

Abnormal immune system response in the brain of women with Fibromyalgia after experimental endotoxin challenge

Christina Mueller^{a,*}, Indonesia Jordan^b, Chloe Jones^b, Prentiss Lawson^c, Jarred W. Younger^a

^a Department of Neurology, Heersink School of Medicine, University of Alabama at Birmingham, Birmingham, AL, USA

^b Department of Psychology, University of Alabama at Birmingham, Birmingham, AL, USA

^c Department of Anesthesiology and Perioperative Medicine, Heersink School of Medicine, University of Alabama at Birmingham, Birmingham, AL, USA

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ABSTRACT

Background: The pathophysiology of fibromyalgia (FM) is thought to include an overactive immune system, leading to central nervous system sensitization, allodynia, and hyperalgesia. We aimed to test this theory using an experimental immune system activation procedure and neuroimaging with magnetic resonance spectroscopic imaging (MRSI).

Methods: Twelve women with FM and 13 healthy women (healthy controls; HC) received 0.3 or 0.4 ng/kg endotoxin and underwent MRSI before and after the infusion. Changes in brain levels of choline (CHO), myo-inositol (MI), N-Acetylaspartate (NAA), and MRSI-derived brain temperature were compared between groups and dosage levels using mixed analyses of variance.

Results: Significant group-by-time interactions in brain temperature were found in the right thalamus. Post-hoc testing revealed that brain temperature increased by 0.55 °C in the right thalamus in FM ($t(10) = -3.483$, $p = 0.006$), but not in HCs ($p > 0.05$). Dose-by-time interactions revealed brain temperature increases in the right insula after 0.4 ng/kg ($t(12) = -4.074$, $p = 0.002$), but not after 0.3 ng/kg ($p > 0.05$). Dose-by-time interactions revealed decreased CHO in the right Rolandic operculum after 0.4 ng/kg endotoxin ($t(13) = 3.242$, $p = 0.006$) but not 0.3 ng/kg. In the left paracentral lobule, CHO decreased after 0.3 ng/kg ($t(9) = 2.574$, $p = 0.030$) but not 0.4 ng/kg. Dose-by-time interactions affected MI in several brain regions. MI increased after 0.3 ng/kg in the right Rolandic operculum ($t(10) = -2.374$, $p = 0.039$), left supplementary motor area ($t(9) = -2.303$, $p = 0.047$), and left occipital lobe ($t(10) = -3.757$, $p = 0.004$), with no changes after 0.4 ng/kg ($p > 0.05$). Group-by time interactions revealed decreased NAA in the left Rolandic operculum in FM ($t(13) = 2.664$, $p = 0.019$), but not in HCs ($p > 0.05$). A dose-by-time interaction showed decreased NAA in the left paracentral lobule after 0.3 ng/kg ($t(9) = 3.071$, $p = 0.013$) but not after 0.4 ng/kg ($p > 0.05$). In the combined sample, there was a main effect of time whereby NAA decreased in the left anterior cingulate ($F[1,21] = 4.458$, $p = 0.047$) and right parietal lobe ($F[1,21] = 5.457$, $p = 0.029$).

Conclusion: We found temperature increases and NAA decreases in FM that were not seen in HCs, suggesting that FM patients may have abnormal immune responses in the brain. The 0.3 and 0.4 ng/kg had differential effects on brain temperature and metabolites, with neither dose effecting a stronger response overall. There is insufficient evidence provided by the study to determine whether FM involves abnormal central responses to low-level immune challenges.

1. Introduction

Fibromyalgia (FM) is a chronic musculoskeletal pain condition with common complaints of fatigue, depressed mood, impaired cognitive performance, and headaches (Wolfe et al., 2016; Vincent et al., 2013). FM affects between 1 and 8% of the general population (Vincent et al.,

2013; Wolfe et al., 1995a; Jones et al., 2015), the majority of whom are women (Wolfe et al., 1995a, 1995b). Abnormal pain processing, central nervous system sensitization, endocrine, and psychosocial factors have all been implicated in FM pathophysiology, but the exact cause is still unknown (Siracusa et al., 2021). One hypothesis is that FM involves abnormal, repeated activation of the immune system in response to

* Corresponding author. CIRC 312, 1719 6th Ave S, Birmingham, AL, 35233, USA.

E-mail address: cm1@uab.edu (C. Mueller).

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unknown threats (Elenkov et al., 2000; Staud, 2015; Meester et al., 2019). Microglia are the resident immune cells of the central nervous system (CNS) tasked with mounting a protective inflammatory response to pathogens both in the brain and the entire body. During neuroinflammation (NI), microglia produce proinflammatory mediators including cytokines, chemokines, and reactive oxygen species aimed at recruiting other immune cells and eliminating pathogens. Although NI is beneficial in the short term, chronic microglial activation may produce many of the classic symptoms associated with FM, such as widespread pain, fatigue, and a general feeling of unwellness (malaise) (Kelley et al., 2003; Dantzer, Kelley; Parnet et al., 2002). It has been demonstrated that FM symptoms, such as pain sensitivity, can be transferred from FM patients to mice via Immunoglobulin G, further supporting the immune system as an important contributor to FM (Goebel et al., 2021). It is still unknown if the pathological trigger in typical FM patients is exogenous or endogenous.

The lipopolysaccharide (LPS) endotoxin makes up most of the outer cell membrane of Gram-negative bacteria. Microglial cells and other immune cells express specialized toll-like receptors (TLRs) that detect pathogens. Systemic endotoxin can enter the brain and bind to TLR-4 on microglia, causing these cells to release pro-inflammatory cytokines such as interleukin (IL)-1 β , IL-6, and tumor necrosis factor (TNF)- α (Hajjar et al., 2002; Morris et al., 2014; Bryant et al., 2010; Mehta et al., 2010). Endotoxin is normally present at very low levels in human blood and does not engender inflammation under those conditions (Nádházi et al., 2002), but levels increase during infection (Glaros et al., 2013), in various diseases (Brenchley et al., 2006; Wiedermann et al., 1999; Raparelli et al., 2017; Zhang et al., 2009; Opal et al., 1999; Manco et al., 2010; Maachi et al., 2004; Terawaki et al., 2010; Emanuele et al., 2010), in aging populations (Glaros et al., 2013), and after consumption of high-fat meals (Erridge et al., 2007).

Endotoxin has been used experimentally for decades to study immune system responses in animal models and humans (Mehta et al., 2010; Jonsjö et al., 2020), revealing that humans are more sensitive to endotoxin than other mammals (Fink, 2014). In humans, administration of endotoxin at the levels used in the current study (0.4 ng/kg) leads to a transient inflammatory response with predictable immune and behavioral consequences, including increased proinflammatory cytokines (e. g., IL-6, TNF- α), increased heart rate, body temperature and white blood cell count, which peaks between 1 and 3 h after administration and resolves within 4 h (Taudorf et al., 2007). Endotoxin infusions at dosages similar to the current study (0.4–0.8 ng/kg) have also been shown to induce allodynia and hyperalgesia in healthy adults (Wegner et al., 2014; Hutchinson et al., 2013/05), and to activate brain microglia in humans (Brown, 2019). Sandiego et al. (2015) used positron emission tomography (PET) with the radioligand [11C]PBR28 to study neuroinflammatory responses to endotoxin. [11C]PBR28 is a ligand for the translocator protein (TSPO) receptor, which is overexpressed on activated microglia. In the study, imaging was performed before and after administration of 1 ng/kg endotoxin in eight healthy volunteers. Imaging revealed 360% increased [11C]PBR28 binding throughout the brain 3 h after endotoxin, indicating microglial activation.

The central aim of the current study was to determine whether FM patients have abnormally sensitive central immune systems compared to healthy controls (HCs) using a low-dose endotoxin immune activation procedure. Whole-brain magnetic resonance spectroscopic imaging (MRSI) was performed before and after immune activation with endotoxin, and differences were assessed between FM patients and controls. MRSI quantifies several brain metabolites related to NI, including choline (CHO, a marker of cellular metabolism that is hypothesized to increase during inflammation), myo-inositol (MI, a glial marker that is hypothesized to increase during inflammation due to glial proliferation), and N-Acetylaspartate (NAA, an axonal marker that may decrease during inflammation).

MRSI-based brain thermometry (MRSt) was also used to detect hyperthermia as a proxy for NI. Increases in brain temperature may

represent increased metabolic demands during NI that overwhelm the brain's cooling mechanisms and cause local temperature increases (Mrozek et al., 2012; Chen et al., 2020/05). Under homeostatic conditions, brain temperature is approximately 0.5–1 °C higher than core body temperature (Henker et al., 1998a), and this balance is maintained to ensure optimal cell functioning. However, during inflammation, brain temperature increases to 1–2 °C above the core body temperature and can vary independently of body temperature (Mrozek et al., 2012; Lunn and Childs, 2012). Unlike PET, MRSI is completely non-invasive and can be performed repeatedly to study brain metabolic changes in response to experimental procedures, without a need to inject radioligands or down-time to allow PET signal decay.

Our hypotheses were that the FM group would show higher increases in brain temperature, CHO, and MI, and greater decreases in NAA, than the HC group. Support for these hypotheses would show that FM involves abnormal central immune responses to low-level inflammatory triggers.

2. Material and methods

2.1. Study design

This study utilized a mixed (group-by-time) design to compare changes in brain metabolites before and after an experimental endotoxin challenge between FM and HCs. Participants underwent MRI 1.5 h before receiving the endotoxin infusion, and 3.5 h after. The following were measured with MRSI in 47 brain regions as primary outcomes: brain temperature, CHO, MI, and NAA.

The study was registered on clinicaltrials.gov (NCT04263454) and involved an experimental immune challenge without random assignment to different conditions or participant blinding. Study procedures were approved by the University of Alabama at Birmingham (UAB) Institutional Review Board and conducted in accordance with the Declaration of Helsinki. Participants provided written informed consent prior to any procedures. A payment of \$50 was provided following completion of the screening visit, and \$600 was provided for the experimental session. Screening and experimental sessions were performed at the Clinical Research Unit (CRU) located at UAB Hospital. Brain imaging was conducted at the UAB Civitan International Imaging Laboratory (CINL). During the experimental session, participants were transported between locations via rideshare.

2.2. Participants

Women with FM aged 18–55 and healthy age-matched women were recruited from the Birmingham community via online advertisements on the UAB Clinical Trials website. We chose to recruit only women because FM disproportionately affects women and our sample size would not have provided the statistical power necessary to analyze sex differences in the endotoxin response. Our laboratory maintains a database of individuals who have indicated an interest in research, from which additional participants were contacted. Participants in the FM group met the revised diagnostic criteria by the American College of Rheumatology (Wolfe et al., 2010, 2016, 2019), endorsed at least eleven out of nineteen positive tender points on a physical exam, and reported an average daily widespread pain severity of at least 6 on a 0–10 scale. HCs reported average daily pain of 1 out of 10 or less. The following exclusion criteria were applied to both groups: i) MRI contraindications; ii) ECG abnormalities (QTc>450ms, QRS>120ms); iii) fever >99.0 °F; iv) hypotension or hypertension (systolic blood pressure outside of 100–140 range, diastolic blood pressure outside of 60–90 range); v) resting heart rate <55bpm; vi) body mass index (BMI) outside of 19.0–39.9 range; vii) current smoker (within 12 months of study); viii) pregnant, planning to become pregnant, or breastfeeding; ix) high alcohol consumption (>1 drink/day or >4 drinks on a single occasion per week); x) use of illicit substances within six months of participation; xi) vaccination within

four weeks of participation; xii) viral or bacterial illness requiring medical attention or antibiotics within three months of participation; xiii) concurrent participation in another research study; xiv) surgical procedure within three months of participating; xv) history of rheumatologic or autoimmune disease (e.g., multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, Hashimoto's thyroiditis); xvi) other major medical conditions (e.g., cancer, blood or clotting disorder, HIV, Hepatitis C infection, diabetes); xvii) regular use of anti-inflammatory or immunomodulatory drugs, xviii); current use of opioid analgesics; xix) Hospital Anxiety and Depression Scale (HADS) (Stern, 2014) depression total score ≥ 13 ; xx) values outside clinical reference ranges on tests of renal function, tri-iodothyronine, thyroxine, thyroid stimulating hormone, complete blood count with differential, and 25-hydroxy vitamin; xxi) detectable rheumatoid factor or antinuclear antibody, xxii) erythrocyte sedimentation rate > 60 mm/h, xxiii) high sensitivity C-reactive protein (hsCRP) > 10 mg/L, and xiv) ongoing litigation or worker's compensation claim.

2.3. Procedures

2.3.1. Screening visit

Initial inclusion and exclusion criteria, including medical history, ACR criteria, HADS score, and MRI safety, were established via brief phone calls. Interested individuals presented in person to the CRU to complete the remaining screening procedures. Participants underwent a urine pregnancy test and ECG. A tender point exam was administered by applying up to 4 kg pressure at a rate of 1 kg per second to the nineteen tender points established by the ACR (Wolfe et al., 2010) with an FPK20 manual algometer (1 cm diameter; Wagner Instruments, Greenwich, CT). Thirty-three cc blood were collected by research nurses and transported to the UAB Hospital laboratory for processing and confirmation of inclusion/exclusion criteria. Participants also completed the Brief Pain Inventory (BPI) (Cleeland and Ryan, 1994) and Fibromyalgia Impact Questionnaire (Bennett, 2005) (FIQ, FM participants only).

2.3.2. Lipopolysaccharide preparation and administration

LPS doses were prepared in the UAB CRU using an aseptic, no-touch technique. LPS (*E. coli* Type 0113 Clinical Center Reference Endotoxin) was manufactured by List Biological Laboratories, Inc. The Lot #94332B1 vials containing 1.0 mcg endotoxin were reconstituted to 1.0mcg/1.0 mL, using sterile water. 0.5 mL was drawn and diluted with 9.5 mL of sterile water to produce a 50 ng/mL solution for dosing. Dosages were drawn at 0.4 ng/kg (FM N = 7; HC N = 7) or 0.3 ng/kg (FM N = 5; HC N = 6) body weight. Finally, the dose was diluted in 30 mL sterile saline for administration.

2.3.3. Experimental session

Participants presented to the UAB CRU and underwent a repeat ECG for safety and were then transported to the CINL for brain imaging. Participants returned to the CRU, where endotoxin was administered via a venous cannula in the antecubital fossa. Doses were infused at a rate of 1 mL/min, with the entire dose given over 30 min.

The endotoxin dose was reduced to 0.3 ng/kg after three FM participants experienced adverse events following 0.4 ng/kg infusions (further information in the Results section). Two standardized meals of 834 kcal and 508 kcal were provided to participants prior to the first MRI session and 1 h after the endotoxin infusion, respectively.

Participants rated their subjective level of fatigue on a scale from 0 (not fatigued) to 10 (severely fatigued) at the following time points relative to the endotoxin infusion: T0 (3.5h before start of endotoxin infusion), T0.5 (start of endotoxin infusion), T1 (0.5h after completion of infusion), T2 (1.5h after completion of infusion), T3 (2.5h after completion of infusion), and T4 (5h after completion of infusion).

In a subset of 4 FM participants and 6 HCs, blood was collected from a venous cannula in the antecubital fossa of the other arm (not used for endotoxin infusion) into EDTA-coated blood collection tubes at the same

time points as fatigue ratings, except that no blood was taken at T0.5 (start of endotoxin infusion). White blood cell count (WBC) with differential was determined immediately after each collection to monitor the systemic immune response to endotoxin.

To control for the effects of circadian rhythms on the study outcomes, participants were scheduled for the sessions at consistent times, arriving between 10:00 and 11:00 a.m. Endotoxin administration took place between 01:30 and 02:30 p.m. and imaging took place within consistent time windows 1.5 h before and 3.5 h after the endotoxin infusion.

2.3.4. Image acquisition

MRI data were collected on a Siemens Magnetom Prisma 3T scanner with 20-channel head coil. Participants laid supine, were provided with hearing protection, and were instructed to keep their eyes closed during the acquisition. A T1-weighted structural brain scan was collected with a magnetization-prepared rapid gradient echo (MPRAGE) sequence with the following parameters: TR: 2000 m, TE: 2.51 m, TI: 901 ms, flip angle: 8°, field-of-view (FoV): 230 × 230 mm, matrix: 256 × 256, 176 slices, 0.9 mm isotropic voxel resolution. Fast 3-dimensional echoplanar spectroscopic imaging (EPSI) with spin-echo excitation was performed following automatic and manual iterative shimming, with the following parameters: TR: 1550 m, TE: 17.6 ms, inversion time (TI) for lipid inversion nulling: 198 m, flip angle: 71°, FoV: 280 × 280 mm, matrix: 50 × 50, 18 slices, 5.6 × 5.6 × 10 mm voxel resolution. The acquisition time for EPSI was 17:48 min. Participants had their body temperature measured in both ears with a Braun Pro 4000 ThermoScan thermometer before and after the MRI.

2.3.5. Image processing

The Metabolite Imaging Data Analysis System (MIDAS) (Maudsley et al., 2006, 2009) was used to process the whole-brain spectroscopy data as previously described (Mueller et al., 2020/06; Mueller et al., 2020; Mueller et al., 2022/04). Preprocessing is fully automated and includes reconstruction of the spectroscopic images, lipid extrapolation and Gibbs ringing correction, corrections for b0 shifts, Eddy current correction, interpolation to 64 × 64 × 32 voxels, smoothing with 5 × 7 mm Gaussian kernel (final voxel size: 5.6 × 5.6 × 5 mm), and automated fitting of the CHO, creatine (CR), MI, and NAA peaks with Gaussian line shapes. Apparent brain temperature was calculated with the following formula: $T_{CR} = -102.61(\Delta_{H2O-CR}) + 206.1$ °C, where Δ_{H2O-CR} is the difference in resonance frequencies between the water and CR peaks (Maudsley et al., 2017/02; Chadzynski et al., 2011). The metabolite and temperature maps were co-registered with the structural images and the structural images were then non-linearly transformed to Montreal Neurological Institute (MNI) space. Finally, the inverse spatial transform was applied to a modified Automated Anatomical Labeling (AAL) atlas (Tzourio-Mazoyer et al., 2002/01) with the PRANA module in MIDAS to obtain atlas regions in subject space. Mean metabolite levels and temperature were extracted from each ROI for statistical analyses.

2.3.6. Statistical analyses

Statistical analyses were conducted with IBM SPSS for Mac version 27. Body temperatures measured in the left and right ear were averaged for each participant. Baseline data were compared between groups using independent-samples t-tests. Pre-scan and post-scan body temperature at the first and second imaging time point were compared separately in the FM and HC group using paired-samples t-tests. For these analyses, $p < 0.05$ (two-tailed) was considered significant.

Brain metabolite levels were expressed as ratios over the local CR concentration. Brain temperatures below 35.0 °C and above 42.0 °C were excluded as an additional data quality control measure, as temperatures outside of this range are not physiological. Mixed analyses of variance (ANOVA) were conducted with time as the within-subjects factor (levels: pre-endotoxin, post-endotoxin) and study group as the between-subject factor (levels: FM, HCs). Endotoxin dose (0.4 ng/kg vs 0.3 ng/kg) and its interaction with time were added as additional

between-subjects factors, and age and the age-by-time interaction were entered as covariates of no interest. The effects of interest were the time-by-group interaction (to test whether FM and HC participants exhibited different responses to endotoxin) and the dose-by-time interaction (to test whether participants receiving 0.3 ng/kg (FM: N = 5; HC: N = 6) versus 0.4 ng/kg (FM: N = 7; HC: N = 7) endotoxin exhibited different responses). Significant interactions were followed up with repeated-measures t-tests assessing the effect of time separately for FM patients and HCs, and with independent-samples t-tests comparing groups at the post-endotoxin time point. The dependent variables were brain temperature, CHO/CR, NAA/CR, and MI/CR in the 47 brain regions. Because of the small sample size and lack of statistical power, all tests were considered significant at $p < 0.05$. However, all tests meeting a more stringent false discovery rate threshold are highlighted. The false discovery rate threshold of 0.05 yielded a corrected p-value threshold of 0.0113.

3. Results

3.1. Participants

Thirty-six participants (FM: N = 20; HC: N = 16) were scheduled and attended the study visit. Seven participants attended the visit but did not receive the endotoxin infusion: one FM participant due to severe headache; one FM patient and one HC due to elevated hsCRP <10 mg/L; one FM patient due to elevated neutrophils; one FM participant due to irregularities on ECG; one HC due to a recent surgery; and one HC due to elevated blood pressure. One FM participant stopped the endotoxin infusion midway due to chest pains and two FM participants experienced adverse events after receiving endotoxin and were withdrawn by the study physician. One FM dataset was excluded due to poor imaging data quality, leaving complete data for 12 FM participants and 13 HCs.

Baseline characteristics are displayed in Table 1. The mean age was 42.6 (SD = 10.2) in the FM group and 41.3 (SD = 10.5) in the HC group, and did not differ between groups ($t(23) = 0.307$, $p = 0.761$). As expected, average self-reported daily pain prior to the study was higher in FM participants (mean = 6.25, SD = 0.45) compared to HCs (mean =

Table 1

Baseline characteristics in the fibromyalgia (FM) and healthy control (HC) groups. Test statistics refer to independent-samples t-tests comparing the group means.

	FM (n = 12)		HC (n = 13)		t	p
	Mean	SD	Mean	SD		
Age (years)	42.6	10.2	41.3	10.5	0.307	0.761
Pain severity (0–10)	6.25	0.45	0.15	0.38	36.782	$<0.001^{***}$
Fatigue severity (0–10)	7.17	1.59	1.00	1.23	10.932	$<0.001^{***}$
Scan 1 (pre-endotoxin)						
pre-scan body temperature (°F)	97.88	0.61	98.43	0.45	-2.561	0.017*
post-scan body temperature (°F)	98.10	0.50	98.32	0.45	-1.136	0.267
Scan 2 (post-endotoxin)						
pre-scan body temperature (°F)	98.8	0.75	98.85	1.10	-0.122	0.904
post-scan body temperature (°F)	98.73	0.64	98.85	0.82	-0.396	0.696
Brief Pain Inventory (BPI)						
Pain Severity	5.23	1.36	0.30	0.47	13.221	$<0.001^{***}$
Pain Interference	5.8	2.23	0.05	0.15	9.956	$<0.001^{***}$
Fibromyalgia Impact Questionnaire (FIQ)	63.64	17.76	-	-		

* $p < 0.05$, *** $p < 0.001$.

0.15, SD = 0.38; $t(23) = 36.782$, $p < 0.001$). Average self-reported fatigue was also higher in the FM group (mean = 7.17, SD = 1.59) compared to HCs (mean = 1.00, SD = 1.23, $t(23) = 10.932$, $p < 0.001$). HCs exhibited higher body temperature (°F) prior to the first MRI (mean = 98.43, SD = 0.45) compared to FM participants (mean = 97.88, SD = 0.61). There were no group differences in average body temperature at either the first MRI or before and after the second MRI. Pre-scan body temperature increased between the first and second MRI in FM ($t(11) = 4.174$, $p = 0.002$) but not in HCs ($t(12) = -1.629$, $p = 0.129$). Post-scan body temperature increased between the first and second MRI in both FM ($t(11) = 2.608$, $p = 0.024$) and HCs ($t(12) = 2.538$, $p = 0.026$).

3.2. Systemic immune response after endotoxin

WBC, lymphocytes, and monocytes before and after the endotoxin infusion are shown in Fig. 1. As can be seen in the figure, there was an increase in WBC and a decrease in lymphocytes and monocytes from baseline levels in both group. The only difference between the groups was a slightly more pronounced decrease in lymphocytes in FM vs. HCs at 1.5 h and 2.5 h after the infusion, which normalized to HC levels at 5 h after the infusion.

Subjective fatigue ratings before and after endotoxin are shown in Fig. 2. Despite different levels of baseline fatigue, increases in average fatigue levels were observed in both groups after the endotoxin infusion.

3.3. Adverse events

Side effects and adverse events were examined each hour during the experimental visit. Ten FM participants and three HCs reported adverse events after receiving endotoxin. The most common events were headaches (N = 8), worsening gastrointestinal complaints (N = 6), dizziness (N = 4), and chills (N = 3). Due to three FM participants experiencing side effects with the 0.4 ng/kg dosage, the study team decided to reduce the dosage by 25% to increase tolerability of the protocol. The three FM patients were further assessed at 24–48 h and 1–2 weeks after study completion. Of these, one patient reported no symptoms 48 h and two weeks post-infusion. Another FM patient reported night sweats and headaches throughout the night after the study day concluded. Twenty-four hours and one week later, she reported no symptoms. The third patient reported a headache of normal severity and tiredness 24 h post-infusion. One week later, she stated that she was feeling better and that it had taken two days for the initial symptoms to stop.

3.4. Changes in brain temperature in response to endotoxin

Significant results including changes in brain temperature are summarized in Table 2. Results from all tested regions of interest are provided in the supplemental materials. There was a significant group-by-time interaction in brain temperature in the right thalamus ($F[1,20] = 6.814$, $p = 0.017$). Post-hoc t-tests revealed that brain temperature increased in the FM group, from 36.54 °C pre-endotoxin to 37.09 °C post-endotoxin administration ($t(10) = 3.483$, $p = 0.006$; see Fig. 3), while brain temperature remained stable at 36.62 °C in HCs ($t(12) = 0.030$, $p = 0.977$). At time point 2, the groups did not significantly differ ($t(27) = 1.962$, $p = 0.06$). Time-by-group interactions in brain temperature were not statistically significant in any other ROIs (all $p > 0.05$).

There were significant dose-by-time interactions in brain temperature in the left precentral gyrus ($F[1,19] = 4.384$, $p < 0.05$), left frontal lobe ($F[1,19] = 4.899$, $p = 0.039$), left Rolandic operculum ($F[1,18] = 4.467$, $p = 0.049$), and right insula ($F[1,19] = 8.673$, $p = 0.008$). Post-hoc repeated-measures t-tests revealed that brain temperature in the right insula did not change in participants receiving the 0.3 ng/kg dose of endotoxin ($t(9) = -1.083$, $p = 0.307$), but increased from 36.55 °C to 37.09 °C (0.54 °C increase, $t(12) = 4.074$, $p = 0.002$) in those receiving the 0.4 ng/kg dose (see Fig. 4). Results from t-tests in the three other

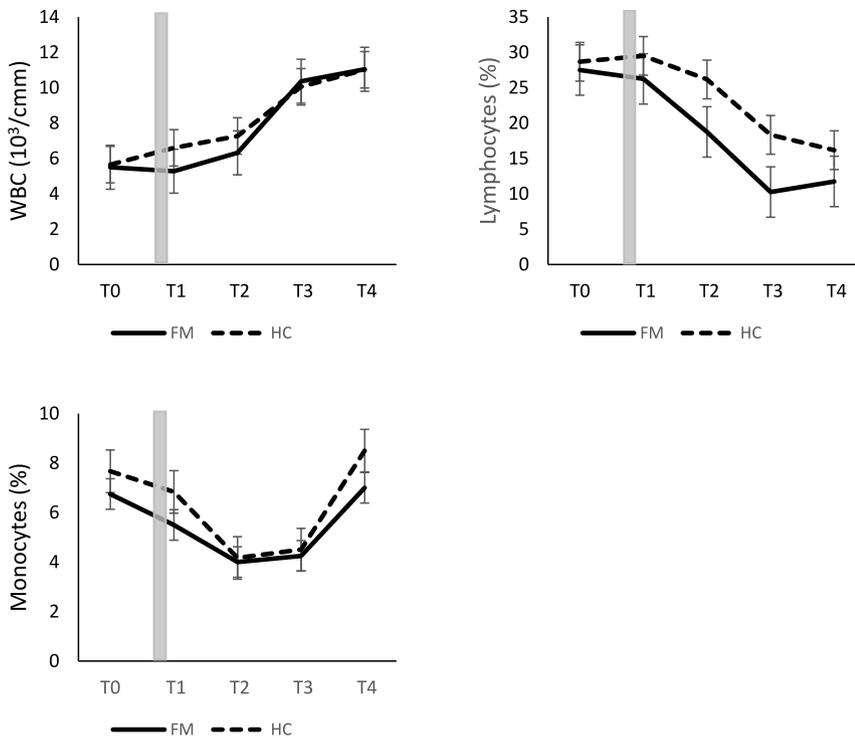


Fig. 1. Mean white blood cell count (WBC), lymphocytes, and monocytes in four fibromyalgia (FM) participants (solid line) and 6 healthy controls (HCs, dashed line) at T0 (3.5h before start of endotoxin infusion), T1 (0.5h after completion of infusion), T2 (1.5h after completion of infusion), T3 (2.5h after completion of infusion), and T4 (5h after completion of infusion). Error bars represent standard errors. Gray bars indicate approximate timing of the 30-min endotoxin infusion. All participants shown received 0.3 ng/kg endotoxin.

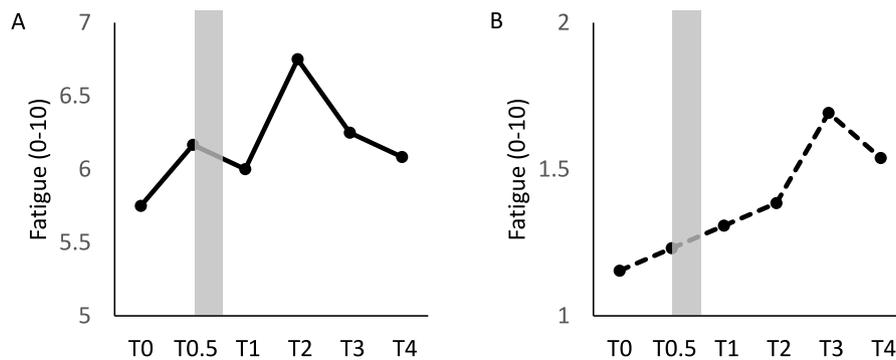


Fig. 2. Mean subjective fatigue ratings before and after the endotoxin infusion (gray bar) in A) Fibromyalgia (N = 12) and B) healthy controls (N = 13). T0 = 3.5h before start of endotoxin infusion, T0.5 = at beginning of infusion, T1 = 0.5h after completion of infusion, T2 = 1.5h after completion of infusion, T3 = 2.5h after completion of infusion), and T4 = 5h after completion of infusion.

brain regions did not reveal significant changes (all $p > 0.05$) in brain temperature. There were no main effects of time on brain temperature in any brain regions that did not show interaction effects (all $p > 0.05$).

3.5. Changes in metabolites in response to endotoxin

3.5.1. Choline

Results from statistical analyses of the metabolite data are summarized in Table 2. There were no significant group-by-time interactions in CHO/CR levels across the brain (all $p > 0.05$). There were significant dose-by-time interactions in the right frontal lobe ($F[1,21] = 6.542, p = 0.018$), right Rolandic operculum ($F[1,21] = 6.278, p = 0.021$), left Rolandic operculum ($F[1,21] = 7.529, p = 0.012$), and left paracentral lobule ($F[1,20] = 4.814, p = 0.040$; Fig. 5). Contrary to our hypotheses, post-hoc repeated-measures t-tests revealed significant decreases in the CHO/CR ratio in the right Rolandic operculum in participants receiving the 0.4 ng/kg dose of endotoxin ($t(13) = -3.242, p = 0.006$) and no

changes in those receiving the 0.3 ng/kg dose ($t(10) = 0.0521, p = 0.613$). There was also a significant decrease of CHO/CR in the left paracentral lobule in those receiving the 0.3 ng/kg dose ($t(9) = -2.574, p = 0.030$) compared to no change in those receiving 0.4 ng/kg ($t(13) = 0.257, p = 0.801$). There were no significant main effects of time in any regions that did not show interaction effects (all $p > 0.05$).

3.5.2. Myo-inositol

There were no significant group-by-time interactions in MI/CR across the tested brain regions (all $p > 0.05$). There were significant dose-by-time interactions in the right Rolandic operculum ($F[1,21] = 5.219, p = 0.033$), left supplementary motor area ($F[1,19] = 6.101, p = 0.023$), left middle cingulate ($F[1,21] = 4.504, p = 0.046$), and left occipital lobe ($F[1,21] = 5.013, p = 0.036$). As can be seen in Fig. 6, post-hoc analyses revealed the expected increase in MI/CR after endotoxin in the group receiving 0.3 ng/kg in the right Rolandic operculum ($t(10) = 2.374, p = 0.039$), left supplementary motor area ($t(9) = 2.303, p =$

Table 2

Group means for brain temperature and metabolites in brain regions showing significant interaction effects or main effects of time.

Temperature (°C)						
		Pre-endotoxin	Post-endotoxin	F	p	Post-hoc tests
Group-by-time						
R Thalamus	FM	36.54	37.09	6.814	0.017*	t(10) = 3.483, p = 0.006** t(12) = 0.030, p = 0.977
	HC	36.62	36.62			
		Pre-endotoxin	Post-endotoxin	F	p	Post-hoc tests
Dose-by-time						
L Precentral	0.3 ng/kg	37.20	36.84	4.384	<0.050*	t(9) = -1.725, p = 0.119 t(12) = 1.248, p = 0.236
	0.4 ng/kg	37.07	37.31			
L Frontal	0.3 ng/kg	37.14	36.85	4.899	0.039*	t(9) = -2.154, p = 0.060 t(12) = 1.308, p = 0.216
	0.4 ng/kg	36.94	37.20			
L Rolandic operculum	0.3 ng/kg	37.16	36.88	4.467	0.049*	t(9) = -1.136, p = 0.285 t(11) = 2.120, p = 0.058
	0.4 ng/kg	37.09	37.43			
R Insula	0.3 ng/kg	37.01	36.72	8.673	0.008 [†]	t(9) = -1.083, p = 0.307 t(12) = 4.074, p = 0.002**
	0.4 ng/kg	36.55	37.09			
CHO/CR						
		Pre-endotoxin	Post-endotoxin	F	p	Post-hoc tests
Dose-by-time						
R Frontal	0.3 ng/kg	0.183	0.166	6.542	0.018*	t(10) = -1.841, p = 0.095 t(13) = 1.503, p = 0.157
	0.4 ng/kg	0.200	0.208			
R Rolandic operculum	0.3 ng/kg	0.190	0.193	6.278	0.021*	t(10) = 0.0521, p = 0.613 t(13) = -3.242, p = 0.006**
	0.4 ng/kg	0.205	0.189			
L Rolandic operculum	0.3 ng/kg	0.176	0.185	7.529	0.012*	t(10) = 2.138, p = 0.058 t(13) = -2.012, p = 0.065
	0.4 ng/kg	0.192	0.183			
L Paracentral lobule	0.3 ng/kg	0.174	0.156	4.814	0.040*	t(9) = -2.574, p = 0.030* t(13) = 0.257, p = 0.801
	0.4 ng/kg	0.170	0.172			
MI/CR						
		Pre-endotoxin	Post-endotoxin	F	p	Post-hoc tests
Dose-by-time						
R Rolandic operculum	0.3 ng/kg	0.537	0.724	5.219	0.033*	t(10) = 2.374, p = 0.039* t(13) = -1.058, p = 0.309
	0.4 ng/kg	0.694	0.591			
L Supplementary Motor Area	0.3 ng/kg	0.266	0.384	6.101	0.023*	t(9) = 2.303, p = 0.047* t(12) = -0.789, p = 0.445
	0.4 ng/kg	0.406	0.388			
L Mid Cingulate	0.3 ng/kg	0.486	0.678	4.504	0.046*	t(10) = 1.798, p = 0.102 t(13) = -0.922, p = 0.373
	0.4 ng/kg	0.569	0.550			
L Occipital	0.3 ng/kg	0.577	0.713	5.013	0.036*	t(10) = 3.757, p = 0.004** t(13) = 0.039, p = 0.969
	0.4 ng/kg	0.684	0.686			
		Pre-endotoxin	Post-endotoxin	F	p	
Time main effects						
L Frontal		0.530	0.614	4.801	0.040*	–
NAA/CR						
		Pre-endotoxin	Post-endotoxin	F	p	Post-hoc tests
Group-by-time						
L Rolandic operculum	FM	1.347	1.226	4.346	<0.05*	t(13) = -2.664, p = 0.019* t(14) = 0.538, p = 0.599
	HC	1.228	1.246			
R Calcarine	FM	1.332	1.331	4.576	0.044*	t(13) = -0.005, p = 0.996 t(14) = 1.811, p = 0.092
	HC	1.297	1.392			
		Pre-endotoxin	Post-endotoxin	F	p	Post-hoc tests
Dose-by-time						
L Paracentral lobule	0.3	1.333	1.237	6.695	0.018*	t(9) = -3.071, p = 0.013* t(13) = -1.407, p = 0.183
	0.4	1.240	1.274			
L Temporal	0.3	1.208	1.281	6.894	0.016*	t(10) = 1.792, p = 0.103 t(13) = -2.125, p = 0.053
	0.4	1.299	1.214			
		Pre-endotoxin	Post-endotoxin	F	p	Post-hoc tests
Time main effects						
L Anterior Cingulate		1.125	1.081	4.458	0.047*	–
R Parietal		1.272	1.251	5.457	0.029*	–

*p < 0.05; **p < 0.01; [†]interaction result survived correction for multiple comparisons.

0.047), and left occipital lobe (t(10) = 3.757, p = 0.004), but not in those receiving the 0.4 ng/kg dose (right Rolandic operculum: t(13) = -1.058, p = 0.309; left supplementary motor area: t(12) = -0.789, p = 0.445; left middle cingulate: t(13) = -0.922, p = 0.373; left occipital lobe: t(13) = 0.039, p = 0.969). There were no significant main effects of time in any regions that did not show interaction effects (all p > 0.05).

3.5.3. N-Acetylaspartate

As can be seen in Fig. 7, NAA/CR showed significant group-by-time interactions in the left Rolandic operculum (F[1,21] = 4.346, p < 0.05) and right calcarine (F[1,21] = 4.576, p = 0.044). In line with our hypotheses, post-hoc t-tests revealed significant decreases in NAA/CR in the left Rolandic operculum in FM participants (t(13) = -2.664, p =

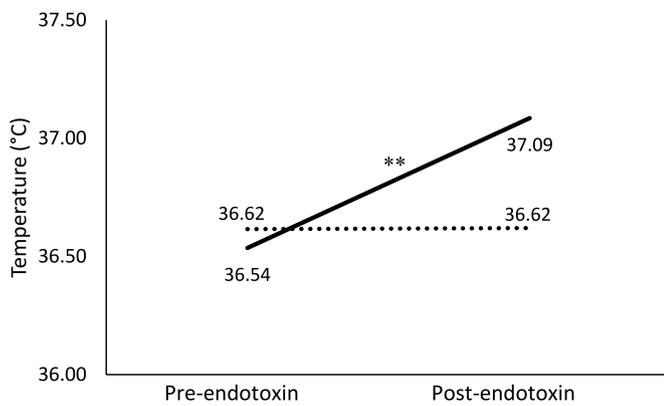


Fig. 3. Changes in brain temperature from pre-endotoxin to post-endotoxin administration in the right thalamus in participants with FM (solid line) and healthy controls (dotted line).

** $p < 0.01$.

0.019), and no changes in HCs ($t(14) = 0.538$, $p = 0.599$). Independent-samples t-tests at the post-endotoxin time point revealed that NAA/CR in the left Rolandic operculum was not different between groups ($t(18.461) = -0.399$, $p = 0.695$). There were no significant changes over time in the right calcarine in either the FM group ($t(13) = -0.005$, $p = 0.996$) or HCs ($t(14) = 1.811$, $p = 0.092$). Independent-samples t-tests revealed no group differences in the right calcarine at post-endotoxin ($t(27) = -0.864$, $p = 0.395$).

NAA/CR also showed significant dose-by-time interactions in the left paracentral lobule ($F[1,20] = 6.695$, $p = 0.018$) and left temporal lobe ($F[1,21] = 6.894$, $p = 0.016$; Fig. 8). Post-hoc tests showed expected significant NAA/CR decreases in the left paracentral lobule in those receiving the 0.3 ng/kg dose of endotoxin ($t(9) = -3.071$, $p = 0.013$) but no significant changes in those receiving the 0.4 ng/kg dose ($t(13) = -1.407$, $p = 0.183$). NAA/CR did not change in the left temporal lobe in those receiving the 0.3 ng/kg dose ($t(10) = 1.792$, $p = 0.103$) or the 0.4 ng/kg dose ($t(13) = -2.125$, $p = 0.053$).

Finally, there were significant main effects of time in the left anterior cingulate, where NAA/CR decreased as expected ($F[1,21] = 4.458$, $p = 0.047$), and in the right parietal lobe, where NAA/CR also decreased ($F[1,21] = 5.457$, $p = 0.029$).

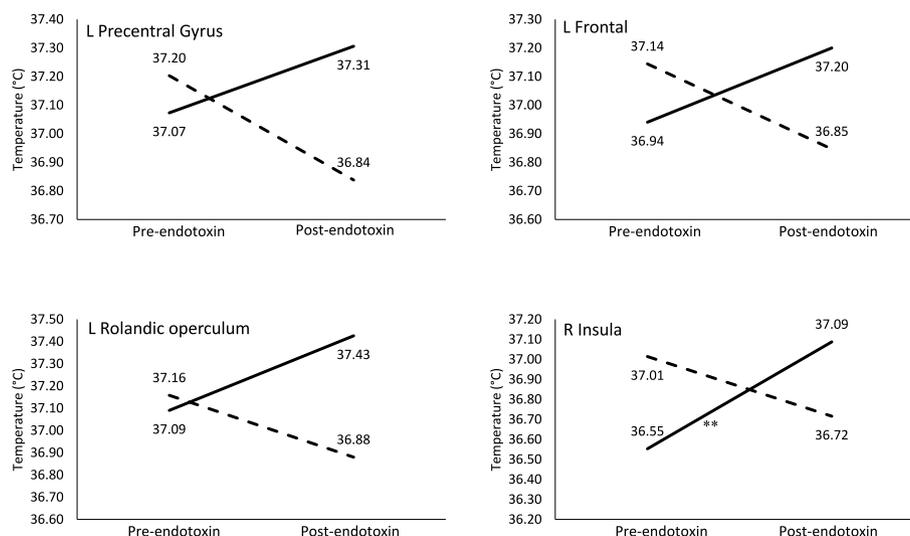


Fig. 4. Significant dose-by-time interactions showing brain temperature changes from pre- to post-endotoxin administration in participants receiving 0.4 kg/kg endotoxin (solid line) versus those receiving 0.3 ng/kg (dashed line). ** $p < 0.01$.

3.6. Stability of MRSt measurements over time

We have previously demonstrated the stability of MRSt-derived brain temperature and metabolites over short time intervals (Sharma et al., 2020; Zhang et al., 2020). However, a large study of traumatic brain injury patients and healthy adults recently demonstrated diurnal brain temperature variations of up to 4.8 °C in healthy adults (range: 36.1–40.9 °C; mean brain temperature $(38.5 \pm 0.4$ °C) (Rzechorzek et al., 2022). Temperature variations appear to be normal and adaptive, since a lack of diurnal rhythms in the traumatic brain injury patients was associated with a 21-fold increase in mortality. To examine the stability of the brain temperature and metabolite measurements in the current study, we examined data in select regions showing group differences from two FM patients and two HCs who were scanned according to the experimental protocol but did not receive endotoxin (Fig. 9). The two FM patients did not receive endotoxin due to ECG abnormalities on the morning of the experimental session, and the HCs were scheduled not to receive endotoxin. As can be seen from the graphs, confidence intervals from scan 1 and scan 2 overlap in all cases, meaning that average temperature and metabolites in the four individuals were stable over time.

4. Discussion

This study was designed to determine if FM involves abnormal central responses to a minor immune challenge. An FM group and HCs were given 0.3 ng/kg or 0.4 ng/kg endotoxin. Brain temperature, MI, CHO, and NAA were assessed before and after the endotoxin administration using a whole-brain MRSI approach. We hypothesized that the FM group would show greater increases of brain temperature, MI, and CHO, and significant decreases of NAA after endotoxin. In general, we did not observe the hypothesized group differences in brain metabolites.

The most significant group difference observed was brain temperature in the right thalamus. In that region, the FM group showed an exaggerated increase of temperature after endotoxin administration compared to HCs. Increased brain temperature in this area may indicate an abnormally increased inflammatory response. We have previously observed elevated brain temperature in myalgic encephalomyelitis/chronic fatigue syndrome in the thalamus, a condition frequently comorbid with FM (Mueller et al., 2020). This region has also been shown to have decreased neuronal volume in individuals with greater FM severity (Liu et al., 2022). The brain temperature increases in the FM group coincided with increases in body temperature. Although

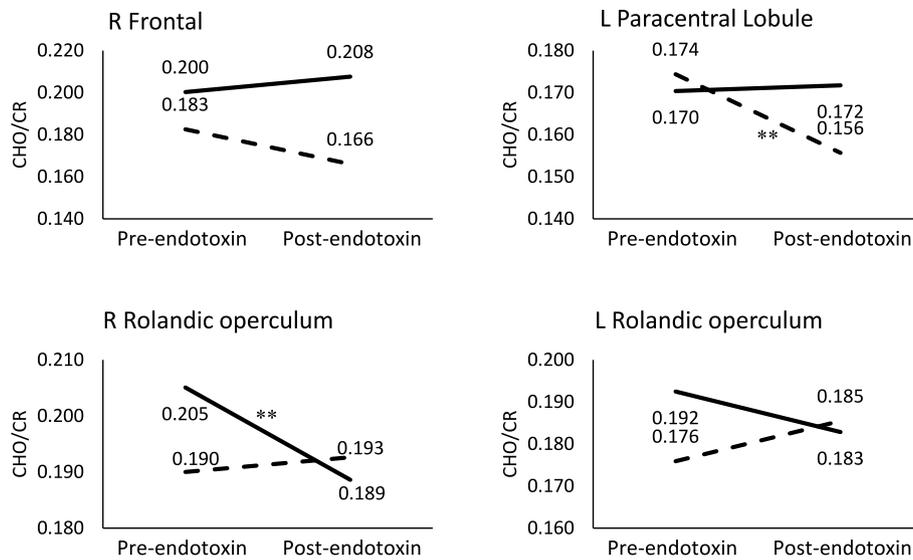


Fig. 5. Changes in CHO/CR from pre-endotoxin to post-endotoxin administration in participants receiving 0.4 ng/kg endotoxin (solid line) versus those receiving 0.3 ng/kg (dashed line). * $p < 0.05$ ** $p < 0.01$.

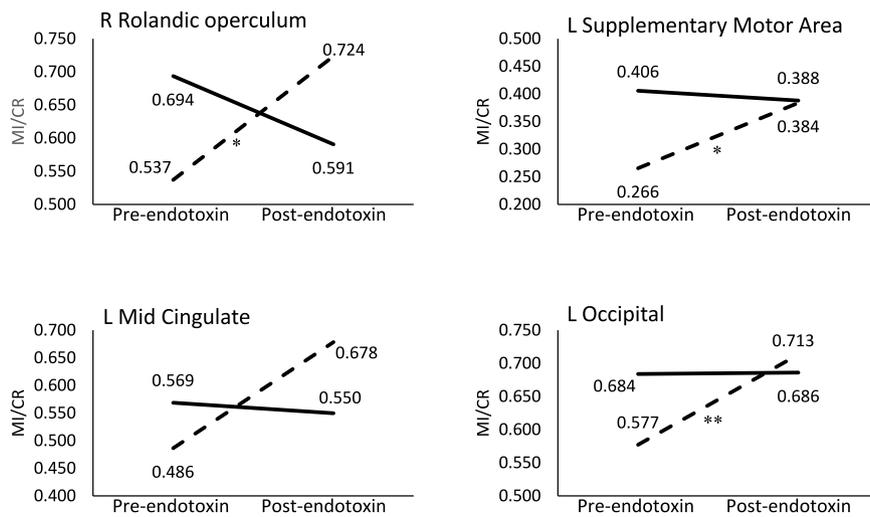


Fig. 6. Changes in MI/CR from pre-endotoxin to post-endotoxin administration in participants receiving 0.4 ng/kg endotoxin (solid line) versus those receiving 0.3 ng/kg (dashed line). * $p < 0.05$ ** $p < 0.01$.

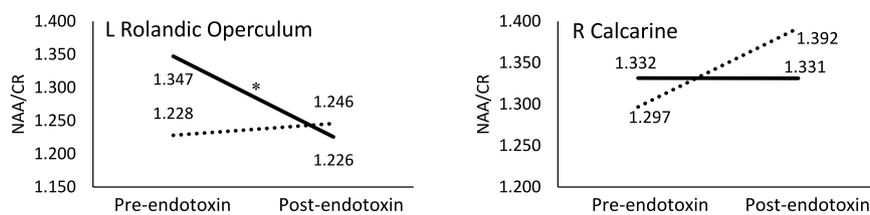


Fig. 7. Changes in NAA/CR from pre-endotoxin to post-endotoxin administration in the left Rolandic operculum and right calcarine in participants with FM (solid line) and healthy controls (HCs, dotted line). * $p < 0.05$.

brain-body temperature discrepancies are common during NI, especially after brain trauma or in patients with progressive neurological disease (Lunn and Childs, 2012; Childs et al., 2005; Rumana et al., 1998; Henker

et al., 1998b; Kirk et al., 2009; Crowder et al., 1996), endotoxin affected both brain and body temperature in our FM group. Because HCs experienced body temperature increases after endotoxin without

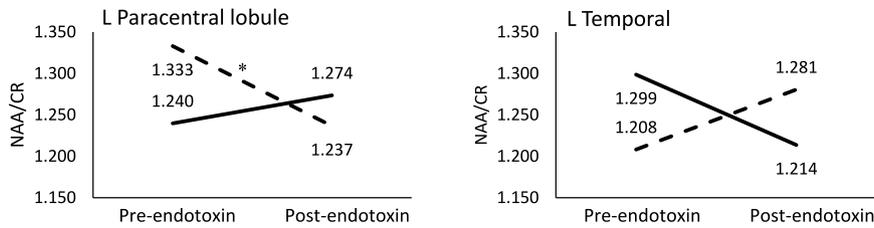


Fig. 8. Changes in NAA/CR from pre-endotoxin to post-endotoxin in the left paracentral lobule and left temporal lobe in participants receiving 0.3 ng/kg of endotoxin (dashed line) and those receiving 0.4 ng/kg (solid line). *p < 0.05.

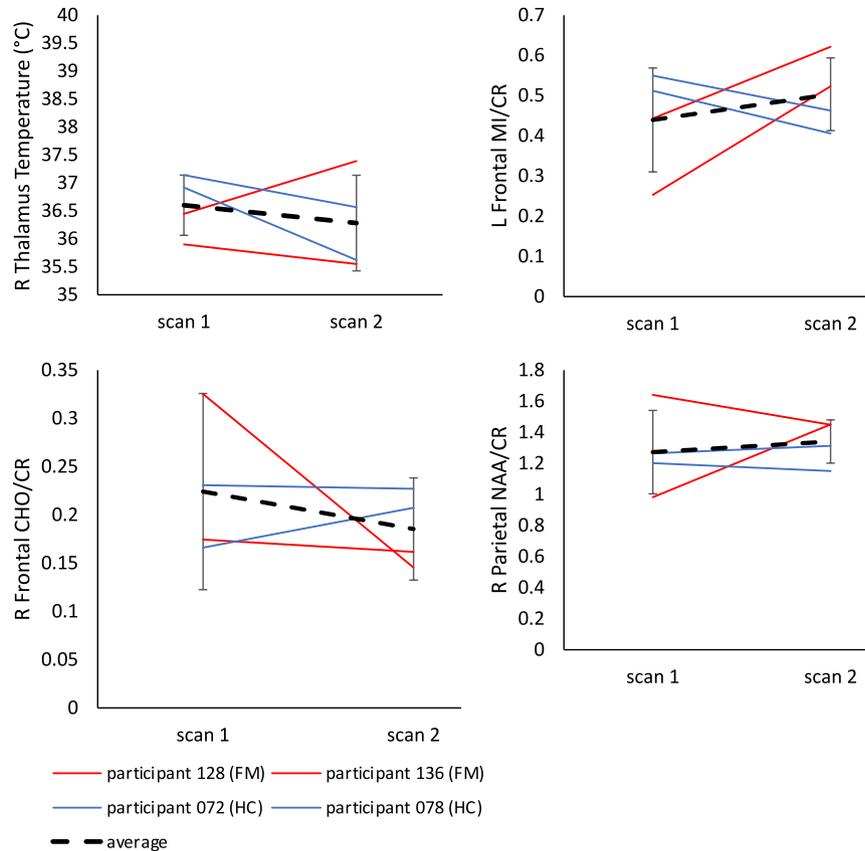


Fig. 9. Temperature, MI/CR, CHO/CHR, and NAA/CR across two scan sessions in four individuals who did not receive endotoxin. Dashed lines represent the average change across the four individuals. Error bars indicate 95% confidence intervals at each time point.

corresponding brain temperature increases, it is possible that mechanisms that normally regulate brain temperature separately from body temperature, such as cerebral blood flow, are defective in FM. This hypothesis could be tested by comparing cerebral blood flow measured with MRI between FM and HCs before and after endotoxin administration (Muir et al., 2014).

We note that brain temperature did not differ between groups at the post-endotoxin time point, meaning that the significant interaction appears to have been driven by pre-endotoxin group differences that changed after the endotoxin administration.

4.1. General response to endotoxin (time main effect)

The FM and HC groups showed similar responses to endotoxin in a few brain regions. MI was significantly increased in the left frontal region after endotoxin. Because MI is a marker of microglia, an acute increase in this metabolite could indicate microglia proliferation or

accumulation.

NAA decreased after endotoxin administration in the left anterior cingulate and right parietal lobe. NAA is a marker of neuronal density and decreased NAA can be a sign of neuroinflammation or neurodegeneration (Moffett et al., 2007). NAA levels can change acutely in response to significant medical conditions such as ischemic stroke (Mazibuko et al., 2020), but it is unknown if neurons can be significantly affected by a relatively low-level immune event. Therefore, it is unclear what NAA changes occurring over a few hours truly represent.

4.2. Dosage differences (dose-by-time effects)

We did not intend to test two different dosages of endotoxin. However, due to adverse responses in some FM patients (faintness, loss of balance, anxiety, and emesis), the dosage was decreased from 0.4 ng/kg to 0.3 ng/kg. This also allowed us to determine if MRSI is sensitive to different levels of acute immune responses. Dosage effects were found

affecting brain temperature, CHO, MI, and NAA in many brain regions, with inconsistent results. For brain temperature, higher dosages were associated with greater signs of inflammation, while metabolite levels were sometimes more affected by the lower dosage, depending on brain region.

Brain temperature increased significantly more in individuals receiving the 0.4 ng/kg dosage in the left precuneus, left frontal lobe, left Rolandic operculum, and right insula. Temperature in these regions was seen to decrease slightly in the individuals receiving the lower 0.3 ng/kg dosage. Decreased temperature may be associated with diurnal rhythms in brain temperature, which has been reported to be greatest in the morning and drop in the afternoon (Rzechorzek et al., 2022).

Dose-by-time interactions were seen with CHO in the right frontal lobe, right Rolandic operculum, left Rolandic operculum, and left paracentral lobule. The CHO results were not consistent. The 0.4 ng/kg dosage was associated with an increase of CHO in the right frontal and left paracentral lobule. Increased CHO is a marker of increased cell turnover that can be over-expressed during neuroinflammation. However, the larger endotoxin dosage was associated with a CHO decrease in the left and right Rolandic operculum (also referred to as the central operculum or subcentral gyrus). We are not aware of any neuroimaging studies reporting this region in the context of FM, or the sickness response. One study reported the right Rolandic operculum to have significantly greater cortical thinning in individuals with higher IL-6 (McCarrey et al., 2014), but CHO was not assessed. The accelerated cortical thinning associated with IL-6 in that study could be due to sustained microglia-mediated neuroinflammation, but more work is needed to determine the significance of that region.

MI showed a dose-by-time effect in the right Rolandic operculum, left supplementary motor area, left mid cingulate, and left occipital lobe. However, the greatest increases of MI were seen with the 0.3 ng/kg dosage rather than the 0.4 ng/kg dosage.

NAA showed a dose-by-time interaction in the left paracentral lobule and left temporal lobe. NAA increased in the 0.4 ng/kg dose group in the left paracentral lobule and decreased in the left temporal lobe. As discussed earlier, it is unknown what acute changes in NAA represent, although decreases are typically interpreted as neuronal loss, whereas increases are thought to reflect neuronal regeneration.

4.3. FM adverse reactions to endotoxin

This study was the first time, to our knowledge, that FM individuals were given endotoxin. We observed the FM group to have greater adverse reactions, including headaches, gastrointestinal complaints, dizziness, and chills, to the endotoxin administration, with ten FM patients reporting adverse events, compared to just three HCs. The adverse events were similar to effects reported in prior studies of healthy individuals, which have included fever, chills, myalgias, headaches, nausea, fatigue, and loss of appetite (Eisenberger et al., 2010; DellaGioia and Hannestad, 2010; van Middendorp et al., 2016; Fullerton et al., 2016). We note that there is no standard measure of sickness symptoms or adverse events in experimental endotoxin studies, so the symptoms reported likely depend on questionnaire items. In the current study, our adverse event assessment specifically included items relating to headaches, nausea, and dizziness, which could have prompted participants to report such symptoms, but not gastrointestinal complaints or chills, which were reported spontaneously by participants.

Because of the severe reactions in our study, the endotoxin dosage was reduced from 0.4 ng/kg to 0.3 ng/kg. We note that both dosages are considered low compared to most human endotoxemia studies (which are typically 0.8 ng/kg or higher). While the adverse events are known reactions to endotoxin administration at higher dosages, it is unknown why FM individuals would experience them at lower dosages. Due to the high number of adverse events in our study, we advise caution when administering endotoxin to individuals with FM or other chronic health conditions that may be sensitive to immune system activation. We also

note that excluding participants with the most severe reactions to endotoxin may have skewed our results toward milder brain responses, and this differentially affected the FM group.

4.4. Limitations

Three limitations will be highlighted. First, the change from 0.4 ng/kg to 0.3 ng/kg was unanticipated. While the dosage change allowed us to explore the effect of dosage on the MRSI signal, the sample size was very low for those analyses. The change of dosage could also introduce enough statistical noise to mask FM versus HC differences. The resulting loss of statistical power means that results must be interpreted with even greater caution than if the same dosage was used throughout the entire study, as originally planned.

Second, it is possible that observed effects were influenced by diurnal patterns, rather than the endotoxin administration. While there are studies showing MRSI stability over time, there is little information about intraday stability. Significant changes in brain temperature across the day using another MRS method, however, have been reported (Rzechorzek et al., 2022). These concerns are somewhat mitigated by the four individuals taking placebo who did not show similar changes, although statistical analysis of this data was not possible. The high number of effects found for dosage also suggests that endotoxin administration rather than time of day was the greater predictor of metabolite changes.

Third, significant influences of participant age and sex on apparent brain temperature have been described (Rzechorzek et al., 2022), which were not tested in the current study due to lack of statistical power. Further, menstrual cycle could have affected brain temperature, and we did not control for this variable. Of the twelve FM participants, three reported taking hormonal contraceptives, and three had had a hysterectomy. Of the 13 HCs, four reported taking hormonal contraceptives, and one had had a hysterectomy. Based on the study's age range (18–55), some participants may also have already completed menopause, but we did not assess this. Future studies could account for these variables to improve the detection of endotoxin-related effects on brain temperature above these factors.

5. Conclusion

We demonstrate here that MRSI is a useful tool for assessing low-level inflammation in the brain. Changes in metabolites were detected at low endotoxin dosages between 0.3 and 0.4 ng/kg. The technique also showed sensitivity to relatively small differences in immune provocation between the two dosages. Using the MRSI technique, however, we saw few differences in endotoxin-mediated metabolite changes between FM and HC. There is insufficient evidence provided by the study to support the hypothesis that FM involves abnormal central responses to low-level immune challenges. Larger samples would be needed for both replication and greater interpretation of effects.

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Declaration of competing interest

Declarations of interest: none.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bbih.2023.100624>.

References

- Bennett, R., 2005. The Fibromyalgia Impact Questionnaire (FIQ): a review of its development, current version, operating characteristics and uses. *Clin. Exp. Rheumatol.* 23 (5 Suppl. 39), S154–S162.
- Brenchley, J.M., Price, D.A., Schacker, T.W., et al., 2006. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat. Med.* 12 (12), 1365–1371. <https://doi.org/10.1038/nm1511>.
- Brown, G.C., 2019. The endotoxin hypothesis of neurodegeneration. *J. Neuroinflammation* 16 (1), 180. <https://doi.org/10.1186/s12974-019-1564-7>.
- Bryant, C.E., Spring, D.R., Gangloff, M., Gay, N.J., 2010. The molecular basis of the host response to lipopolysaccharide. *Nat. Rev. Microbiol.* 8 (1), 8–14. <https://doi.org/10.1038/nrmicro2266>.
- Chadzynski, G.L., Bender, B., Groeger, A., Erb, M., Klose, U., 2011. Tissue specific resonance frequencies of water and metabolites within the human brain. *J. Magn. Reson.* 212 (1), 55–63. <https://doi.org/10.1016/j.jmr.2011.06.009>.
- Chen, H.-L., Yamada, K., Sakai, K., Lu, C.-H., Chen, M.-H., Lin, W.-C., 2020. Alteration of brain temperature and systemic inflammation in Parkinson's disease. *Neurol. Sci.* 41 (5), 1267–1276. <https://doi.org/10.1007/s10072-019-04217-3>.
- Childs, C., Vail, A., Protheroe, R., King, A.T., Dark, P.M., 2005. Differences between brain and rectal temperatures during routine critical care of patients with severe traumatic brain injury. *Anaesthesia* 60 (8), 759–765. <https://doi.org/10.1111/j.1365-2044.2005.04193.x>.
- Cleeland, C.S., Ryan, K.M., 1994. Pain assessment: global use of the brief pain inventory. *Ann. Acad. Med. Singapore* 23 (2), 129–138.
- Crowder, C.M., Tempelhoff, R., Theard, M.A., Cheng, M.A., Todorov, A., Dacey Jr., R.G., 1996. Jugular bulb temperature: comparison with brain surface and core temperatures in neurosurgical patients during mild hypothermia. *J. Neurosurg.* 85 (1), 98–103. <https://doi.org/10.3171/jns.1996.85.1.0098>.
- Dantzer, R., Kelley, K.W., 2007. Twenty years of research on cytokine-induced sickness behavior. *Brain Behav. Immun.* 21 (2), 153–160. <https://doi.org/10.1016/j.bbih.2006.09.006>.
- DellaGioia, N., Hannestad, J., 2010. A critical review of human endotoxin administration as an experimental paradigm of depression. *Neurosci. Biobehav. Rev.* 34 (1), 130–143. <https://doi.org/10.1016/j.neubiorev.2009.07.014>.
- Eisenberger, N.I., Berkman, E.T., Inagaki, T.K., Rameson, L.T., Mashal, N.M., Irwin, M.R., 2010. Inflammation-induced anhedonia: endotoxin reduces ventral striatum responses to reward. *Biol. Psychiatr.* 68 (8), 748–754. <https://doi.org/10.1016/j.biopsych.2010.06.010>.
- Elenkov, I.J., Wilder, R.L., Chrousos, G.P., Vizi, E.S., 2000. The sympathetic nerve—an integrative interface between two supersystems: the brain and the immune system. *Pharmacol. Rev.* 52 (4), 595–638.
- Emanuele, E., Orsi, P., Boso, M., et al., 2010. Low-grade endotoxemia in patients with severe autism. *Neurosci. Lett.* 471 (3), 162–165. <https://doi.org/10.1016/j.neulet.2010.01.033>.
- Erridge, C., Attina, T., Spickett, C.M., Webb, D.J., 2007. A high-fat meal induces low-grade endotoxemia: evidence of a novel mechanism of postprandial inflammation. *Am. J. Clin. Nutr.* 86 (5), 1286–1292. <https://doi.org/10.1093/ajcn/86.5.1286>.
- Fink, M.P., 2014. Animal models of sepsis. *Virulence* 5 (1), 143–153. <https://doi.org/10.4161/viru.26083>.
- Fullerton, J.N., Segre, E., De Maeyer, R.P., Maini, A.A., Gilroy, D.W., 2016. Intravenous endotoxin challenge in healthy humans: an experimental platform to investigate and modulate systemic inflammation. *J. Vis. Exp.* 111. <https://doi.org/10.3791/53913>.
- Glaros, T.G., Chang, S., Gilliam, E.A., Maitra, U., Deng, H., Li, L., 2013. Causes and consequences of low grade endotoxemia and inflammatory diseases. *Front. Biosci. (Schol Ed)* 5 (2), 754–765. <https://doi.org/10.2741/s405>.
- Goebel, A., Krock, E., Gentry, C., et al., 2021. Passive transfer of fibromyalgia symptoms from patients to mice. *J. Clin. Invest.* 131, 131. <https://doi.org/10.1172/jci144201>.
- Hajjar, A.M., Ernst, R.K., Tsai, J.H., Wilson, C.B., Miller, S.L., 2002. Human Toll-like receptor 4 recognizes host-specific LPS modifications. *Nat. Immunol.* 3 (4), 354–359. <https://doi.org/10.1038/ni777>.
- Henker, R.A., Brown, S.D., Marion, D.W., 1998a. Comparison of brain temperature with bladder and rectal temperatures in adults with severe head injury. *Neurosurgery* 42 (5), 1071–1075. <https://doi.org/10.1097/00006123-199805000-00071>.
- Henker, R.A., Brown, S.D., Marion, D.W., 1998b. Comparison of brain temperature with bladder and rectal temperatures in adults with severe head injury. *Neurosurgery* 42 (5), 1071–1075. <https://doi.org/10.1097/00006123-199805000-00071>.
- Hutchinson, M.R., Buijs, M., Tuke, J., et al., 2013. Low-dose endotoxin potentiates capsaicin-induced pain in man: evidence for a pain neuroimmune connection. *Brain Behav. Immun.* 30, 3–11. <https://doi.org/10.1016/j.bbih.2013.03.002>.
- Jones, G.T., Atzeni, F., Beasley, M., Fluss, E., Sarzi-Puttini, P., Macfarlane, G.J., 2015. The prevalence of fibromyalgia in the general population: a comparison of the American College of Rheumatology 1990, 2010, and modified 2010 classification criteria. *Arthritis Rheumatol.* 67 (2), 568–575. <https://doi.org/10.1002/art.38905>.
- Jonsjö, M.A., Åström, J., Jones, M.P., et al., 2020. Patients with ME/CFS (Myalgic Encephalomyelitis/Chronic Fatigue Syndrome) and chronic pain report similar level of sickness behavior as individuals injected with bacterial endotoxin at peak inflammation. *Brain, Behav. Immun. Health* 2, 100028. <https://doi.org/10.1016/j.bbih.2019.100028>.
- Kelley, K.W., Bluthé, R.M., Dantzer, R., et al., 2003. Cytokine-induced sickness behavior. *Brain Behav. Immun.* 17 (Suppl. 1), S112–S118. [https://doi.org/10.1016/s0889-1591\(02\)00077-6](https://doi.org/10.1016/s0889-1591(02)00077-6).
- Kirk, D., Rainey, T., Vail, A., Childs, C., 2009. Infra-red thermometry: the reliability of tympanic and temporal artery readings for predicting brain temperature after severe traumatic brain injury. *Crit. Care* 13 (3), R81. <https://doi.org/10.1186/cc7898>.
- Liu, H.Y., Lee, P.L., Chou, K.H., et al., 2022. Brain morphometry changes with fatigue severity in fibromyalgia. *Clin. Exp. Rheumatol.* <https://doi.org/10.55563/clinexp Rheumatol/zviutu>.
- Lunn, K.W., Childs, C., 2012. A systematic review of differences between brain temperature and core body temperature in adult patients with severe traumatic brain injury. *JBI Libr. Syst. Rev.* 10 (24), 1410–1451. <https://doi.org/10.11124/01938924-201210240-00001>.
- Maachi, M., Pièroni, L., Bruckert, E., et al., 2004. Systemic low-grade inflammation is related to both circulating and adipose tissue TNFalpha, leptin and IL-6 levels in obese women. *Int. J. Obes. Relat. Metab. Disord.* 28 (8), 993–997. <https://doi.org/10.1038/sj.ijo.0802718>.
- Manco, M., Putignani, L., Bottazzo, G.F., 2010. Gut microbiota, lipopolysaccharides, and innate immunity in the pathogenesis of obesity and cardiovascular risk. *Endocr. Rev.* 31 (6), 817–844. <https://doi.org/10.1210/er.2009-0030>.
- Maudsley, A.A., Darkazanli, A., Alger, J.R., et al., 2006. Comprehensive processing, display and analysis for in vivo MR spectroscopic imaging. *NMR Biomed.* 19 (4), 492–503. <https://doi.org/10.1002/nbm.1025>.
- Maudsley, A.A., Domenici, C., Govind, V., et al., 2009. Mapping of brain metabolite distributions by volumetric proton MR spectroscopic imaging (MRSI). *Magn. Reson. Med.* 61 (3), 548–559. <https://doi.org/10.1002/mrm.21875>.
- Maudsley, A.A., Goryawala, M.Z., Sheriff, S., 2017. Effects of tissue susceptibility on brain temperature mapping. *Neuroimage* 146, 1093–1101. <https://doi.org/10.1016/j.neuroimage.2016.09.062>.
- Mazibuko, N., Tuura, R.O., Sztriha, L., et al., 2020. Subacute changes in N-acetylaspartate (NAA) following ischemic stroke: a serial mr spectroscopy pilot study. *Diagnostics* 10 (7). <https://doi.org/10.3390/diagnostics10070482>.
- McCarrey, A.C., Pacheco, J., Carlson, O.D., et al., 2014. Interleukin-6 is linked to longitudinal rates of cortical thinning in aging. *Transl. Neurosci.* 5 (1), 1–7. <https://doi.org/10.2478/s13380-014-0203-0>.
- Meester, I., Rivera-Silva, G.F., González-Salazar, F., 2019. Immune system sex differences may bridge the gap between sex and gender in fibromyalgia. *Front. Neurosci.* 13, 1414. <https://doi.org/10.3389/fnins.2019.01414>.
- Mehta, N.N., McGillicuddy, F.C., Anderson, P.D., et al., 2010. Experimental endotoxemia induces adipose inflammation and insulin resistance in humans. *Diabetes* 59 (1), 172–181. <https://doi.org/10.2337/db09-0367>.
- Moffett, J.R., Ross, B., Arun, P., Madhavarao, C.N., Namboodiri, A.M., 2007. N-Acetylaspartate in the CNS: from neurodiagnostics to neurobiology. *Prog. Neurobiol.* 81 (2), 89–131. <https://doi.org/10.1016/j.pneurobio.2006.12.003>.
- Morris, M.C., Gilliam, E.A., Li, L., 2014. Innate immune programming by endotoxin and its pathological consequences. *Front. Immunol.* 5, 680. <https://doi.org/10.3389/fimmu.2014.00680>.
- Mrozek, S., Vardon, F., Geeraerts, T., 2012. Brain temperature: physiology and pathophysiology after brain injury. *Anesthesiol Res. Pract.* 2012, 989487. <https://doi.org/10.1155/2012/989487>.
- Mueller, C., Lin, J.C., Sheriff, S., Maudsley, A.A., Younger, J.W., 2020. Evidence of widespread metabolite abnormalities in Myalgic encephalomyelitis/chronic fatigue syndrome: assessment with whole-brain magnetic resonance spectroscopy. *Brain Imag. Behav.* 14 (2), 562–572. <https://doi.org/10.1007/s11682-018-0029-4>.
- Mueller, C., Lin, J.C., Thannickal, H.H., et al., 2020. No evidence of abnormal metabolic or inflammatory activity in the brains of patients with rheumatoid arthritis: results from a preliminary study using whole-brain magnetic resonance spectroscopic imaging (MRSI). *Clin. Rheumatol.* 39 (6), 1765–1774. <https://doi.org/10.1007/s10067-019-04923-5>.
- Mueller, C., Baird, J.F., Motl, R.W., 2022. Whole-brain metabolic abnormalities are associated with mobility in older adults with multiple sclerosis. *Neurorehabilitation Neural Repair* 36 (4–5), 286–297. <https://doi.org/10.1177/15459683221076461>.
- Muir, E.R., Watts, L.T., Tiwari, Y.V., Bresnen, A., Shen, Q., Duong, T.Q., 2014. Quantitative cerebral blood flow measurements using MRI. In: Milner, R. (Ed.), *Cerebral Angiogenesis: Methods and Protocols*. Springer, New York, pp. 205–211.
- Nádházi, Z., Takáts, A., Offenmüller, K., Bertók, L., 2002. Plasma endotoxin level of healthy donors. *Acta Microbiol. Immunol. Hung.* 49 (1), 151–157. <https://doi.org/10.1556/AMicr.49.2002.1.15>.
- Opal, S.M., Scannon, P.J., Vincent, J.L., et al., 1999. Relationship between plasma levels of lipopolysaccharide (LPS) and LPS-binding protein in patients with severe sepsis and septic shock. *J. Infect. Dis.* 180 (5), 1584–1589. <https://doi.org/10.1086/315093>.
- Parnet, P., Kelley, K.W., Bluthé, R.M., Dantzer, R., 2002. Expression and regulation of interleukin-1 receptors in the brain. Role in cytokines-induced sickness behavior. *J. Neuroimmunol.* 125 (1–2), 5–14. [https://doi.org/10.1016/s0165-5728\(02\)00022-x](https://doi.org/10.1016/s0165-5728(02)00022-x).
- Raparelli, V., Basili, S., Carnevale, R., et al., 2017. Low-grade endotoxemia and platelet activation in cirrhosis. *Hepatology* 65 (2), 571–581. <https://doi.org/10.1002/hep.28853>.
- Rumana, C.S., Gopinath, S.P., Uzura, M., Valadka, A.B., Robertson, C.S., 1998. Brain temperature exceeds systemic temperature in head-injured patients. *Crit. Care Med.* 26 (3), 562–567. <https://doi.org/10.1097/00003246-199803000-00032>.
- Rzechorzek, N.M., Tripleton, M.J., Chappell, F.M., et al., 2022. A daily temperature rhythm in the human brain predicts survival after brain injury. *Brain* 145 (6), 2031–2048. <https://doi.org/10.1093/brain/awab466>.

- Sandiego, C.M., Gallezot, J.D., Pittman, B., et al., 2015. Imaging robust microglial activation after lipopolysaccharide administration in humans with PET. *Proc. Natl. Acad. Sci. U. S. A* 112 (40), 12468–12473. <https://doi.org/10.1073/pnas.1511003112>.
- Sharma, A.A., Nenert, R., Mueller, C., Maudsley, A.A., Younger, J.W., Szaflarski, J.P., 2020. Repeatability and reproducibility of in-vivo brain temperature measurements. *Original research. Front. Hum. Neurosci.* (573), 14. <https://doi.org/10.3389/fnhum.2020.598435>.
- Siracusa, R., Paola, R.D., Cuzzocrea, S., Impellizzeri, D., 2021. Fibromyalgia: pathogenesis, mechanisms, diagnosis and treatment options update. *Int. J. Mol. Sci.* (8), 22. <https://doi.org/10.3390/ijms22083891>.
- Staud, R., 2015. Cytokine and immune system abnormalities in fibromyalgia and other central sensitivity syndromes. *Curr. Rheumatol. Rev.* 11 (2), 109–115. <https://doi.org/10.2174/1573397111666150619094819>.
- Stern, A.F., 2014. The hospital anxiety and depression scale. *Occup. Med.* 64 (5), 393–394. <https://doi.org/10.1093/occmed/kqu024>.
- Taudorf, S., Krabbe, K.S., Berg, R.M., Pedersen, B.K., Møller, K., 2007. Human models of low-grade inflammation: bolus versus continuous infusion of endotoxin. *Clin. Vaccine Immunol.* 14 (3), 250–255. <https://doi.org/10.1128/cvi.00380-06>.
- Terawaki, H., Yokoyama, K., Yamada, Y., et al., 2010. Low-grade endotoxemia contributes to chronic inflammation in hemodialysis patients: examination with a novel lipopolysaccharide detection method. *Ther. Apher. Dial.* 14 (5), 477–482. <https://doi.org/10.1111/j.1744-9987.2010.00815.x>.
- Tzourio-Mazoyer, N., Landeau, B., Papathanassiou, D., et al., 2002. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage* 15 (1), 273–289. <https://doi.org/10.1006/nimg.2001.0978>.
- van Middendorp, H., Kox, M., Pickkers, P., Evers, A.W., 2016. The role of outcome expectancies for a training program consisting of meditation, breathing exercises, and cold exposure on the response to endotoxin administration: a proof-of-principle study. *Clin. Rheumatol.* 35 (4), 1081–1085. <https://doi.org/10.1007/s10067-015-3009-8>.
- Vincent, A., Lahr, B.D., Wolfe, F., et al., 2013. Prevalence of fibromyalgia: a population-based study in olmsted county, Minnesota, utilizing the rochester epidemiology project. *Arthritis Care Res.* 65 (5), 786–792. <https://doi.org/10.1002/acr.21896>.
- Wegner, A., Elsenbruch, S., Maluck, J., et al., 2014. Inflammation-induced hyperalgesia: effects of timing, dosage, and negative affect on somatic pain sensitivity in human experimental endotoxemia. *Brain Behav. Immun.* 41, 46–54. <https://doi.org/10.1016/j.bbi.2014.05.001>.
- Wiedermann, C.J., Kiechl, S., Dunzendorfer, S., et al., 1999. Association of endotoxemia with carotid atherosclerosis and cardiovascular disease: prospective results from the Bruneck Study. *J. Am. Coll. Cardiol.* 34 (7), 1975–1981. [https://doi.org/10.1016/s0735-1097\(99\)00448-9](https://doi.org/10.1016/s0735-1097(99)00448-9).
- Wolfe, F., Ross, K., Anderson, J., Russell, I.J., Hebert, L., 1995a. The prevalence and characteristics of fibromyalgia in the general population. *Arthritis Rheum.* 38 (1), 19–28.
- Wolfe, F., Ross, K., Anderson, J., Russell, I.J., 1995b. Aspects of fibromyalgia in the general population: sex, pain threshold, and fibromyalgia symptoms. *J. Rheumatol.* 22 (1), 151–156.
- Wolfe, F., Clauw, D.J., Fitzcharles, M.A., et al., 2010. The American College of Rheumatology preliminary diagnostic criteria for fibromyalgia and measurement of symptom severity. *Arthritis Care Res.* 62 (5), 600–610. <https://doi.org/10.1002/acr.20140>.
- Wolfe, F., Clauw, D.J., Fitzcharles, M.A., et al., 2016. Revisions to the 2010/2011 fibromyalgia diagnostic criteria. *Semin. Arthritis Rheum.* 46 (3), 319–329. <https://doi.org/10.1016/j.semarthrit.2016.08.012>. Dec 2016.
- Wolfe, F., Butler, S.H., Fitzcharles, M., et al., 2019. Revised chronic widespread pain criteria: development from and integration with fibromyalgia criteria. *Scand. J. Pain* 20 (1), 77–86. <https://doi.org/10.1515/sjpain-2019-0054>.
- Zhang, R., Miller, R.G., Gascon, R., et al., 2009. Circulating endotoxin and systemic immune activation in sporadic amyotrophic lateral sclerosis (sALS). *J. Neuroimmunol.* 206 (1–2), 121–124. <https://doi.org/10.1016/j.jneuroim.2008.09.017>.
- Zhang, Y., Taub, E., Mueller, C., et al., 2020. Reproducibility of whole-brain temperature mapping and metabolite quantification using proton magnetic resonance spectroscopy. *NMR Biomed.* 33 (7), e4313 <https://doi.org/10.1002/nbm.4313>.