Original Experimental

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Experimental partial-night sleep restriction increases pain sensitivity, but does not alter inflammatory plasma biomarkers

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Abstract

Objectives – Disturbed sleep and chronic pain are public health concerns. Sleep disturbances seem to influence inflammation and may contribute to the increased pain sensitivity after sleep restriction (SR), such as after night work. The primary objective of this study was to determine the effects of SR on pain sensitivity and on relevant markers of inflammation. A secondary objective was to determine if SR affected pain sensitivity and inflammatory responses differently in men and women.

Methods – A paired crossover design with block randomization was applied. Subjects were instructed to follow their habitual sleep (HS) rhythm for two nights (HS condition) and to delay their bedtime to shorten their sleep with 50% for two nights (SR condition). Thirty-nine healthy volunteers between 19 and 44 years old participated (21 women). Experimental pain sensitivity was tested with heat-, electrical-, and pressure pain thresholds (PPTs); electrical temporal summation threshold; pinprick pain; suprathreshold heat pain tolerance; and rating of suprathreshold heat and cold pain. The following markers of inflammation were measured in plasma from a blood sample taken between 10:00 and 12:00: C-reactive protein, fractalkine, tumor necrosis factor, interleukin -8, and monocyte chemoattractant protein-1.

Results – Most subjects did not comply with the SR instructions. Total sleep time during SR was on average 2.6 h shorter than during HS. Therefore, the SR condition was re-defined to be "at least 40% reduction in the time in bed (TIB) the last night." The HS condition was re-defined to "at least 85% of normally reported TIB." SR produced higher suprathreshold heat pain sensitivity and cold pressor pain, compared to HS, but no significant change in electrical pain threshold, electrical temporal summation threshold, PPT, or any of the measured immune parameters. Sex-stratified analyses indicated that the effect on heat pain only occurred in women and that the effect on cold pressor pain was significant only in men.

Conclusions – The present findings indicate that heat and cold pressor pain were rated higher following SR, whereas pain thresholds remained unchanged. We did not find an effect of SR on biomarkers of inflammation. The findings should be cautiously interpreted given the poor adherence to the SR condition.

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1 Introduction

Sleep disturbances and chronic pain are comorbid disorders that constitute major public health concerns. Several longitudinal studies indicate that disturbed sleep is associated with a higher risk for musculoskeletal pain conditions [1–5]. Micro-longitudinal studies of the day-to-day associations between sleep and pain have found causal interaction between short sleep duration and pain complaints [6–8]. Finally, several experimental sleep restriction (SR) studies have reported that disturbed sleep (total or partial SR or sleep fragmentation) reduces pain thresholds and elevate the subjective response to experimentally

induced suprathreshold pain stimuli [9–14]. A recent systematic review and meta-analysis supported detrimental effects of sleep disturbance on pain sensitivity in healthy individuals [15]. Another recent systematic review suggested that the hyperalgesic effect of sleep disturbance was greater on females than on males [16].

Plasma cytokines and chemokines are biomarkers of potential inflammatory mechanisms that may explain the sleep–pain association [17]. SR may dysregulate immune function by amplifying proinflammatory mediators at night and in the early morning, when cortisol is at its lowest. Acute total and short-term partial sleep deprivation have been demonstrated to result in elevated levels of high-sensitivity C-reactive protein (CRP) [18], a marker of the "acute-phase response" in response to an inflammatory stimulus [19]. Notably, the sleep hormone melatonin that peaks in the evening, reduces interleukin (IL)-1, IL-6, IL-8, and tumor necrosis factor (TNF) levels, though it has no significant effect on CRP [20]. A systematic review found evidence of an association between short sleep duration and higher levels of CRP [21]. TNF seems to increase after experimental sleep deprivation [22,23].

In addition to CRP and TNF, a range of chemokines seem to be associated with sleep loss and may play a role in the associations between sleep and pain [24]. There seems to be an interplay between sleeplessness and CRP on risk of chronic musculoskeletal pain [25]. Proinflammatory cytokines such as IL-6 and TNF were associated with enhanced heat pain sensitivity also in a forced awakening randomized clinical trial [23]. Restricting sleep to 4 h a night for 10 days resulted in elevated levels of IL-6, and this increase was strongly associated with an increase in pain ratings [26]. IL-6 is also produced in muscle, which may influence its levels [27]. Sleep disturbances further exacerbate inflammatory signaling. Studies of obstructive sleep apnea patients have found associations with elevated serum fractalkine, IL-8, fractalkine (C-X3-C motif chemokine ligand 1 [CX3CL1]), and monocyte chemoattractant protein 1 (MCP-1; CCL2), compared to controls [28–30]. In mild obstructive sleep apnea, cytokines such as IL-1β, IL-6, and TNF-α also exhibit time-dependent fluctuations, with significantly higher levels in the morning (post-sleep) compared to the evening (pre-sleep) [31]. In chronic insomnia, there seems to be an imbalance of pro-inflammatory cytokines, including interferon gamma (IFN-γ) [32].

Most of the studies on SR and inflammatory markers are studies with strict control of sleep conditions in a sleep laboratory. The present study aimed to apply SR intervention on top of real-life sleep conditions. Hence a protocol with self-administered SR at home, combined with experimental testing of pain sensitivity in the laboratory, was designed. This is of relevance to shift workers, who experience partial sleep loss.

Several studies have reported that men and women differ in responses to pain with women showing higher pain sensitivity and lower pain tolerance [33,34]. Recently, a systematic review found that sex moderates the effect of sleep disturbance on heat pain [16]. Despite evidence of sex differences in both experimental and clinical pain, relationships between sex, sleep loss, and pain remains unclear. Hence, the present study also determined if the associations between SR and pain sensitivity differed between men and women.

The primary objectives of the present study were to determine the effect of SR on (i) pain sensitivity and (ii) pain-relevant markers of inflammation. A secondary objective was to determine if sex moderated these effects.

2 Methods

2.1 Subjects

Forty-one subjects (18–45 years) were recruited through advertisements at nearby universities.

Participants were healthy according to self-report. We sought to exclude subjects with conditions that are known to affect pain responses or regular sleep. Exclusion criteria were chronic somatic pain (pain intensity ≥3/10 lasting ≥3 months during the last 2 years); moderate or heavy headache ≥2 days per month on average; history of psychiatric, neurological, heart, or lung disease; regular use of medication including over-the-counter analgesics; pregnancy; breast feeding; diagnosed sleep disorder; shift work with regular night shifts; Epworth sleepiness scale score ≥11 [35]; Pittsburg sleep quality index (PSQI) score ≥7 [36], and consummation of any alcohol in the last 24 h. In addition, we excluded subjects with a history of allergic reaction to salt-rich creams that were used with electroencephalography recordings (results not reported in this manuscript).

Two subjects withdrew after the pretesting session. One subject withdrew after the first experimental session but was still included in the analysis. The remaining 39 participants (21 women, 18 men) were between 19 and 44 years old (mean ± SD; 25 ± 6 years).

2.2 Design

The study was a paired cross-over design with two experimental sessions with different sleep conditions (Figure 1),

	Week 1							Week 2						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	P				HS/SR		E					HS/SR		E
Diary	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Actigraphy	→													

Figure 1: Design and sleep measurements. P: pre-test session. E: experimental session, testing pain sensitivity in the laboratory and blood sampling. HS/SR: planned habitual sleep or sleep restriction the two nights before testing in randomized order. Diary: smartphone-based sleep diary. Actigraphy: continuous (24/7) actigraphy measurements over 3 weeks. Note: day numbers are unrelated to calendar days.

comparing a session after two consecutive nights with habitual sleep (HS) with a session after two consecutive nights with 50% SR (block randomized order). Individual 50% sleep length was calculated based on HS patterns assessed by PSQI. Based on an instructed wake-up time at 07:00 for all subjects, individual bedtimes were calculated for the SR condition. Figure 1 shows an overview of the design and sleep measurements.

2.3 Instructions on sleeping

Subjects were given written sleep instructions: "The last two nights before the lab experiment, you should (if HS condition) sleep your usual sleep duration, e.g., 8 h and therefore go to bed at, e.g., 23:00 both tomorrow night and the day after tomorrow, or (if SR condition) sleep half your normal sleep length, i.e., 4 h and therefore go to bed at 03:00. Please get up at 07:00 both tomorrow and on the day of the lab experiment." Hence, sleep instruction was based on time in bed (TIB).

2.4 Sleep measurements

Subjects slept at home and sleep was monitored by a smartphone-based sleep diary [37], and by wrist actigraphy on the non-dominant arm (AX3, Axivity Ltd, Newcastle, UK) from 1 week before until 1 week after each experimental session (omgui.sourceforge.net) [38].

2.5 Procedure

Subjects were familiarized to the experimental procedures approximately 1 week before the first experimental session. The experimental sessions were identical except for the sleep condition, lasted for 150 min, and took place approximately 1 week apart (Figure 1). Each participant was tested by the same female experimenter, who was blinded with respect to the sleep condition. Experiments started between 08:00 and 09:00. Subjective sleepiness was rated on the Karolinska Sleepiness Scale (KSS) [39]. Objective alertness was measured as response speed (inverse response time) of the 10 min psychomotor vigilance test (PVT) [40] (custom-written C++ program, STAMI, Norway), before the experimental pain stimuli. The inverse response time has been shown to be sensitive to sleep loss [40]. At the end of each experimental session blood and saliva samples were collected.

2.6 Experimental pain stimuli

A series of standardized pain-sensitivity tests were delivered: contact heat pain, pinprick pain, electrical pain, cold pressor pain, and pressure pain. Cutaneous heat stimuli (3 × 3 cm Pathway ATS Thermode, Medoc Ltd, Ramat Yishai, Israel) of increasing intensity were delivered to the volar side of the forearm until the *heat-pain threshold* (HPT) and *heat-pain tolerance threshold* was reached (repeated 3× and averaged). Suprathreshold heat pain (*pain6)* was determined analogous to Granot et al. to the other forearm [41] until the temperature that equaled a visual analogue scale (VAS) rating of 6/10 cm (pain6; 4–9 cm was accepted).

Pinprick pain was delivered by a MCR pinprick stimulator (MRC systems Heidelberg, Germany), which consist of a flat contact area of 0.25 mm diameter. A series of 20 stimuli (force 256 mN) was delivered at 10–15 s inter-stimulus-interval [10]. Each stimulus was rated on a 0–10 VAS (0 indicating "no pain" and 10 indicating "most intense pain imaginable").

Electrical pain was delivered by an electrode consisting of two felt pads soaked in NaCl strapped to the skin overlying the tibia bone with a Velcro strap. The pain detection threshold in milliamperes (mA) was assessed three times by delivering stimuli of ascending intensity from "no sensation" until the first sensation of pain. The average of the three pain thresholds was used. Temporal summation was determined as the mA value corresponding to the fifth stimulus in a series of five electrical pain stimuli (2 Hz) being painful (NRS > 0).

Cold pressor pain was assessed by immersing one hand into a 7°C water bath for 2 min, with fingers spread [14]. The pain was indicated on a 0–10 numerical rating scale at 30 s intervals with the same end points as VAS.

Pressure was induced by a handheld pressure algometer with a probe size of 1 cm^2 (Force One FDIX,

Wagner Instruments, Greenwich, CT, USA) to both trapezius muscles, at 1/3 the distance along an imaginary line between the C7 spinous process and the acromion (rate 50 kPa/s), until the pressure pain threshold (PPT) was reached. More details of the experimental pain stimuli have been published [42].

2.7 Blood samples

Venous blood was collected using BD Vacutainer® Plasma Preparation Tubes (BD PPT™, 9.0 mg K_2EDTA, BD, Franklin Lakes, USA) after completion of the experimental procedures (between 10:00 and 12:00). Tubes were inverted immediately on a tilt tray and centrifuged for 10 min at 1,500 × *g*. After centrifugation, plasma was immediately transferred into cryo tubes, frozen, and stored until analysis at −80°C.

2.8 Luminex immunoassay analysis of inflammatory markers

We chose to explore a standard panel with the most common inflammatory markers for pain to elucidate whether their potential as mediators between sleep and pain [43–45]. Plasma samples were analyzed using magnetic beads pre-coupled with antibodies for the detection of human CRP (eBioscience™ ProcartaPlex Human CRP Simplex, Bender MedSystems GmbH, Vienna, Austria). The following inflammatory markers were analyzed in plasma: epithelial neutrophil-activating protein 78 (ENA-78), IFN-γ, interleukins (IL1-β, IL-4, IL-6, IL-8, IL-10), MCP-1, TNF, and fractalkine (CX3CL1) (Bio-Plex Pro™ Human Chemokine Assay, Bio-Rad Laboratories, Hercules, CA, USA). Prior to incubation with beads, plasma samples were diluted 1:500 for the CRP assay, and 1:4 for the remaining. Assays were analyzed on a MAGPIX™ Multiplex Reader (Luminex Corporation, Austin, TX, USA) using Bio-Plex Manager MP Software, and inflammation marker concentrations were obtained from analysis of raw data using Bio-Plex Data Pro software (Bio-rad Laboratories, Inc., USA).

2.9 Statistical analysis

Sleep condition was the independent variable (HS vs SR). Sleep measures (hour in bed, total sleep time (TST), sleepiness, and alertness) were compared between sleep conditions with Wilcoxon non-parametric paired *t*-test. Dependent variables were concentrations of inflammatory markers, HPT, heat pain tolerance threshold, PPT, and pain6 and were analyzed in mixed model analyses with random intercept and unstructured covariance structure. In exploratory analyses, sex-stratified analyses were performed. The criterion for statistical significance was $p < 0.05$, two-tailed. Statistical analyses were done in Stata 18 (StataCorp, College Station, Texas, USA).

3 Results

3.1 Sleep measurements

Most of the participants did not comply with the 50% SR during the last two nights preceding the experiment (Table 1). Hence, we re-defined the inclusion criteria slightly. First, from the original “last two nights before the experiment” to “the last night before the experiment.” Second, instead of the PSQI-reported hours of sleep per night being the reference, the median measured TIB over the 3-week recording period (Figure 1) was calculated as a reference for the individual’s HS length. The revised SR condition

Table 1: Number of subjects with original (two last nights before experiment) and revised (last night before experiment) SR criteria, e.g., eight subjects would have been included in the SR condition, if the original criterion of SR had been kept. With the revised criteria, 28 subjects were included in the SR condition

	HS	SR	
	≥85% of median TIB for normal sleep	≥50% reduction of TIB	≥40% reduction of TIB
Two last nights before experiment	23[a]	8[a]	18
Last night before experiment	26[b]	15	28[b]

HS: habitual sleep; SR: sleep restriction; TIB: time in bed. [a]Criteria of the original experimental design/protocol. [b]Revised criteria used in the analyses.

was then defined as ≥40% reduction of TIB the last night before the experimental testing, whereas the revised HS condition as ≥85% of TIB the last night before the experimental testing, both referenced to the measured median TIB.

Table 2 shows the sleep characteristics of the participants included in the study; 26 participants met the criteria the night before the HS experimental session and 28 participants met the criteria the last night before the SR experimental session. Twenty-one participants met the criteria before both experimental sessions. The 33 participants that met the criteria for at least one of the sleep conditions were included in the analysis. Of the 33 included participants, 19 were women and 14 men. Only data from sessions in agreement with the criteria described above were used. Compared to the HS condition, average TIB and TST were significantly shorter in the SR condition. TST during SR was on average 2.6 h shorter than during HS. Subjective and objective sleepiness, measured by KSS and PVT response speed, respectively, showed that subjects were significantly sleepier after SR, compared to after HS.

3.2 Pain sensitivity and inflammatory markers

Participants were more sensitive to suprathreshold heat pain stimuli and cold pressor pain after SR. Both the heat pain tolerance threshold and the threshold for pain6 was lower after SR, compared to after HS (Table 3). However, pinprick pain, electrical pain threshold, electrical temporal summation threshold, PPT, or HPT did not differ between sleep conditions. We also analyzed data using an intention to treat approach (including subjects that did not adhere to the SR protocol according to the defined criteria), i.e., including all the original 39 participants. The differences between sleep conditions for heat pain tolerance threshold and pain6 were still significant using this approach (data not shown).

Of the biomarkers, only CRP, fractalkine, TNF, IL-8, and MCP-1 were analyzed statistically, as the remaining biomarkers were below the detection threshold. There were no statistically significant differences between sleep conditions for any of the analyzed inflammation markers in plasma (Table 3).

3.3 Sex-stratified analyses

Exploratory analyses addressed whether the effect of SR on pain sensitivity differed between men and women. For women there was a statistically significant reduction in pain6 and heat pain tolerance threshold after SR (p = 0.004 and p = 0.033, respectively), indicating increased pain sensitivity (Table S1, Supplementary material), whereas cold pressor pain was rated higher after SR in men (Table S2, Supplementary material).

The relationship between SR and inflammation markers was also examined with sex-stratified analyses. The results did not show any differences in inflammation markers after SR for either women or men (Tables S1 and S2, Supplementary material).

4 Discussion

The results indicated that partial SR increased heat pain and cold pressor pain ratings, whereas pinprick pain and pain thresholds for pressure, electrical, or heat pain, did not change compared with HS, for 33 healthy men and women. Partial SR was in this study operationalized as at least 40% reduction of self-reported TIB the preceding night. There was no difference in the measured inflammatory markers between sleep conditions. Some sex differences were observed, effects on pain6 (the temperature that equaled VAS rating of 6/10 cm) and heat pain tolerance threshold after SR were seen in women, but not in men;

Table 2: Sleep characteristics in HS and SR of the 33 participants included in the study

	HS		SR		
	Mean	**95% CI**	**Mean**	**95% CI**	***p*-value**
TIB (h)	7.40	(7.11, 7.69)	3.69	(3.53, 3.85)	0.0017
TST (h)	5.82	(5.46, 6.18)	3.08	(2.87, 3.29)	0.0008
Subjective sleepiness (KSS), 1–9	3.15	(2.62, 3.68)	6.79	(6.42, 7.15)	0.0001
PVT, response speed (s^{-1})	3.02	(2.92, 3.12)	2.83	(2.71, 2.95)	0.0057

TIB: time in bed (wake-up time minus lights off), actigraph-validated. TST: total sleep time (TIB minus sleep onset latency and awakenings after sleep onset). KSS: Karolinska sleepiness scale (1–9). Response speed was measured by the 10 min PVT. *p*-values are from Wilcoxon Signed Rank sum test.

Table 3: Results from the analysis of experimental pain stimuli and inflammation markers after HS and SR for the 33 participants

	HS (n = 26)		SR (n = 28)		
Experimental pain stimuli	**Mean**	**95% CI**	**Mean**	**95% CI**	***p*-value**
PPT (kPa)	27.5	(21, 33.9)	24.3	(19.8, 28.7)	0.403
HPT (°C)	42.4	(41.5, 43.4)	41.9	(40.9, 42.8)	0.095
Pain6 (°C)	46.2	(45.6, 46.7)	45.8	(45, 46.6)	**0.004**
Heat pain tolerance threshold (°C)	48.8	(48.2, 49.5)	48.4	(47.5, 49.3)	**0.044**
Electrical pain threshold (mA)	6.2	(5, 7.5)	5.1	(4.1, 6.1)	0.15
Electrical temp. summ. threshold (mA)	3.3	(2.5, 4)	2.8	(2.1, 3.6)	0.61
Cold pressor pain (NRS)	8.2	(7.6, 8.8)	8.7	(8.3, 9.2)	**0.016**
Pinprick pain (VAS)	0.31	(0.1, 0.5)	0.68	(0, 1.4)	0.316
Inflammatory markers					
CRP (mg/L)	0.65	(0.37, 0.92)	0.59	(0.32, 0.86)	0.723
Fractalkine (pg/mL)	118	(103, 133)	109	(96, 122)	0.96
TNF (pg/mL)	6.05	(5.09, 7.02)	5.59	(4.95, 6.23)	0.242
MCP-1 (pg/mL)	19.5	(16.5, 22.6)	19.8	(16, 23.6)	0.691
IL-8 (pg/mL)	4.35	(3.78, 4.92)	3.91	(3.52, 4.3)	0.272

Inflammation markers analyzed in blood plasma sampled in conjunction with experimental pain stimulation: CRP, C-reactive protein; fractalkine (CX3CL1), C-X3-C motif chemokine ligand 1; TNF, tumor necrosis factor; MCP-1, monocyte chemoattractant protein-1; IL-8, interleukin-8.
Bold values are significant at the $p < 0.05$ level.

and cold pressor pain was rated higher after SR in men, but not in women.

The increased sensitivity to experimental suprathreshold heat pain after SR is in line with several previous studies [9,16,42,46,47]. We did not find SR-related increased sensitivity to pressure pain. Heat and pressure pain detection thresholds were not affected by SR in the present study and this is in line with findings by Neverdahl et al. [48].

Our results indicate that the increased heat pain sensitivity after SR was limited to women. Eichhorn et al. found that HPTs decreased in women, but not men, after one night of total sleep deprivation [49]. This is in line with a recent systematic review with meta-analysis that concluded that the hyperalgesic effect of sleep disturbance is greater on females than on males, and that sex-based differences should be considered when investigating the effects of sleep disturbance on pain sensitivity [16]. The reductions in suprathreshold heat pain (pain6) and heat pain tolerance for women after SR in this study were, although statistically significant, quite modest [49].

In a study of effects of 24 h total sleep deprivation on perceived pain during a 2 min *cold pressor test*, Larson and Carter found no differences between men and women [50]. The type of pain test (cold pressor test vs heat pain test) and the type of SR (24 h total sleep deprivation vs ≥40% reduction in HS) may explain the discrepancies between the studies. The effect of partial SR on pain sensitivity may be weaker in men, and this study may not have enough power to show an effect on men. The speculation that sex differences may vary across outcome measures is further supported by other experimental studies [49,51] and a recent review [52], indicating that females may exhibit sleep-related impairment in endogenous pain modulation, but not men.

Our results indicate that one night of approximately 40% SR is not sufficient to produce pronounced changes in the circulating inflammation markers measured in this study. Other studies have found changes in inflammation after SR; however, the results vary. Lower IL-8 levels were recorded in firefighters who had a 4 h sleep each night, when compared to participants who had 8 h sleep opportunity each night for 3 days and 2 nights of simulated physical firefighting work. However, no change in IL-6 and TNF was found [53]. Sauvet et al. found a decrease in TNF and an increase in MCP-1, after 6 days of SR with 4 h sleep opportunity, but no change in IL-8 or IL-6 [54]. Chennaoui et al. found that 34–36 h of total sleep deprivation induced a significant increase in TNF plasma levels but had no effect on IL-6 and CRP plasma levels [22]. A study of nurses by Bjorvatn et al. found that short sleep duration (<6 h) was associated with lower levels of IL-1 and higher levels of TNF [55]. CRP levels were higher after 4.2 h sleep for 10 consecutive days compared to 8.2 h sleep [18]. That many inflammatory mediators follow a diurnal pattern may also have affected our findings. Proinflammatory cytokines, including IFN-γ, TNF, and IL-1β, peak during the night and early morning, coinciding with the lowest plasma cortisol levels [56].

Activation of inflammatory systems is a potential mediator of sleep and pain relationships, as immune responses

may moderate nociceptive pathways. Inflammatory systems are complex with a plethora of signals acting on a large repertoire of receptors. Likewise, sensory systems and pain encompass many types of receptors and neurons at many levels and pathways in the central nervous system. Hence, cytokines may play many roles in pain perception [57]. A systematic review and meta-analysis of cohort studies and experimental sleep deprivation found that sleep disturbance and long sleep duration (>7–8 h), but not short sleep duration, are associated with increases in several markers of inflammation [21]. This could indicate that it is not short sleep duration *per se* that influences inflammation, and that sleep quality may be equally important. Heffner et al. found that poorer sleep quality was associated with higher IL-6 levels and pain in patients with chronic lower back pain [58]. Inflammation may link short or disturbed sleep to chronic pain; however, causality is yet to be demonstrated [59]. However, the present study found only small and statistically insignificant effects.

4.1 Strengths and limitations

The paired crossover design is a strength of the present study. The study also included both subjective and objective measures of sleep. The experimenter administering the tests was blinded with respect to the sleep condition. Another strength is that standard pain stimulation tests were used. The SR was self-administered and done at home and assessed by actigraphy, which are both a strength and a limitation. It is a strength because the participant is in his/her natural environment. Stricter follow-up may have improved compliance with the SR protocol but could have led to a limitation because too much influence would have been imposed on voluntary participation.

A limitation in this study is that only 33 participants were included in the analysis due to lack of compliance with the SR protocol. Participants were instructed to sleep 50% of their HS time by extending their bedtime; however, this was apparently difficult for the participants as only 15 participants managed to reduce their sleep time by 50%. This finding illustrates the challenges of home-based self-administered SR. A cutoff at 40%, and not 50% as instructed, was chosen to allow some flexibility if subjects went to bed, e.g., 10 min too early. Due to lack of compliance, it was not possible to restrict inclusion to two full nights of SR, hence some of the participants only had one night's SR. We used per-protocol instead of intention-to-treat analysis. This may introduce a bias as participants that were unable to adhere to protocol were excluded from the analysis. The mean reduction in TST is only 2.6 h for the SR compared to the HS the night before testing.

A major limitation is the small group size and lack of power, particularly in the sex stratified analyses. However, we did record clear-cut effects on sleepiness (KSS) and PVT. Hence, the SR did produce substantial effects. Future studies should have adequate statistical power to do separate analyses in both men and women.

Partial SR for only one night is probably not sufficient to cause changes in inflammation and pain sensitivity at least in men, although some complied with the original protocol and got two nights partial SR. The time frame of 10:00–12:00 for blood sample collection is somewhat broad, given the known diurnal fluctuations in inflammation markers in healthy individuals [56]. The main cause of the variable blood sampling time was variable duration of the experiment, particularly caused by the time it took to obtain correct montage of the EEG electrodes.

However, the fact that we found increased heat pain sensitivity in women after SR highlights the important relationship between sleep and pain and may indicate that women are more vulnerable than men for consequences of SR. We did not synchronize SR and testing with menstrual cycle which influences several components of inflammation. Hence, we cannot rule out that testing took place on days at different cycle phases that cancel out potential effects of SR. Experimental pain sensitivity has been found to vary across the menstrual cycle [60].

The present findings indicate that suprathreshold measures of pain sensitivity are more sensitive to SR than pain thresholds. Sensitivity analyses suggested that the increased pain sensitivity to heat pain was limited to the female participants. Whether low-grade inflammation may explain the association between pain and disturbed sleep is still unknown, but we did not find an effect of SR on our selected biomarkers of inflammation. The findings should be cautiously interpreted given the poor adherence to the SR condition.

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Research ethics: Research involving human subjects complied with all relevant national regulations, institutional policies and is in accordance with the tenets of the Helsinki Declaration (as amended in 2013), and has been approved by the authors' equivalent regional research ethical committee (approval no. 2012/199).

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References

[1] Sivertsen B, Lallukka T, Salo P, Pallesen S, Hysing M, Krokstad S, et al. Insomnia as a risk factor for ill health: results from the large population-based prospective HUNT Study in Norway. J Sleep Res. 2014;23(2):124–32.

[2] Canivet C, Ostergren PO, Choi B, Nilsson P, Sillen U, Moghadassi M, et al. Sleeping problems as a risk factor for subsequent musculoskeletal pain and the role of job strain: results from a one-year follow-up of the Malmo Shoulder Neck Study Cohort. Int J Behav Med. 2008;15(4):254–62.

[3] Kaila-Kangas L, Kivimaki M, Harma M, Riihimaki H, Luukkonen R, Kirjonen J, et al. Sleep disturbances as predictors of hospitalization for back disorders – a 28-year follow-up of industrial employees. Spine. 2006;31(1):51–6.

[4] Gupta A, Silman AJ, Ray D, Morriss R, Dickens C, MacFarlane GJ, et al. The role of psychosocial factors in predicting the onset of chronic widespread pain: results from a prospective population-based study. Rheumatology (Oxford, England). 2007;46(4):666–71.

[5] Skarpsno ES, Nilsen TIL, Hagen K, Mork PJ. Long-term changes in self-reported sleep quality and risk of chronic musculoskeletal pain: the HUNT study. J Sleep Res. 2021;30(6):e13354.

[6] Katsifaraki M, Nilsen KB, Christensen JO, Waersted M, Knardahl S, Bjorvatn B, et al. Sleep duration mediates abdominal and lower-extremity pain after night work in nurses. Int Arch Occup Environ Health. 2019;92(3):415–22.

[7] Katsifaraki M, Nilsen KB, Christensen JO, Wærsted M, Knardahl S, Bjorvatn B, et al. Pain complaints after consecutive nights and quick returns in Norwegian nurses working three-shift rotation: an observational study. BMJ Open. 2020;10(9):e035533.

[8] Alsaadi SM, McAuley JH, Hush JM, Lo S, Lin CW, Williams CM, et al. Poor sleep quality is strongly associated with subsequent pain intensity in patients with acute low back pain. Arthritis Rheumatol. 2014;66(5):1388–94.

[9] Schuh-Hofer S, Baumgartner U, Treede RD. Effect of sleep deprivation on the electrophysiological signature of habituation to noxious laser stimuli. Eur J Pain. 2015;19(8):1197–209.

[10] Schuh-Hofer S, Wodarski R, Pfau DB, Caspani O, Magerl W, Kennedy JD, et al. One night of total sleep deprivation promotes a state of generalized hyperalgesia: a surrogate pain model to study the relationship of insomnia and pain. Pain. 2013;154(9):1613–21.

[11] Matre D, Hu L, Viken LA, Hjelle IB, Wigemyr M, Knardahl S, et al. Experimental sleep restriction facilitates pain and electrically induced cortical responses. Sleep. 2015;38(10):1607–17.

[12] Faraut B, Leger D, Medkour T, Dubois A, Bayon V, Chennaoui M, et al. Napping reverses increased pain sensitivity due to sleep restriction. PLoS One. 2015;10(2):e0117425.

[13] Tiede W, Magerl W, Baumgartner U, Durrer B, Ehlert U, Treede RD. Sleep restriction attenuates amplitudes and attentional modulation of pain-related evoked potentials, but augments pain ratings in healthy volunteers. Pain. 2010;148(1):36–42.

[14] Matre D, Knardahl S, Nilsen KB. Night-shift work is associated with increased pain perception. Scand J Work Environ Health. 2017;43(3):260–8.

[15] Chang JR, Fu S-N, Li X, Li SX, Wang X, Zhou Z, et al. The differential effects of sleep deprivation on pain perception in individuals with or without chronic pain: a systematic review and meta-analysis. Sleep Med Rev. 2022;66:101695.

[16] Rouhi S, Topcu J, Egorova-Brumley N, Jordan AS. The impact of sleep disturbance on pain perception: a systematic review examining the moderating effect of sex and age. Sleep Med Rev. 2023;71:101835.

[17] Babiloni AH, De Koninck BP, Beetz G, De Beaumont L, Martel MO, Lavigne GJ. Sleep and pain: recent insights, mechanisms, and future directions in the investigation of this relationship. J Neural Transm. 2020;127:647–60.

[18] Meier-Ewert HK, Ridker PM, Rifai N, Regan MM, Price NJ, Dinges DF, et al. Effect of sleep loss on C-reactive protein, an inflammatory marker of cardiovascular risk. J Am Coll Cardiol. 2004;43(4):678–83.

[19] Morley JJ, Kushner I. Serum C-reactive protein levels in disease. Ann N Y Acad Sci. 1982;389(1):406–18.

[20] Cho JH, Bhutani S, Kim CH, Irwin MR. Anti-inflammatory effects of melatonin: a systematic review and meta-analysis of clinical trials. Brain Behav Immun. 2021;93:245–53.

[21] Irwin MR, Olmstead R, Carroll JE. Sleep disturbance, sleep duration, and inflammation: a systematic review and meta-analysis of cohort studies and experimental sleep deprivation. Biol Psychiatry. 2016;80(1):40–52.

[22] Chennaoui M, Sauvet F, Drogou C, Van Beers P, Langrume C, Guillard M, et al. Effect of one night of sleep loss on changes in tumor necrosis factor alpha (TNF-α) levels in healthy men. Cytokine. 2011;56(2):318–24.

[23] Irwin MR, Olmstead R, Bjurstrom MF, Finan PH, Smith MT. Sleep disruption and activation of cellular inflammation mediate heightened pain sensitivity: a randomized clinical trial. Pain. 2023;164(5):1128–37.

[24] Cook AD, Christensen AD, Tewari D, McMahon SB, Hamilton JA. Immune cytokines and their receptors in inflammatory pain. Trends Immunol. 2018;39(3):240–55.

[25] Skarpsno ES, Mork PJ, Nilsen TIL, Steingrímsdóttir ÓA, Zwart JA, Nilsen KB. The interplay between sleeplessness and high-sensitivity

C-reactive protein on risk of chronic musculoskeletal pain. Longitudinal data from the Tromsø Study. Sleep. 2019;42(9):zsz127.
[26] Haack M, Sanchez E, Mullington JM. Elevated inflammatory markers in response to prolonged sleep restriction are associated with increased pain experience in healthy volunteers. Sleep. 2007;30(9):1145–52.
[27] Tingstad RH, Norheim F, Haugen F, Feng YZ, Tunsjø HS, Thoresen GH, et al. The effect of toll-like receptor ligands on energy metabolism and myokine expression and secretion in cultured human skeletal muscle cells. Sci Rep. 2021;11(1):24219.
[28] Afsar GC, Oruc O, Sarac S, Topçuoğlu ÖB, Salturk C, Tepetam FM, et al. Fractalkine in obstructive sleep apnea patients. Sleep Breath. 2017;21(2):355–9.
[29] Li X, Hu R, Ren X, He J. Interleukin-8 concentrations in obstructive sleep apnea syndrome: a systematic review and meta-analysis. Bioengineered. 2021;12(2):10650–65.
[30] Kim J, Lee CH, Park CS, Kim BG, Kim SW, Cho JH. Plasma levels of MCP-1 and adiponectin in obstructive sleep apnea syndrome. Arch Otolaryngol – Head Neck Surg. 2010;136(9):896–9.
[31] Yang H, Engeland CG, King TS, Sawyer AM. The relationship between diurnal variation of cytokines and symptom expression in mild obstructive sleep apnea. J Clin Sleep Med. 2020;16(5):715–23.
[32] Aghelan Z, Karima S, Abtahi SH, Khazaie H, Salimi Y, Khodarahmi R. The imbalance of circulating pro-inflammatory cytokines interleukin-12 and interferon gamma and anti-inflammatory cytokine interleukin-1ra in chronic insomnia disorder. Sleep Med. 2023;112:194–6.
[33] Pieretti S, Di Giannuario A, Di Giovannandrea R, Marzoli F, Piccaro G, Minosi P, et al. Gender differences in pain and its relief. Ann Ist Super Sanita. 2016;52(2):184–9.
[34] Bartley EJ, Fillingim RB. Sex differences in pain: a brief review of clinical and experimental findings. Br J Anaesth. 2013;111(1):52–8.
[35] Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep. 1991;14(6):540–5.
[36] Buysse DJ, Reynolds 3rd CF, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res. 1989;28(2):193–213.
[37] Carney CE, Buysse DJ, Ancoli-Israel S, Edinger JD, Krystal AD, Lichstein KL, et al. The consensus sleep diary: standardizing prospective sleep self-monitoring. Sleep. 2012;35(2):287–302.
[38] Borazio M, Berlin E, Kücükyildiz N, Scholl P, Van Laerhoven K, eds. Towards benchmarked sleep detection with wrist-worn sensing units. 2014 IEEE International Conference on Healthcare Informatics (ICHI). IEEE; 2014.
[39] Åkerstedt T, Gillberg M. Subjective and objective sleepiness in the active individual. Int J Neurosci. 1990;52(1–2):29–37.
[40] Basner M, Dinges DF. Maximizing sensitivity of the psychomotor vigilance test (PVT) to sleep loss. Sleep. 2011;34(5):581–91.
[41] Granot M, Weissman-Fogel I, Crispel Y, Pud D, Granovsky Y, Sprecher E, et al. Determinants of endogenous analgesia magnitude in a diffuse noxious inhibitory control (DNIC) paradigm: do conditioning stimulus painfulness, gender and personality variables matter? Pain. 2008;136(1–2):142–9.
[42] Matre D, Andersen MR, Knardahl S, Nilsen KB. Conditioned pain modulation is not decreased after partial sleep restriction. Eur J Pain. 2015;20(3):408–16.
[43] Moen G-H, Moen A, Schistad EI, Gjerstad J. Local up-regulation of interferon-γ (IFN-γ) following disc herniation is involved in the inflammatory response underlying acute lumbar radicular pain. Cytokine. 2017;97:181–6.
[44] DeVon HA, Piano MR, Rosenfeld AG, Hoppensteadt DA. The association of pain with protein inflammatory biomarkers: a review of the literature. Nurs Res. 2014;63(1):51–62.
[45] Huo LW, Ye YL, Wang GW, Ye YG. Fractalkine (CX3CL1): a biomarker reflecting symptomatic severity in patients with knee osteoarthritis. J Investig Med. 2015;63(4):626–31.
[46] Ødegård SS, Omland PM, Nilsen KB, Stjern M, Gravdahl GB, Sand T. The effect of sleep restriction on laser evoked potentials, thermal sensory and pain thresholds and suprathreshold pain in healthy subjects. Clin Neurophysiol. 2015;126(10):1979–87.
[47] Ree A, Nilsen KB, Knardahl S, Sand T, Matre D. Sleep restriction does not potentiate nocebo-induced changes in pain and cortical potentials. Eur J Pain. 2020;24(1):110–21.
[48] Neverdahl JP, Uglem M, Matre D, Hansen JO, Engstrøm M, Tronvik E, et al. Pain thresholds and suprathreshold pain after sleep restriction in migraine – a blinded crossover study. Cephalalgia. 2022;42(6):110–80.
[49] Eichhorn N, Treede RD, Schuh-Hofer S. The role of sex in sleep deprivation related changes of nociception and conditioned pain modulation. Neuroscience. 2018;387:191–200.
[50] Larson RA, Carter JR. Total sleep deprivation and pain perception during cold noxious stimuli in humans. Scand J Pain. 2016;13:12–6.
[51] Haack M, Engert LC, Besedovsky L, Goldstein MR, Devine JK, Dang R, et al. Alterations of pain pathways by experimental sleep disturbances in humans: central pain-inhibitory, cyclooxygenase, and endocannabinoid pathways. Sleep. 2023;46(6). doi: 10.1093/sleep/zsad061.
[52] Herrero Babiloni A, Brazeau D, Jodoin M, Theis-Mahon N, Martel MO, Lavigne GJ, et al. The impact of sleep disturbances on endogenous pain modulation: a systematic review and meta-analysis. J Pain. 2024;25(4):875–901.
[53] Wolkow A, Ferguson SA, Vincent GE, Larsen B, Aisbett B, Main LC. The impact of sleep restriction and simulated physical firefighting work on acute inflammatory stress responses. PLoS One. 2015;10(9):e0138128.
[54] Sauvet F, Drogou C, Bougard C, Arnal PJ, Dispersyn G, Bourrilhon C, et al. Vascular response to 1 week of sleep restriction in healthy subjects. A metabolic response? Int J Cardiol. 2015;190:246–55.
[55] Bjorvatn B, Axelsson J, Pallesen S, Waage S, Vedaa O, Blytt KM, et al. The association between shift work and immunological biomarkers in nurses. Front Public Health. 2020;8:415.
[56] Cutolo M, Straub RH. Circadian rhythms in arthritis: hormonal effects on the immune/inflammatory reaction. Autoimmun Rev. 2008;7(3):223–8.
[57] Babiloni AH, De Koninck BP, Beetz G, De Beaumont L, Martel MO, Lavigne GJ. Sleep and pain: recent insights, mechanisms, and future directions in the investigation of this relationship. J Neural Transm. 2020;127(4):647–60.
[58] Heffner KL, France CR, Trost Z, Ng HM, Pigeon WR. Chronic low back pain, sleep disturbance, and interleukin-6. Clin J Pain. 2011;27(1):35–41.
[59] Haack M, Simpson N, Sethna N, Kaur S, Mullington J. Sleep deficiency and chronic pain: potential underlying mechanisms and clinical implications. Neuropsychopharmacology. 2020;45(1):205–16.
[60] Fillingim RB, King CD, Ribeiro-Dasilva MC, Rahim-Williams B, Riley III JL. Sex, gender, and pain: a review of recent clinical and experimental findings. J Pain. 2009;10(5):447–85.