	0.64 (0.93)	<0.001
Anti-inflammatory medication, %	14.9	18.1	0.11
β-blocking agents, %	6.6	9.0	0.08
Other heart medication, %	8.7	13.7	0.002
Depression and anxiety factors			
Lifetime depressive or anxiety disorder, %	71.4	81.9	<0.001
Antidepressant medication, %	21.7	25.8	0.07
Biological stress systems function			
HPA axis			
AUCg, nmol/L/h	18.8 (6.9)	19.6 (7.3)	0.08
AUCi, nmol/L/h	2.1 (6.1)	2.0 (6.7)	0.83
Mean evening level, nmol/L, median (IQR)	4.6 (3.3–6.3)	5.0 (3.5–7.0)	0.02
Cortisol suppression ratio, median (IQR)	2.5 (1.8–3.4)	2.3 (1.8–3.2)	0.22
IMS			
Basal CRP, mg/L, median (IQR)	1.1 (0.5–2.7)	1.2 (0.5–2.8)	0.44
Basal IL-6, pg/mL, median (IQR)	0.7 (0.5–2.6)	0.7 (0.5–1.3)	0.12
Basal TNF-α, pg/mL, median (IQR)	0.8 (0.6–1.1)	0.8 (0.6–1.1)	0.98
Basal summary index	−0.06 (0.99)	0.04 (1.1)	0.07
LPS summary index	−0.02 (0.99)	−0.02 (1.1)	0.98
ANS			
Heart rate, bpm	72 (9.7)	72 (9.9)	0.75
PEP, ms	120 (17.5)	118 (18.4)	0.06
SDNN, ms	69.4 (26.3)	65.9 (23.5)	0.01
RSA, ms	46.1 (26.8)	42.0 (21.7)	0.004
Adverse life events			
Total number of adverse life events	0.6 (0.9)	0.8 (1.1)	0.007

*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. Smoking/alcohol drinking: reference group consists of non-smokers/non-alcohol drinkers. p Values are for illustrative purposes only and are uncorrected for multiple testing.
ANS, autonomic nervous system; AUCg, area under the curve with respect to the ground; AUCi, area under the curve with respect to the increase; CRP, C reactive protein; HPA, hypothalamic-pituitary-adrenal; IL-6, interleukin 6; IMS, immune system; LPS, lipopolysaccharide; MET, one's resting metabolic rate multiplied by minutes of physical activity per week; PEP, pre-ejection period; RSA, respiratory sinus arrhythmia; SDNN, SD of the normal-to-normal interval; TNF-α, tumour necrosis factor α.

stress systems predicts chronic pain development. Only one previous longitudinal study examined the association of biological stress systems function with chronic pain onset. This study showed that HPA axis alterations predicted new-onset chronic widespread pain after 15 months.[5] In that study, a psychosocially at-risk group was selected based on their somatic symptoms and illness behaviour.[5 36] Perhaps these subjects were more vulnerable to HPA axis changes than subjects in our study. The distinct findings might also be explained by measurement differences, for example, in the assessment of pain, depression or cortisol.[37] Several cross-sectional studies showed biological alterations in chronic pain[3 8] also in the Netherlands Study of Depression and Anxiety sample.[9 12] Our current finding of no associations with chronic pain onset might therefore suggest

Table 2 Associations* between biological stress systems function and onset of chronic multisite musculoskeletal pain

	Onset of chronic pain HR (95% CI)	p Value	N†
HPA-axis			
AUCg[a]			1339
Sociodemographic adjusted‡	1.08 (0.97 to 1.21)	0.16	
Lifestyle and disease adjusted§	1.10 (0.98 to 1.24)	0.10	
Depression and anxiety adjusted¶	1.08 (0.97 to 1.22)	0.17	
AUCi[a]			1339
Sociodemographic adjusted‡	0.99 (0.88 to 1.11)	0.81	
Lifestyle and disease adjusted§	1.00 (0.89 to 1.12)	0.98	
Depression and anxiety adjusted¶	0.99 (0.88 to 1.11)	0.80	
Mean evening level[a]			1454
Sociodemographic adjusted‡	1.09 (0.97 to 1.22)	0.14	
Lifestyle and disease adjusted§	1.10 (0.97 to 1.24)	0.14	
Depression and anxiety adjusted¶	1.09 (0.96 to 1.23)	0.19	
Cortisol suppression ratio[a]			1375
Sociodemographic adjusted‡	0.98 (0.87 to 1.09)	0.67	
Lifestyle and disease adjusted§	0.99 (0.88 to 1.11)	0.79	
Depression and anxiety adjusted¶	1.00 (0.89 to 1.12)	0.94	
IMS			
CRP[b]			2016
Sociodemographic adjusted‡	1.00 (0.91 to 1.11)	0.97	
Lifestyle and disease adjusted§	0.95 (0.85 to 1.05)	0.30	
Depression and anxiety adjusted¶	0.94 (0.85 to 1.05)	0.29	
IL-6[b]			2016
Sociodemographic adjusted‡	1.08 (0.98 to 1.18)	0.11	
Lifestyle and disease adjusted§	1.04 (0.94 to 1.14)	0.48	
Depression and anxiety adjusted¶	1.04 (0.95 to 1.15)	0.40	
TNF-α[b]			2002
Sociodemographic adjusted‡	1.00 (0.91 to 1.11)	0.95	
Lifestyle and disease adjusted§	0.98 (0.89 to 1.08)	0.65	
Depression and anxiety adjusted¶	0.98 (0.88 to 1.08)	0.66	
IMS			
Basal summary index[b]			1994
Sociodemographic adjusted‡	1.04 (0.95 to 1.15)	0.39	
Lifestyle and disease adjusted§	0.99 (0.88 to 1.10)	0.78	
Depression and anxiety adjusted¶	0.99 (0.89 to 1.10)	0.82	
LPS summary index[b,c]			862
Sociodemographic adjusted‡	1.15 (0.95 to 1.39)	0.17	
Lifestyle and disease adjusted§	1.11 (0.91 to 1.34)	0.32	
Depression and anxiety adjusted¶	1.08 (0.89 to 1.32)	0.42	
ANS			
Heart rate[d]			1951
Sociodemographic adjusted‡	1.03 (0.94 to 1.13)	0.56	
Lifestyle and disease adjusted§	1.02 (0.92 to 1.12)	0.74	
Depression and anxiety adjusted¶	1.01 (0.92 to 1.12)	0.78	
PEP[d]			1937
Sociodemographic adjusted‡	0.93 (0.84 to 1.03)	0.14	
Lifestyle and disease adjusted§	0.93 (0.84 to 1.02)	0.13	
Depression and anxiety adjusted¶	0.94 (0.85 to 1.04)	0.24	
SDNN[d]			1951
Sociodemographic adjusted‡	0.94 (0.85 to 1.04)	0.22	
Lifestyle and disease adjusted§	0.95 (0.86 to 1.05)	0.34	
Depression and anxiety adjusted¶	0.96 (0.87 to 1.06)	0.44	
RSA[d,e]			1951
Sociodemographic adjusted‡	0.92 (0.83 to 1.03)	0.14	
Lifestyle and disease adjusted§	0.93 (0.84 to 1.04)	0.19	
Depression and anxiety adjusted¶	0.95 (0.85 to 1.06)	0.35	

*Using Cox regression analyses; HR=HR per 1 SD increase; ln-transformed variables were used for evening cortisol, postdexamethasone cortisol and basal inflammatory markers.
†Numbers of subjects do not total 2039 because of exclusion of subjects with missing or incomplete data.
‡Adjusted for sex, age, years of education (and [a] cortisol sampling factors; [c] laboratory site; [e] respiration rate).
§Additionally adjusted for alcohol intake, smoking, body mass index, number of chronic diseases and physical activity (and [b] use of anti-inflammatory medication; [d]use of β blocking agents or other heart medication)
¶Additionally adjusted for lifetime diagnoses of depressive and anxiety disorders and use of antidepressants.
ANS, autonomic nervous system; AUCg, area under the curve with respect to the ground; AUCi, area under the curve with respect to the increase; CRP, C reactive protein; HPA, hypothalamic-pituitary-adrenal; IMS, immune system; LPS, lipopolysaccharide; PEP, pre-ejection period; RSA, respiratory sinus arrhythmia; SDNN, SD of the normal-to-normal interval; TNF-α, tumour necrosis factor α.

Table 3 Association* between adverse life events and onset of chronic multisite musculoskeletal pain (n=2039)

	Onset of chronic pain HR (95% CI)	p Value
Number of adverse life events		
Sociodemographic†	1.16 (1.06 to 1.27)	0.001
Lifestyle and disease‡	1.15 (1.06 to 1.26)	0.002
Depression and anxiety§	1.14 (1.04 to 1.24)	0.005

*Using Cox regression analyses; HR=HR per 1 unit increase.
†Adjusted for sex, age, 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.

that these biological changes are consequences rather than risk factors for developing this condition. Chronic pain itself, by acting as a chronic stressor, with associated symptoms such as sleep disturbance, fatigue, depressed mood, and lower physical activity levels may be the cause of these biological alterations at a later stage.[2]

In line with previous hypotheses[19 38] we demonstrated a significant effect of baseline adverse life events on chronic pain onset. This effect was neither modulated by biological variables, nor by depression or anxiety. This last finding is in contrast to previous studies showing negative life events to be relevant mainly in patients with prior psychiatric history.[17] We found strongest effects for 'another serious life event', which might reflect more a subjective component than the more objective enumeration of the other events (1–12). Perhaps subjects with pain vulnerability are more likely to report negative life experiences.[39 40] However, our findings might also suggest that exposure to psychosocial stress triggers the development of a chronic pain state.

We assessed biological variables in 1 day, whereas HPA axis, ANS and IMS functioning fluctuates from day-to-day. Missing data does not seem differential for LPS-stimulated inflammation,[12] but may be slightly differential for HPA axis markers.[31] Moreover, pain symptoms may fluctuate and therefore some subjects might have been misclassified. Our classification of chronic multisite musculoskeletal pain was pain in the extremities, the back and the neck in the prior 6 months. Previous studies mostly defined chronic widespread pain, a cardinal symptom of the fibromyalgia syndrome, as axial and bilateral pain above and below the waist.[41] With this somewhat 'less stringent' definition, we may have included some patients with milder pain. However, the majority of patients with multisite pain do not fulfil the 'widespread' criteria, but still suffer from high pain intensity, significant psychological distress and severe limitations in daily living.[42] Therefore, setting somewhat broader parameters for studying underlying mechanisms of chronic pain might be useful.[42 43]

Strong aspects of this study are the longitudinal design, the large sample size, and the assessment of a wide range of biological variables in relation to chronic pain onset while adjusting for a number of covariates. Moreover, interaction effects were tested with life events and with psychopathology that are known to influence biological stress systems functioning. Thus, if dysregulated biological stress systems would play an important role in the onset of chronic pain, our study should have been able to show this, especially since our cross-sectional findings did indicate biological alterations in chronic pain.[9 12]

In conclusion, this study does not support the hypothesis that dysregulated biological stress systems precede the development

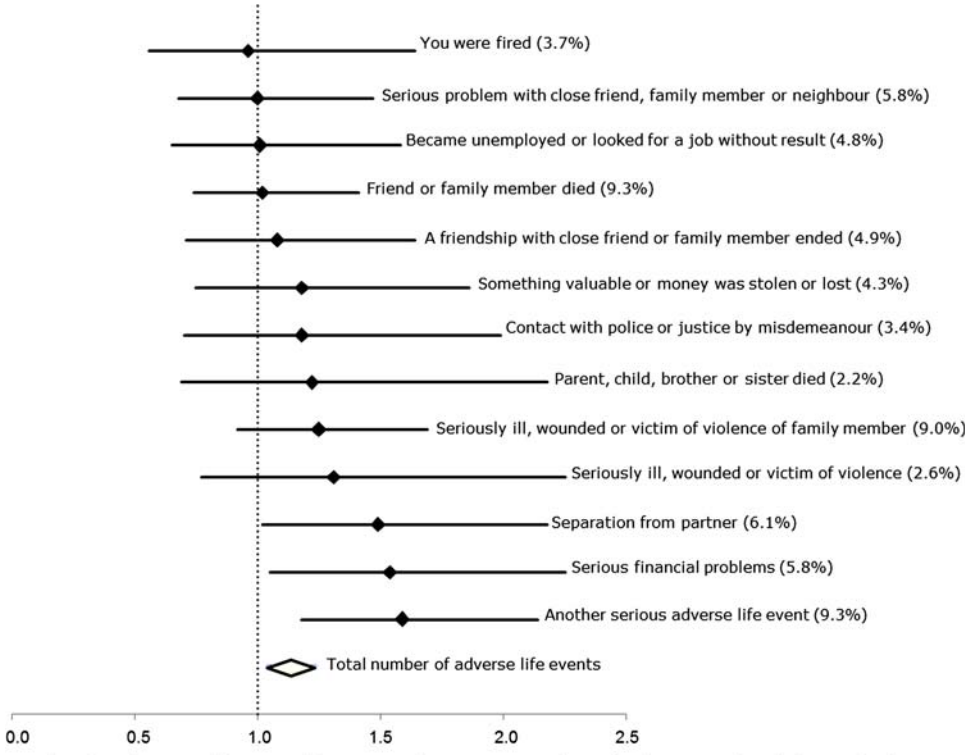

Figure 1 Associations* between each adverse life event and onset of chronic multisite musculoskeletal pain.

Hazard ratio's and 95% CI of the onset of chronic pain after six years. Prevalence of each event is indicated between brackets. *Using Cox's regression analyses separate for each event, adjusted for sex, age, years of education, alcohol intake, smoking, body mass index, number of chronic diseases, physical activity, lifetime diagnoses of depressive and anxiety disorders and use of antidepressants. Total number of adverse life events: HR per 1 additional life event.

of chronic pain, which might suggest that biological changes are consequences rather than causes of the condition. Adverse life events may play a role in triggering the development of chronic pain.

Author affiliations
[1]Department of Psychiatry and EMGO Institute for Health and Care Research, VU University Medical Center, Amsterdam, The Netherlands
[2]Musculoskeletal Research Collaboration (Epidemiology Group), University of Aberdeen, Aberdeen, UK
[3]Department of Clinical and Health Psychology, Utrecht University, Utrecht, The Netherlands
[4]Department of Biological Psychology and EMGO Institute for Health and Care Research, VU University Medical Center, Amsterdam, The Netherlands
[5]Department of Rehabilitation Medicine, VU University Medical Center, Amsterdam, The Netherlands

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REFERENCES

1 Gupta A, Silman AJ. Psychological stress and fibromyalgia: a review of the evidence suggesting a neuroendocrine link. *Arthritis Res Ther* 2004;6:98–106.
2 Adler GK, Geenen R. Hypothalamic-pituitary-adrenal and autonomic nervous system functioning in fibromyalgia. *Rheum Dis Clin North Am* 2005;31:187–202, xi.
3 Maletic V, Raison CL. Neurobiology of depression, fibromyalgia and neuropathic pain. *Front Biosci* 2009;14:5291–338.
4 Woolf CJ. Central sensitization: implications for the diagnosis and treatment of pain. *Pain* 2011;152:S2–15.
5 McBeth J, Silman AJ, Gupta A, *et al*. Moderation of psychosocial risk factors through dysfunction of the hypothalamic-pituitary-adrenal stress axis in the onset of chronic widespread musculoskeletal pain: findings of a population-based prospective cohort study. *Arthritis Rheum* 2007;56:360–71.
6 Heim C, Ehlert U, Hellhammer DH. The potential role of hypocortisolism in the pathophysiology of stress-related bodily disorders. *Psychoneuroendocrinology* 2000;25:1–35.
7 Raison CL, Miller AH. When not enough is too much: the role of insufficient glucocorticoid signaling in the pathophysiology of stress-related disorders. *Am J Psychiatry* 2003;160:1554–65.
8 McBeth J, Chiu YH, Silman AJ, *et al*. Hypothalamic-pituitary-adrenal stress axis function and the relationship with chronic widespread pain and its antecedents. *Arthritis Res Ther* 2005;7:R992–1000.
9 Generaal E, Vogelzangs N, Macfarlane GJ, *et al*. Reduced hypothalamic-pituitary-adrenal axis activity in chronic multi-site musculoskeletal pain: partly masked by depressive and anxiety disorders. *BMC Musculoskelet Disord* 2014;15:227.
10 Tak LM, Cleare AJ, Ormel J, *et al*. Meta-analysis and meta-regression of hypothalamic-pituitary-adrenal axis activity in functional somatic disorders. *Biol Psychol* 2011;87:183–94.
11 Kwok YH, Hutchinson MR, Gentgall MG, *et al*. Increased responsiveness of peripheral blood mononuclear cells to in vitro TLR 2, 4 and 7 ligand stimulation in chronic pain patients. *PLoS One* 2012;7:e44232.
12 Generaal E, Vogelzangs N, Macfarlane GJ, *et al*. Basal inflammation and innate immune response in chronic multisite musculoskeletal pain. *Pain* 2014;155:1605–12.
13 Cohen H, Neumann L, Shore M, *et al*. Autonomic dysfunction in patients with fibromyalgia: application of power spectral analysis of heart rate variability. *Semin Arthritis Rheum* 2000;29:217–27.
14 Raj SR, Brouillard D, Simpson CS, *et al*. Dysautonomia among patients with fibromyalgia: a noninvasive assessment. *J Rheumatol* 2000;27:2660–5.
15 Reyes Del Paso GA, Garrido S, Pulgar A, *et al*. Aberrances in autonomic cardiovascular regulation in fibromyalgia syndrome and their relevance for clinical pain reports. *Psychosom Med* 2010;72:462–70.
16 Barakat A, Vogelzangs N, Licht CM, *et al*. Dysregulation of the autonomic nervous system and its association with the presence and intensity of chronic widespread pain. *Arthritis Care Res (Hoboken)* 2012;64:1209–16.
17 Lampe A, Doering S, Rumpold G, *et al*. Chronic pain syndromes and their relation to childhood abuse and stressful life events. *J Psychosom Res* 2003;54:361–7.
18 Anderberg UM, Marteinsdottir I, Theorell T, *et al*. The impact of life events in female patients with fibromyalgia and in female healthy controls. *Eur Psychiatry* 2000;15:295–301.
19 Gupta A, Silman AJ, Ray D, *et al*. The role of psychosocial factors in predicting the onset of chronic widespread pain: results from a prospective population-based study. *Rheumatology (Oxford)* 2007;46:666–71.
20 Penninx BW, Beekman AT, Smit JH, *et al*. The Netherlands Study of Depression and Anxiety (NESDA): rationale, objectives and methods. *Int J Methods Psychiatr Res* 2008;17:121–40.
21 Von Korff M, Ormel J, Keefe FJ, *et al*. Grading the severity of chronic pain. *Pain* 1992;50:133–49.
22 Von Korff M. Epidemiologic and survey methods: chronic pain assessment. In: Turk DC, Melzack R, eds. *Handbook of pain assessment*, 2nd edn. New York: Guilford Press, 2001:603–18.
23 Vreeburg SA, Hoogendijk WJ, van PJ, *et al*. Major depressive disorder and hypothalamic-pituitary-adrenal axis activity: results from a large cohort study. *Arch Gen Psychiatry* 2009;66:617–26.
24 Pruessner JC, Kirschbaum C, Meinlschmid G, *et al*. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology* 2003;28:916–31.
25 de Geus EJ, Willemsen GH, Klaver CH, *et al*. Ambulatory measurement of respiratory sinus arrhythmia and respiration rate. *Biol Psychol* 1995;41:205–27.
26 Willemsen GH, de Geus EJ, Klaver CH, *et al*. Ambulatory monitoring of the impedance cardiogram. *Psychophysiology* 1996;33:184–93.
27 Licht CM, de Geus EJ, Zitman FG, *et al*. Association between major depressive disorder and heart rate variability in the Netherlands Study of Depression and Anxiety (NESDA). *Arch Gen Psychiatry* 2008;65:1358–67.
28 Brugha T, Bebbington P, Tennant C, *et al*. The List of Threatening Experiences: a subset of 12 life event categories with considerable long-term contextual threat. *Psychol Med* 1985;15:189–94.
29 Brugha TS, Cragg D. The List of Threatening Experiences: the reliability and validity of a brief life events questionnaire. *Acta Psychiatr Scand* 1990;82:77–81.
30 Spinhoven P, Elzinga BM, Hovens JG, *et al*. The specificity of childhood adversities and negative life events across the life span to anxiety and depressive disorders. *J Affect Disord* 2010;126:103–12.
31 Vreeburg SA, Kruijtzer BP, van Pelt J, *et al*. Associations between sociodemographic, sampling and health factors and various salivary cortisol indicators in a large sample without psychopathology. *Psychoneuroendocrinology* 2009;34:1109–20.
32 Grossman P, Kollai M. Respiratory sinus arrhythmia, cardiac vagal tone, and respiration: within- and between-individual relations. *Psychophysiology* 1993;30:486–95.
33 Babor TF, Higgins-Biddle JC, Saunders JB, *et al*. *The alcohol use disorders identification test: guidelines for use in primay care*. Geneva, Switzerland: World Health Organization, 2001.
34 Ainsworth BE, Bassett DR Jr, Strath SJ, *et al*. Comparison of three methods for measuring the time spent in physical activity. *Med Sci Sports Exerc* 2000;32:S457–64.
35 Robins LN, Wing J, Wittchen HU, *et al*. The Composite International Diagnostic Interview. An epidemiologic Instrument suitable for use in conjunction with different diagnostic systems and in different cultures. *Arch Gen Psychiatry* 1988;45:1069–77.
36 McBeth J, Macfarlane GJ, Benjamin S, *et al*. Features of somatization predict the onset of chronic widespread pain: results of a large population-based study. *Arthritis Rheum* 2001;44:940–6.

37 Hansen AM, Garde AH, Persson R. Sources of biological and methodological variation in salivary cortisol and their impact on measurement among healthy adults: a review. *Scand J Clin Lab Invest* 2008;68:448–58.
38 Skillgate E, Vingard E, Josephson M, *et al*. Life events and the risk of low back and neck/shoulder pain of the kind people are seeking care for: results from the MUSIC-Norrtalje case-control study. *J Epidemiol Community Health* 2007;61:356–61.
39 De Moja CA. Causality, linearity, and circularity of relations between stressful life events and pain experience. *Percept Mot Skills* 1995;80:85–6.
40 McBeth J, Morris S, Benjamin S, *et al*. Associations between adverse events in childhood and chronic widespread pain in adulthood: are they explained by differential recall? *J Rheumatol* 2001;28:2305–9.
41 Wolfe F, Smythe HA, Yunus MB, *et al*. The American College of Rheumatology 1990 Criteria for the Classification of Fibromyalgia. Report of the Multicenter Criteria Committee. *Arthritis Rheum* 1990;33:160–72.
42 Carnes D, Parsons S, Ashby D, *et al*. Chronic musculoskeletal pain rarely presents in a single body site: results from a UK population study. *Rheumatology (Oxford)* 2007;46:1168–70.
43 Davies HT, Crombie IK, Macrae WA. Where does it hurt? Describing the body locations of chronic pain. *Eur J Pain* 1998;2:69–80.

Biological stress systems, adverse life events and the onset of chronic multisite musculoskeletal pain: a 6-year cohort study

Ellen Generaal, Nicole Vogelzangs, Gary J Macfarlane, Rinie Geenen, Johannes H Smit, Eco J C N de Geus, Brenda W J H Penninx and Joost Dekker

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