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Neurobiology of Pain 20 (2026) 100215

Contents lists available at ScienceDirect

Neurobiology of Pain

journal homepage: www.sciencedirect.com/journal/neurobiology-of-pain

Original Research

Relationship between brain metabolites and chronic pain mechanisms in knee osteoarthritis pre- and post-total knee replacement

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ARTICLE INFO

Keywords:
Magnetic resonance spectroscopy
Knee osteoarthritis
Chronic pain
Metabolite
Quantitative sensory testing
Total knee arthroplasty

ABSTRACT

Impaired descending modulation and heightened pain sensitivity are thought to contribute to pain in knee osteoarthritis (KOA) and its persistence after total knee arthroplasty (TKA). These mechanisms can be assessed using quantitative sensory testing (QST), including pressure pain thresholds (PPT), conditioned pain modulation (CPM), and mechanical temporal summation (MTS). Magnetic resonance spectroscopy (MRS) enables non-invasive quantification of neurometabolites, including gamma-aminobutyric acid (GABA), glutamate + glutamine (Glx), myoinositol (Myo), and choline (tCho), which are suggested to modulate pain perception. This study investigated region-specific neurometabolite levels and their associations with pain mechanisms in individuals with KOA before and after TKA. Single-voxel MRS quantified neurometabolites in the anterior cingulate cortex, anterior insula, posterior insula, and somatosensory cortex in 20 female KOA patients and 19 pain-free controls. We found that anterior cingulate GABA was significantly lower in KOA patients prior to TKA compared to controls ($p = 0.012$). Prior to TKA, significant metabolite-QST interactions included: anterior cingulate Myo was associated with improved descending modulation ($p = 0.0017$; interaction $p < 0.001$), whereas controls showed the opposite in the anterior insula ($p = 0.014$; interaction $p = 0.012$). Following TKA, increases in somatosensory Myo ($p = 0.016$) and tCho ($p = 0.031$) were associated with worsened pain sensitivity (lower pain thresholds), whereas increases in anterior cingulate Myo ($p = 0.018$) and anterior insula tCho ($p = 0.027$) were associated with improved descending modulation and pain sensitization relative to pre-TKA, respectively. These results provide a foundation for future studies investigating neurochemical contributions to pain mechanisms and their potential relevance for pain phenotyping.

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https://doi.org/10.1016/j.ynpai.2026.100215
Received 9 January 2026; Received in revised form 21 April 2026; Accepted 24 April 2026
Available online 1 May 2026

1. Introduction

Knee osteoarthritis (KOA) is a leading cause of chronic pain worldwide and poses significant challenges in pain management (Neogi, 2013). Total knee arthroplasty (TKA) is the final treatment option for KOA after non-surgical interventions prove ineffective (Bjordal et al., 2006; Roos and Juhl, 2012; Sharma, 2021; Zhang, 2004). The complexity of pain in KOA is further underscored by the mismatch between joint damage observed on x-ray and pain levels reported through self-assessment or quantitative sensory testing (QST) (Cooper et al., 2000; Dieppe et al., 1997; Finan et al., 2013; Hannan et al., 2000). While limited sensitivity on radiographs may contribute to this discrepancy, 15–40% of patients report persistent pain following TKA (Beswick et al., 2012; Bourne et al., 2010; Werner and Kongsgaard, 2014), suggesting pain is more complex. Females disproportionately affected by post-operative pain and report 37% higher rates of moderate to severe pain 2–5 years post-TKA compared to males (Singh et al., 2008). One interpretation of this discrepancy is that centrally mediated pain mechanisms (i.e., processes involving the brain and spinal cord) may amplify pain beyond what is caused by osteoarthritic tissue damage (Phillips and Clauw, 2011). Here, we examine nociplastic pain features as one possible contributor to pain in KOA.

Nociplastic pain, defined as altered nociceptive processing in the absence of clear evidence of tissue damage or somatosensory system lesion, may contribute to the pain experience in knee osteoarthritis (IASP, 2017; Kosek et al., 2016; Nijs et al., 2023). Osteoarthritis is classified as a chronic secondary musculoskeletal pain condition characterized by peripheral tissue pathology, in which nociceptive and nociplastic processes may coexist rather than representing a primary nociplastic pain condition (IASP, 2017; Kidd, 2012; O'Neill and Felson, 2018; Wylde et al., 2013); as such, we use the term nociplastic pain to describe this coexistence and context.

Signs of nociplastic pain in KOA include heightened pain sensitivity and reduced capacity for descending pain modulation (Clauw and Hassett, 2017; Clauw and Witter, 2009; Imamura et al., 2008; Lee et al., 2011; Wessel, 1995). These alterations parallel clinical features – such as widespread pain, hyperalgesia, allodynia, and impaired conditioned pain modulation – commonly observed in conditions involving nociplastic pain mechanisms, such as fibromyalgia (Bradley et al., 2004; Clauw, 2014; Sluka and Clauw, 2016). Proton Magnetic resonance spectroscopy (MRS) studies have identified regional differences in gamma-aminobutyric acid (GABA), glutamate and glutamine (Glx), myoinositol (Myo), and total choline (tCho) between individuals with fibromyalgia and healthy controls (Fayed et al., 2010; Foerster et al., 2012; Harris et al., 2009; Jung et al., 2021; Pomares et al., 2020). These findings point to disrupted cortical inhibition (GABA), heightened excitation (Glx), and evidence of neuroinflammation (Myo, tCho) in chronic pain. More specifically in KOA, elevated Myo and reduced GABA levels in the anterior cingulate cortex has been associated to greater pain severity (El-Najjar et al., 2020; Reckziegel et al., 2016). Weerasekera et al. also reported persistently increased thalamic Myo levels in KOA patients both before and after TKA, relative to pain-free controls (Weerasekera et al., 2021).

Previous MRS studies on KOA pain have assessed only a single brain region. Therefore, it is unknown whether these findings are region-specific or across the brain. Given the complexity of chronic pain, understanding the regional and whole brain alterations important for guiding targeted treatments. For example, the anterior cingulate cortex (ACC) is involved in the affective-motivational and cognitive-evaluative dimensions of pain, including attention, unpleasantness, and behavioral regulation (Hsieh et al., 1999; Sun et al., 2023); the anterior insula combines interoceptive and affective information and contributes to the emotional salience and anticipation of pain (Geuter et al., 2017; Gu et al., 2012); the posterior insula integrates sensory signals with interoceptive awareness to represent perceived pain intensity (Horing and Büchel, 2022); and the primary somatosensory cortex (S1) encodes the sensory-discriminative features of pain, including its location, intensity, and duration (Sun et al., 2023).

In this study, we used single-voxel MRS, including both GABA-edited and conventional short echo-time MRS, to measure GABA, Glx, Myo, and tCho in the anterior cingulate cortex, anterior insula, posterior insula, and somatosensory cortex. We examined female KOA patients prior to TKA and at least 3 months following TKA, as well as age-matched pain-free controls. We tested for group differences in (1) metabolite levels and (2) QST metrics between patients and controls, and between pre- and post-TKA timepoints. Exploratory analyses assessed (3) group-specific associations between metabolite levels and QST measures (pre-TKA vs. controls), and (4) correlations between changes in metabolites and changes in QST metrics following TKA (pre–post TKA).

2. Methods

2.1. Participants

Twenty female participants (aged 40–75 years) with end-stage KOA awaiting TKA were recruited from surgical waitlists. KOA diagnosis was confirmed by clinical and radiographic evaluation. All participants had exhausted non-surgical pain management options (e.g., physiotherapy, weight optimization, NSAIDs) and had experienced chronic knee pain for at least six months, consistent with criteria for surgical eligibility and chronic pain definitions (Alberta Health Services et al., 2024). Fourteen patients returned for a follow-up visit at least three months after TKA.

Nineteen pain-free female control participants in the same age range were recruited from the community. Control participants had no known arthritis in any joint, no history of joint replacement or knee surgery, no chronic pain, and did not experience knee pain at rest or during walking. Only females were included in this pilot study to control for sex-related differences in pain perception (Singh et al., 2008).

Exclusion criteria for all participants included arthritis diagnosis in other joint, other chronic pain conditions or joint diagnoses, neurological disorders (e.g., multiple sclerosis and epilepsy), significant mental health disorders (e.g., psychosis), contraindications to MRI, physical impairment hindering participants from remaining motionless during MRI, inability to speak, understand, and read English, and current use of GABA-targeting medications (e.g., Gabapentin) or central sensitization-targeting drugs (e.g., Duloxetine). Other pain medications (such as Ibuprofen and Acetaminophen) were permitted. All participants provided written informed consent before involvement in the study, and this study was approved by the institutional research ethics board (REB20-0019).

2.2. Questionnaires

To characterize the study sample, all participants completed the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) (Bellamy et al., 1988), Central Sensitization Inventory (CSI) (Mayer et al., 2012), Pain Catastrophizing Scale (PCS) (Sullivan et al., 1995), Beck Depression Inventory (BDI) (Beck et al., 1961), Profile of Mood States (POMS) (Shahid et al., 2012), Short Form McGill Pain Questionnaire (SF-MPQ) (Melzack, 1987), 36-Item Short-Form Health Survey (SF-36) (Ware and Sherbourne, 1992), and the painDETECT questionnaire (PD-Q) (Freynhagen et al., 2006). As per recommended reporting standards for chronic pain research (Dworkin et al., 2008), these questionnaires capture participant-reported pain characteristics, including pain intensity, physical functioning, and emotional functioning. KOA participants to rate symptoms specifically related to their affected knee. Participants completed questionnaires via REDCap (Harris et al., 2019), a secure online database, within one week before their visit. Questionnaires not completed beforehand were completed during the study visit.

2.3. Quantitative sensory testing (QST)

During the study visit, three standardized tests that have previously been used to characterize nociplastic pain mechanisms in KOA (Finan et al., 2013) were used:

(1) Pain pressure thresholds (PPT) to assess pain sensitivity (Rolke et al., 2006; Wessel, 1995);
(2) Conditioned pain modulation (CPM) to measure descending inhibitory control of pain (Yarnitsky, 2010; Yarnitsky et al., 2008); and
(3) Mechanical temporal summation (MTS) to measure wind-up, a hallmark feature of central sensitization (Petersen et al., 2015; Woolf, 2011).

Participants were familiarized with all equipment and procedures before each test.

2.3.1. Pain pressure threshold (PPT)

PPT quantifies the minimum pressure required to elicit pain (i.e., pain thresholds). Pressure was applied using an algometer (NorthStar Echo algometer with 1 cm^2 tip attachment, JTech Medical). Pressure was applied at a constant rate of approximately 10 N/s to reduce testing duration and participant discomfort while maintaining a standardized application rate (Mutlu and Ozdincler, 2015). Measurements were taken at the trapezius contralateral to the affected knee (i.e., a site distal from the affected knee) to assess widespread hyperalgesia (Finan et al., 2013). Three trials, with 30 s of rest between each trial were performed and averaged.

2.3.2. Conditioned pain modulation (CPM)

CPM assesses descending inhibitory control of pain by measuring the degree to which a secondary noxious stimulus alters the perception of the first stimulus. In this test, participants submerged their ipsilateral hand to the affected knee in a 4°C water bath (VWR International Refrigerated Open Bath, ANOVA Precision Cooker Pro), and then the PPT of the contralateral trapezius was determined, as above) (Yarnitsky, 2010). This was repeated for a total of three trials, with 2 min rest in between each trial, with participants allowing their hand to warm up between trials. CPM was calculated by subtracting the pressure required to elicit pain (PPT) from the pressure applied during the conditioned pain modulation test (i.e., while the ipsilateral hand was in cold water).

2.3.3. Mechanical temporal summation (MTS)

MTS quantifies the increased perception of pain with repeated

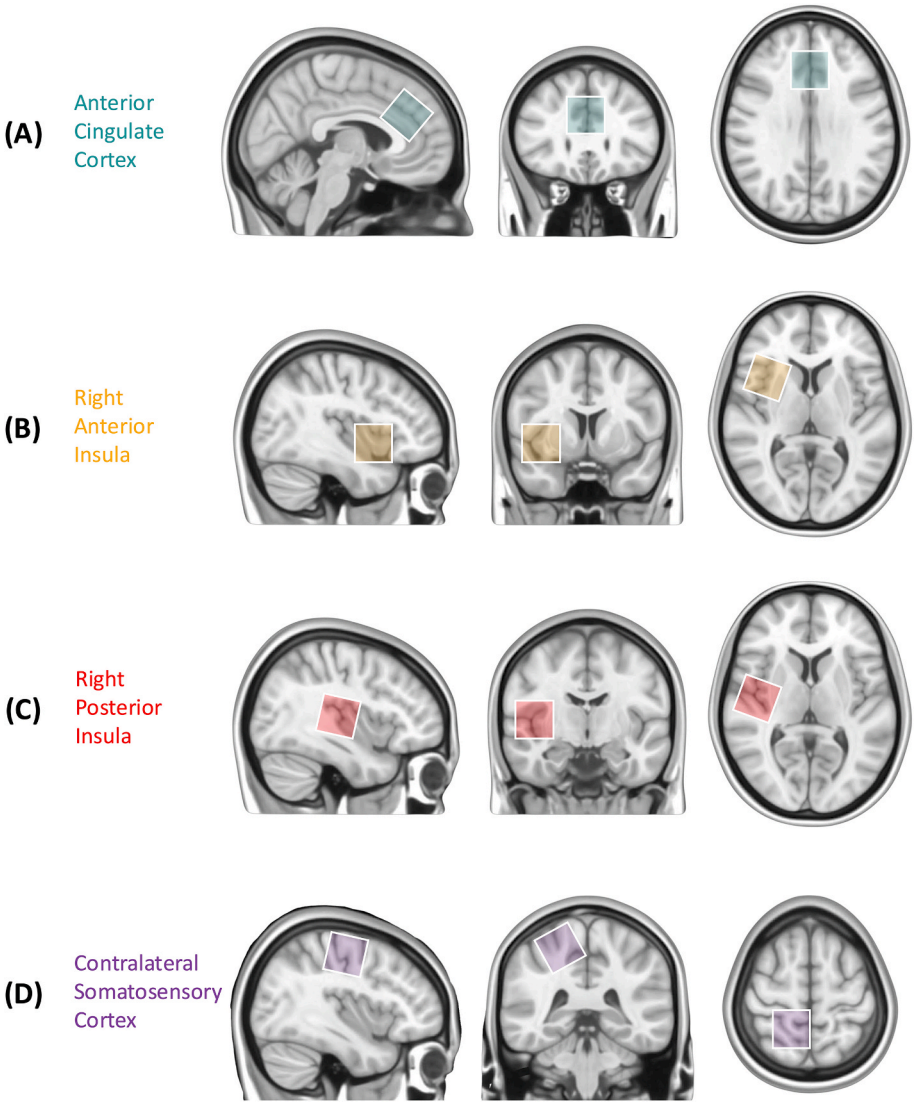

Fig. 1. Location of 3×3×3cm3 voxels in the (A) anterior cingulate cortex, (B) right anterior insula, (C) right posterior insula, and (D) somatosensory cortex, where the voxel was placed in the hemisphere contralateral to the knee affected with osteoarthritis.

mechanical stimuli and is thought to reflect central sensitization and altered pain processing, characteristic of chronic pain (Woolf, 2011). MTS was quantified as the ratio of discomfort levels from a single weighted pinprick (256 mN on the index finger) to a train of 10 pinpricks at 1 Hz using a metronome. Participants rated pain using a visual-analogue scale with the Wong-Baker FACES® Pain Rating Scale as a secondary reference. The Wong-Baker FACES® Pain Rating Scale has demonstrated strong reliability and feasibility in adult populations, including those with acute and chronic pain, (Herr et al., 2004), supporting the reliability of the visual-analogue scale.

2.4. MRS acquisition

Single voxel MRS data were collected from four brain regions: the anterior cingulate cortex, the anterior insular cortex, the posterior insular cortex, and the somatosensory cortex (Fig. 1). MR data including MRS and anatomical T1-weigted images were collected using a 3 T 750 W General Electric scanner using a 32-channel receiver head coil. T1-weighted images were acquired using a BRAVO sequence (TR/TE = 7.4 ms/2.7 ms, 1 mm^3 isotropic voxels) to guide voxel placement and for subsequent tissue segmentation for metabolite quantification. GABA data were collected using a macromolecule-suppressed GABA-edited MEschcher-Garwood Point RESolved Spectroscopy (MEGA-PRESS) acquisition (TR/TE = 1800 ms/80 ms, 20 ms editing pulses at 1.5 ppm and 1.9 ppm, $3\times3\times3cm^3$ voxels, 256 averages). Glx, Myo, and tCho were quantified from a short-echo time Point RESolved Spectroscopy (PRESS) sequence (TR/TE = 1800 ms/30 ms, $3\times3\times3cm^3$ voxels, 64 averages) using the same voxel placement as the GABA-edited sequence.

Voxel placement was guided using standardized anatomical landmarks on each participant's T1-weighted image (Fig. 1). All placements were performed by the same investigator who developed the placement protocol to ensure consistency across participants. For longitudinal scans, voxel placement from the pre-TKA visit was referenced during the post-TKA scan to visually guide placement and maintain consistency between sessions.

2.5. MRS processing and analysis

MEGA-PRESS data for the quantification of GABA were processed using the open-source software package, Gannet 3.3.1 (Edden et al., 2014) in MATLAB (R2022b). Preprocessing included coil combination, line broadening, Fourier transformation, spectral registration, and subtraction to generate the edited difference spectrum. Tissue correction was done within Gannet including co-registering MRS voxels to participant T1-weighted structural images and segmenting the voxels using Statistical Parametric Mapping (SPM12; The FIL Methods Group, London, UK) to determine voxel fractions of gray matter (GM), white matter (WM), and cerebral spinal fluid (CSF) (Ashburner and Friston, 2005). Quantification accounted for tissue-specific water density, T1 and T2 water relaxation (Near et al., 2021). We report GABA including the "α-correction" (Harris et al., 2015b), which accounts for the higher concentration of GABA in grey matter (GM) compared to white matter (WM) ($\alpha = 0.5$, which assumes the concentration of GABA in GM is twice that of WM). Outliers were excluded if the creatine frequency, phase, or full-width at half maximum (FWHM) exceeded three standard deviations from the group mean. Spectra were visually inspected and excluded if subtraction artifacts were unresolvable, the GABA fit error exceeded 15%, or the creatine FWHM exceeded 12 Hz, consistent with current quality standards for edited MRS.

PRESS data was preprocessed using FID-A, a MATLAB based software (Simpson et al., 2017). The automated preprocessing pipeline includes coil combination, removal of bad averages, frequency drift correction, and zero-order phase correction. Outputs from FID-A were then processed and quantified with LCModel software version 6.3 (Provencher, 2001). The basis set was simulated using FID-A based on sequence timings and the shape of radiofrequency pulses used during our acquisition. Our basis set included alanine, aspartate, choline, glycerophosphocholine, phosphocholine, creatine, phosphocreatine, GABA, glutamate, glutamine, lactate, inositol, N-acetylaspartate, N-acetylaspartylglutamate, scyllo-inositol, glutathione, glucose, taurine, β-hydroxybutyrate, citrate, ethanol, glycine, phosphoethanolamine. LCModel default macromolecules were also included. Tissue correction (again including tissue-specific T1, T2, and water density correction) was performed to report absolute metabolite concentrations (Near et al., 2021). Data were excluded if visual inspection indicated substantial artifacts, the signal-to-noise (SNR) ratio was less than 15 and/or the full width at half maximum (FWHM) of the NAA peak was greater than 20 Hz.

2.6. Statistical analysis

All statistical analyses were performed in R version 4.2.3 (R Core Team, 2021) and RStudio version 2023.06.1+524 (Posit team, 2025) and used a significance level of $p < 0.05$. Shapiro-Wilks tests checked for normality of continuous data elements. Means and standard deviations (SD) are reported for normally distributed data, while medians and interquartile ranges [IQR] are reported for non-normally distributed data.

All QST values were directionally transformed where necessary (e.g., multiplying PPT and CPM by −1) so that higher values consistently reflect greater sensitivity. Directional transformations of QST measures were applied to facilitate consistent interpretation across metrics (produced identical statistical results, with only the direction of coefficients reversed). We have verified the results were not affected by this simple transformation. This transformation improved interpretability across QST metrics and ensured alignment in the direction of effects when modeling relationships with brain metabolites.

As this was a pilot study and all analyses were exploratory these were not corrected for multiple comparisons.

2.6.1. Differences in metabolite levels

For normally distributed data, independent t-tests were used to test for metabolite (GABA, Glx, Myo, and Cho) differences between groups (patients pre-TKA and controls), and paired t-tests were used to measure changes pre-post TKA. Effect sizes were calculated as Cohen's d for group comparisons and Cohen's dz for paired longitudinal comparisons to assist interpretation of the magnitude of observed effects and to inform sample size calculations for future studies. When data were non-normally distributed, Mann-Whitney U tests were used for group differences, and Wilcoxon signed-rank tests were used to test for changes pre-post TKA. Uncorrected and Holm-Bonferroni-adjusted p-values (to account for multiple comparisons) are reported.

2.6.2. Differences in QST metrics

Between-group differences in QST metrics – PPT, CPM, MTS – were assessed using independent samples t-tests for comparisons between groups. Within-subject changes from pre- to post-TKA were evaluated using paired samples t-tests. For non-normally distributed data, Mann-Whitney U tests and Wilcoxon signed-rank tests were used, respectively.

2.6.3. Exploratory associations between metabolites and QST measures

Exploratory, hypothesis-generating analyses were conducted to examine relationships between neurometabolite concentrations and QST measures across regions of interest. Given the large number of comparisons, formal multiple-comparison correction was not applied. Associations were evaluated using both cross-sectional and longitudinal approaches.

2.6.3.1. Cross-Sectional associations. Multiple linear regression analyses examined the relationships between QST measures, metabolites and group in each region;

*QST ~ β0 + β1*Metabolite + β2*Group + β3*Metabolite*Group.*

We did not run models without a group term, as QST scores are well established to differ significantly between KOA patients and controls (Finan et al., 2013). If the interaction term was significant, relationships were explored further using univariate regression plots to visually demonstrate how QST-metabolite relationships differ between KOA patients and controls.

2.6.3.2. Longitudinal associations. Pearson correlations were used to examine the relationships between changes in metabolite levels (GABA, Glx, Myo, and tCho) and changes in QST measures (PPT, CPM, and MTS). Change scores were calculated as post-TKA minus pre-TKA values.

3. Results

3.1. Demographics and participant characteristics

The mean age of the twenty KOA patients was 66 ± 4.7 (57–75) years, and of the nineteen control participants was 65.5 ± 4.7 (59–76) years (Table 1). The median time and inter-quartile range (IQR) between TKR surgery and the follow-up study visit was 100.5 days [95.5, 105.8]. Table 2 summarizes health and pain questionnaires used to describe the participants; Table 2A describes the controls and pre-TKA samples and Table 2B shows the same demographic between the pre- and post-TKA samples.

3.2. Differences in metabolite levels

Data quality metrics and proportions of MRS data included in the analysis following quality assessment are summarized in Table 3.

Table 1
Participant demographic information. Data reported as mean (SD) or N (%), as applicable.

Demographics	Controls (n = 19)	KOA (n = 20)
Age (years)		
Mean (SD)	65.5 (4.9)	66 (4.7)
Range	59 – 76	57 – 75
Body Mass Index		
Mean (SD)	23.7 (4.5)	27.7 (4.0)
Range	18.6 – 33.9	20.6—33.2
Ethnicity, n (%)		
Caucasian	17 (89%)	17 (85%)
South Asian	2 (11%)	0 (0%)
Indigenous	0 (0%)	1 (5%)
Other/mixed	0 (0%)	2 (10%)
Current Marital Status n (%)		
Married	9 (47%)	11 (55%)
Single	1 (5%)	1 (5%)
Common-law	1 (5%)	1 (5%)
Widow/widower	5 (26%)	2 (10%)
Separated or divorced	3 (16%)	5 (25%)
Prefer not to answer	0 (0%)	0 (0%)
Education, n (%)		
Professional school degree (e.g., PhD, law, medicine, ministry)	0 (0%)	3 (15%)
Master's Degree	5 (26%)	3 (15%)
Bachelor's Degree	8 (42%)	2 (10%)
Technical/Trades School	4 (21%)	5 (25%)
High school	2 (11%)	7 (35%)
Did no complete high school	0 (0%)	0 (0%)
Current Employment Status, n (%)		
Full-time	2 (11%)	5 (25%)
Part-time	3 (16%)	3 (15%)
Retired	14 (74%)	12 (60%)
Handedness, R/L	17 (89%) / 2 (11%)	19 (95%) / 1 (5%)
Affected Knee R/L	–	9 (45%) / 11 (55%)

Table 2
Health and pain questionnaires to describe the sample. Data were reported as median [IQR] as all questionnaire measures were non-normally distributed. Scoring ranges are reported.

A)	Pain Characteristics	Controls (n = 19) Median [IQR]	KOA (n = 20) Median [IQR]
	WOMAC		
	Pain [0 – 20]	0 [0 – 0.5]	9 [7.8 – 11]
	Function [0 – 68]	0 [0 – 1]	31 [24.8 – 41.5]
	Stiffness [0 – 8]	0 [0 – 1]	4 [3 – 5]
	Overall [0 – 96]	1 [0 – 2.5]	42.5 [34.8 – 59]
	PD-Q		
	NRS current pain [0 – 10]	0 [0 – 0]	4 [2.8 – 5.5]
	NRS strongest pain [0 – 10]	1 [0.5 –2]	8 [7.8 – 9]
	NRS average pain [0 – 10]	0 [0 – 1]	6 [5 – 7]
	Neuropathic pain score [0 – 38]	1 [0 – 2]	12.5 [8.5 – 15.5]
	CSI		
	Total [0 – 100]	18 [15 – 24.5]	28.5 [26.5 – 40.2]
	PCS		
	Total [0 – 52]	8 [3.5 – 10.5]	11.5 [5.8 – 19.2]
	BDI		
	Total [0 – 40]	3 [1 – 5.5]	8 [5 – 15.5]
	POMS		
	Total Mood Disturbance [-24 – 94]	−27 [-31.5 – −15]	2 [-13 – 18.75]
	SF-MPQ		
	VAS general pain [0 – 100]	1 [0 – 10.2]	64 [53 – 70]
	SF-36		
	Mental Health [0 – 100]	84.1 [76.4 – 87.2]	59.2 [47.1 – 68.7]
	Physical Functioning [0 – 100]	97.4 [93.4 – 98.0]	46.0 [28.6 – 52.6]
B)	**Pain Characteristics**	**Pre-TKA (n = 14)**	**Post-TKA (n = 14)**
	WOMAC		
	Pain [0 – 20]	8.5 [8 – 10.8]	4 [2 – 5.8]
	Function [0 – 68]	31 [25.5 – 40.2]	10 [7 – 19.8]
	Stiffness [0 – 8]	4 [3 – 5]	3 [2 – 3.8]
	Overall [0 – 96]	42.5 [36.2 – 57.2]	17.5 [11.2 – 29.5]
	painDETECT		
	NRS current pain [0 – 10]	5 [3.2 – 6.5]	1 [0.2 – 2]
	NRS strongest pain [0 – 10]	8 [8 – 9]	5.5 [2.2 – 6]
	NRS average pain [0 – 10]	6 [5 – 7]	2.5 [1 – 3.8]
	Neuropathic pain score [0 – 38]	13 [9.2 – 15]	9 [7.2 – 12.2]
	CSI		
	Total [0 – 100]	27.5 [25.5 – 36.5]	21.5 [19 – 25.5]
	PCS		
	Total [0 – 52]	13.5 [7.2 – 24.2]	4 [2 – 6]
	BDI		
	Total [0 – 40]	7 [5 – 11.8]	3.5 [2 – 5.8]
	POMS		
	Total Mood Disturbance [-24 – 94]	−2 [-16.5 – 7]	−15 [-20.5 – −7.8]
	SF-MPQ		
	VAS general pain [0 – 100]	64 [55 – 69]	21 [8.5 – 26.5]
	SF-36		
	Mental Health [0 – 100]	60.5 [55.1—67.7]	74.1 [64.1 – 78.0]
	Physical Functioning [0 – 100]	48.7 [34.5—52.6]	60.5 [55—64.1]

WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index; PD-Q: painDETECT; CSI: Central Sensitization Inventory; PCS: Pain Catastrophizing Scale; BDI: Beck Depression Inventory; POMS: Profile of Mood States; SF-MPQ: Short-Form McGill Pain Questionnaire; SF-36: 36-Item Short-Form Health Survey; NRS: Numeric Rating Scale; VAS: visual analog scale.

Frequency drift for all GABA acquisitions was less than 12 Hz, which is below the threshold known to impact GABA editing efficiency (Harris et al., 2014; Hui et al., 2021), thereby supporting the reliability of the metabolite estimates. All metabolite distributions met normality assumptions. Comparable overlap patterns between groups and across timepoints support consistent voxel placement both cross-sectionally and longitudinally (Fig. 2).

GABA levels in the anterior cingulate cortex were significantly lower in KOA patients pre-TKA compared to controls (uncorrected p = 0.012; Table 4**A**); this difference remained statistically significant after for

Table 3
Number of MRS datasets collected and analyzed after quality assessment for each voxel and acquisition type. For the included data, spectral quality metrics are reported as mean (SD), including mean linewidth measurements (reported as the full width half maximum, FWHM, of the NAA peak for PRESS and of the Cr peak for GABA-edited, Hz), signal to noise ratio (SNR), voxel tissue fractions (fGM, fWM, and fCSF), Cramér-Rao lower bounds (CRLB) for PRESS metabolites (Glx, Myo, and tCho), and fir error for GABA-edited spectra. Quality metrics were extracted using LCModel and FID-A for PRESS and Gannet for GABA-edited data.

	Anterior Cingulate		**Anterior Insula**		**Posterior Insula**		**Somatosensory Cortex**	
	PRESS	*GABA-edited*	*PRESS*	*GABA-edited*	*PRESS*	*GABA-edited*	*PRESS*	*GABA-edited*
Controls								
Acquired (n)	19	19	19	19	19	19	19	19
Analyzed (n)	18	17	15	18	17	19	19	19
fGM	0.43 (0.03)	0.43 (0.03)	0.47 (0.06)	0.48 (0.06)	0.39 (0.05)	0.39 (0.05)	0.29 (0.04)	0.29 (0.04)
fWM	0.35 (0.05)	0.35 (0.05)	0.40 (0.09)	0.37 (0.09)	0.49 (0.05)	0.50 (0.05)	0.57 (0.06)	0.57 (0.06)
fCSF	0.22 (0.04)	0.22 (0.05)	0.14 (0.03)	0.14 (0.04)	0.12 (0.03)	0.11 (0.03)	0.14 (0.04)	0.14 (0.04)
FWHM (Hz)	13.0 (2.3)	10.1 (2.3)	14.0 (1.9)	12.6 (1.1)	13.7 (2.2)	12.2 (1.4)	8.54 (0.9)	9.0 (0.5)
SNR	129.6 (15.2)	127.9 (29.5)	116.2 (12.0)	111.4 (20.4)	129.5 (15.5)	128.8 (25.7)	152.4 (25.6)	145.9 (22.8)
CRLB Glx (%)	7.44 (2.97)	–	6.33 (1.23)	–	8.00 (1.58)	–	6.05 (1.22)	–
CRLB Myo (%)	12.61 (11.56)	–	13.00 (3.98)	–	12.18 (6.52)	–	9.00 (2.87)	–
CRLB tCho (%)	3.67 (1.50)	–	3.60 (0.63)	–	4.41 (1.18)	–	2.89 (0.57)	–
GABA fit error (%)	–	11.87 (4.12)	–	10.35 (3.20)	–	9.88 (4.17)	–	6.23 (1.50)
Pre-TKA								
Acquired (n)	20	20	20	20	20	20	18	19
Analyzed (n)	18	15	17	18	20	19	18	18
fGM	0.47 (0.03)	0.47 (0.03)	0.51 (0.06)	0.52 (0.05)	0.41 (0.05)	0.41 (0.05)	0.29 (0.04)	0.30 (0.04)
fWM	0.31 (0.05)	0.31 (0.05)	0.33 (0.10)	0.33 (0.09)	0.47 (0.07)	0.47 (0.08)	0.58 (0.06)	0.57 (0.07)
fCSF	0.22 (0.05)	0.22 (0.05)	0.15 (0.06)	0.16 (0.06)	0.12 (0.05)	0.12 (0.05)	0.13 (0.05)	0.14 (0.05)
FWHM (Hz)	14.9 (3.0)	11.3 (2.1)	16.2 (3.2)	13.5 (2.2)	13.0 (2.3)	11.8 (1.5)	8.26 (1.00)	9.2 (0.8)
SNR	122.5 (20.0)	111.9 (20.1)	108.5 (21.3)	99.6 (10.0)	130.7 (26.7)	119.8 (29.5)	160.2 (25.1)	148.3 (26.4)
CRLB Glx (%)	10.00 (4.49)	–	7.53 (2.07)	–	7.55 (2.96)	–	5.61 (1.82)	–
CRLB Myo (%)	15.72 (8.53)	–	13.06 (5.99)	–	14.50 (5.81)	–	8.44 (1.76)	–
CRLB tCho (%)	4.28 (1.36)	–	3.82 (1.19)	–	4.00 (1.08)	–	2.56 (0.62)	–
GABA fit error (%)	–	13.15 (5.82)	–	11.11 (3.39)	–	9.41 (2.7)	–	6.25 (1.65)
Post-TKA								
Acquired (n)	14	14	14	14	14	14	14	14
Analyzed (n)	12	13	14	13	11	13	14	14
fGM	0.45 (0.04)	0.45 (0.04)	0.49 (0.04)	0.49 (0.04)	0.41 (0.04)	0.41 (0.04)	0.30 (0.03)	0.30 (0.03)
fWM	0.33 (0.07)	0.33 (0.06)	0.38 (0.06)	0.38 (0.06)	0.48 (0.04)	0.47 (0.05)	0.57 (0.05)	0.57 (0.05)
fCSF	0.22 (0.06)	0.22 (0.06)	0.13 (0.04)	0.13 (0.04)	0.11 (0.04)	0.11 (0.04)	0.13 (0.03)	0.13 (0.03)
FWHM (Hz)	14.2 (3.0)	10.5 (1.8)	14.5 (2.2)	12.6 (0.7)	13.9 (3.5)	11.8 (1.0)	8.6 (0.7)	9.0 (0.6)
SNR	124.4 (10.6)	111.3 (22.0)	112.8 (15.1)	105.7 (10.6)	122.1 (14.5)	111.4 (13.3)	159.5 (29.9)	143.0 (29.6)
CRLB Glx (%)	7.25 (2.26)	–	8.07 (2.06)	–	7.82 (2.96)	–	5.79 (1.82)	–
CRLB Myo (%)	14.58 (6.57)	–	16.14 (6.53)	–	10.82 (2.68)	–	9.07 (1.76)	–
CRLB tCho (%)	3.25 (0.62)	–	4.07 (0.83)	–	4.73 (2.28)	–	2.71 (0.62)	–
GABA fit error (%)	–	14.65 (5.22)	–	10.43 (2.29)	–	9.76 (3.06)	–	6.77 (1.49)

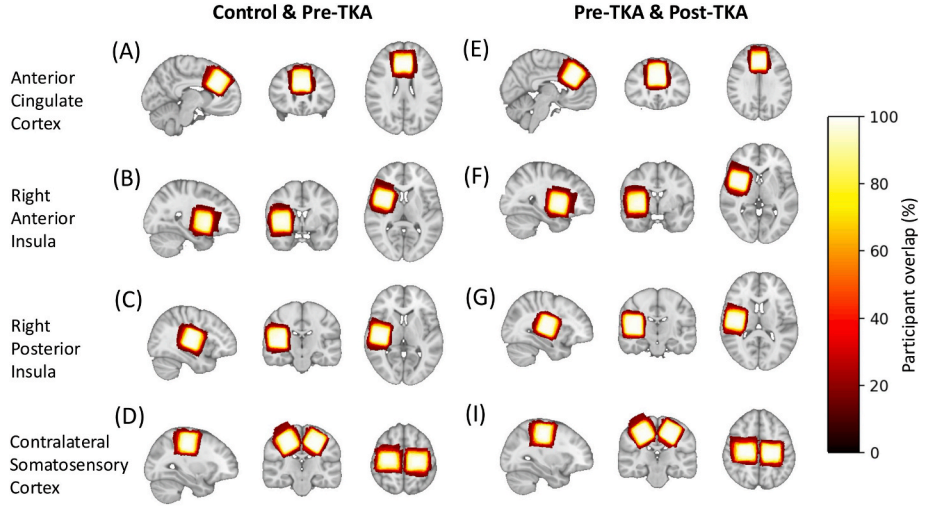

Fig. 2. Voxel overlap. For visualisation, we illustrate voxel overlap for the two analyses: Pre-TKA and Controls in the left panel and pre-TKA and post-TKA in right panel for each of the 4 voxels. Note, the intensity scale reflects the percentage of participants for each group as the somatosensory voxel was contralateral to the affected knee.

multiple comparisons across 4 voxels. No significant within-subject change in GABA was observed following TKA ($p = 0.206$; Fig. 3, Table 4**B**). No other statistically significant group differences or longitudinal changes were detected for Glx, Myo, or tCho across brain regions (Table 4). Creatine-referenced ratios are reported in the supplement (Supplementary **Table S1**) and demonstrate consistency with

Table 4
Summary of regional metabolite concentrations. A) Testing for group differences between KOA patients to healthy controls (unpaired t-tests, unpaired Cohen's d) and B) Testing for pre- and post-TKA changes (paired t-tests, paired Cohen's dz). Significant p-values are bolded and denoted with an asterisk (*).

A)	Metabolite Concentration (mmol/L)	Controls (n = 19) mean (SD)	KOA (pre-TKA, n = 20) mean (SD)	p-values	Cohen's d
	Anterior Cingulate				
	Cortex	1.01 (0.32)	0.75 (0.23)	**0.012***	0.94
	GABA	12.12	11.34	0.282	0.36
	Glx	(1.88)	(2.39)	0.225	0.41
	Myo	5.82 (1.34)	5.24 (1.44)	0.182	−0.45
	tCho	2.05 (0.27)	2.18 (0.31)		
	Anterior Insular Cortex				
	GABA	1.19 (0.38)	1.07 (0.39)	0.392	0.29
	Glx	13.99	13.88	0.898	0.05
	Myo	(2.06)	(2.93)	0.330	−0.35
	tCho	4.93 (1.03)	5.40 (1.58)	0.395	−0.31
		2.15 (0.21)	2.26 (0.48)		
	Posterior Insular				
	Cortex	1.07 (0.42)	1.11 (0.44)	0.769	−0.10
	GABA	11.40	11.88	0.434	−0.26
	Glx	(1.64)	(2.05)	0.062	0.41
	Myo	5.5 (1.43)	5.53 (1.44)	0.710	−0.12
	tCho	1.86 (0.26)	1.83 (0.29)		
	Somatosensory Cortex				
	GABA	1.68 (0.25)	1.75 (0.43)	0.553	−0.20
	Glx	9.33 (1.13)	9.46 (0.81)	0.702	−0.18
	Myo	4.26 (1.01)	4.32 (0.68)	0.844	−0.14
	tCho	1.78 (0.11)	1.75 (0.15)	0.420	−0.62
B)	**Metabolite Concentration**	**Pre-TKA (n = 14)**	**Post-TKA (n = 14)**	**p-values**	**Cohen's dz**
	Anterior Cingulate				
	Cortex	0.73 (0.27)	0.94 (0.33)	0.206	0.43
	GABA	10.75	12.82 (2.8)	0.112	0.50
	Glx	(2.23)	4.74 (1.24)	0.951	0.02
	Myo	4.71 (1.37)	2.29 (0.23)	0.121	0.51
	tCho	2.17 (0.27)			
	Anterior Insular Cortex				
	GABA	1.04 (0.33)	1.20 (0.27)	0.262	0.34
	Glx	13.28	12.68	0.555	−0.18
	Myo	(2.70)	(2.27)	0.502	−0.21
	tCho	4.84 (1.26)	4.47 (1.05)	0.475	−0.22
		2.18 (0.39)	2.09 (0.29)		
	Posterior Insular				
	Cortex	1.08 (0.52)	1.09 (0.33)	0.913	0.03
	GABA	11.79	12.72	0.394	0.27
	Glx	(2.13)	(2.09)	0.781	0.09
	Myo	4.45 (1.01)	4.57 (1.82)	0.920	−0.03
	tCho	1.91 (0.26)	1.90 (0.25)		
	Somatosensory Cortex				
	GABA	1.65 (0.34)	1.52 (0.36)	0.288	−0.32
	Glx	9.61 (1.01)	9.31 (0.86)	0.189	−0.40
	Myo	4.41 (1.05)	4.27 (0.48)	0.608	−0.15
	tCho	1.82 (0.12)	1.85 (0.16)	0.428	0.24

GABA: gamma-aminobutyric acid; Glx: total glutamate and glutamine; Myo: myoinositol; tCho: total choline.

the primary analyses using water-referenced metabolite concentrations.

3.3. Difference in QST metrics

Compared to controls, KOA patients showed evidence of nociplastic pain mechanisms, with significantly lower pain thresholds at a remote site (PPT) and greater MTS, consistent with enhanced central sensitization (Table 5**A**). CPM did not differ significantly between groups, although values were lower in KOA patients.

At the postoperative follow-up visit, objective QST measures remained largely unchanged relative to preoperative values, including PPT, CPM, and MTS (Table 5**B**), despite improvements in self-reported pain.

3.4. Exploratory associations between metabolites and QST measures

Exploratory analyses were conducted to examine associations between neurometabolite concentrations and QST measures across regions of interest. These analyses were hypothesis-generating, and given the large number of comparisons performed, results are presented using uncorrected p-values and should be interpreted cautiously.

3.4.1. Cross-Sectional associations

Fig. 4 displays univariate regression plots for models that showed significant metabolite-by-group interactions in the full regression model (QST ~ β*Metabolite*Group; interaction $p < 0.05$), with QST measures directionally adjusted such that higher values reflect worse pain processing. For the plots in Fig. 4**A and B**, the interaction terms were significant in all cases (See **Supplementary Table S2**). In Fig. 4**A-B**, the plotted p-values are the univariate associations within each group. Specifically, higher Myo was associated with better descending pain modulation (CPM) in KOA patients in the anterior cingulate cortex ($p = 0.0017$; Fig. 4**A**), and with worse descending pain modulation in controls in the anterior insula ($p = 0.014$; Fig. 4**B**). Full multivariate regression coefficients, including all β-values and significance levels, are presented in **Supplementary Table S2**.

3.4.2. Longitudinal associations

There were significant associations between changes in metabolite concentrations and changes in QST metrics following TKA. Recall, changes in QST measures were directionally adjusted such that more negative values reflect improvement in pain processing, whereas more positive values reflect worsening. In the somatosensory cortex of the patient group, larger increases in Myo and tCho showed larger increases in PPT (Myo, $R = 0.68$, $p = 0.016$; Fig. 5**A**) and (tCho, $R = 0.62$, $p = 0.031$; Fig. 5**B**). Larger increases in anterior cingulate Myo were significantly associated with improved descending pain modulation, reflected by more negative CPM values ($R = –0.69$, $p = 0.018$; Fig. 5**C**). In the anterior insula, larger increases in tCho were associated with reduced central sensitization, reflected by decreases in MTS ($R = –0.66$, $p = 0.027$; Fig. 5**D**). Full correlation results, including non-significant associations, are presented in *Supplementary Fig. S1*.

4. Discussion

This study quantified Glx, GABA, Myo and Cho as neurometabolites reflecting excitation, inhibition, and inflammation, respectively, in KOA, as these metabolites have been previously linked to chronic pain (Petrou et al., 2012; Phillips and Clauw, 2011; Pigott et al., 2023). It is the first study in KOA to use single-voxel MRS to examine of four brain regions involved in different aspects of pain processing across both sensory and affective networks.

4.1. Differences in metabolite levels

GABA levels in the anterior cingulate cortex were significantly lower in KOA patients compared to pain-free controls. This finding is similar to previous MRS studies in KOA showing lower GABA in the ACC is correlated with higher pain intensity, though these previous studies did not detect differences in GABA between controls and patients (El-Najjar et al., 2020; Reckziegel et al., 2016). Those previous studies also did not use *J*-difference editing to measure GABA. In contrast, the present study used macromolecule-suppressed GABA editing to improve specificity to GABA (Edden et al., 2012; Harris et al., 2015a). GABA specificity is physiologically relevant; macromolecule-suppressed GABA acquisitions show stronger correlations with behaviour compared to more typical GABA+ editing methods that include macromolecular signal contamination (Mikkelsen et al., 2018) likely due to interindividual differences in macromolecule contributions in the GABA+ signal (Harris et al., 2015b). However, macromolecule-suppressed GABA has a smaller signal

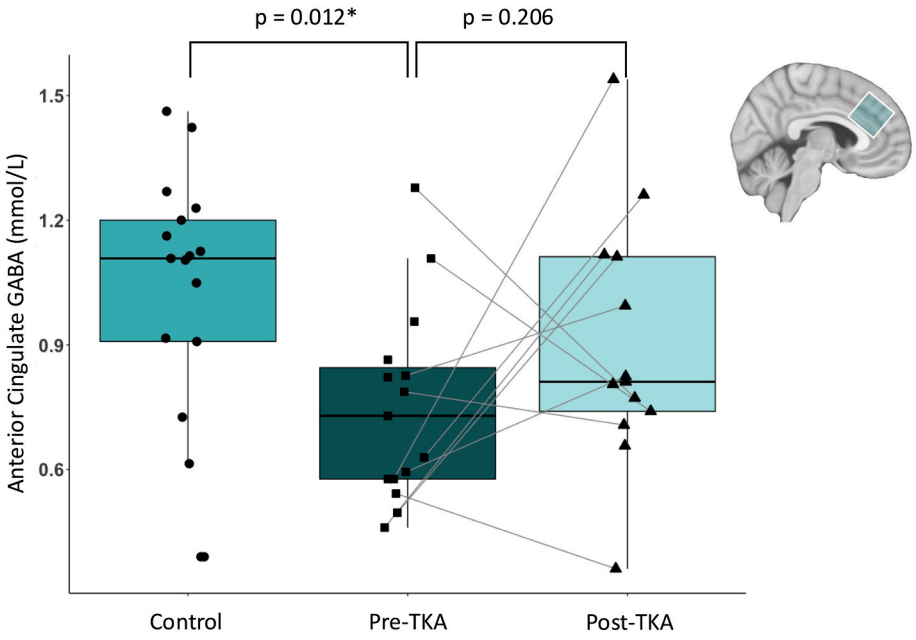

Fig. 3. GABA levels (mmol/L) in the anterior cingulate cortex in healthy controls (left), knee osteoarthritis patients before TKA (middle), and after TKA (right). P-values reflect an unpaired *t*-test comparing controls and pre-TKA groups and a paired *t*-test comparing pre-TKA and post-TKA longitudinally. Boxes represent median and interquartile ranges with whiskers showing the maximum and minimum. Individual participants are connected by lines when both pre- and post data was available.

Table 5
Summary of quantitative sensory testing (QST) pain measures (not transformed), with data presented as median [IQR]. Group differences were tested between A) control and KOA groups (Mann-Whitney U tests for unpaired samples) and B) pre- and post-TKA groups (Wilcoxon tests for paired samples); Significant p-values are bolded and denoted with an asterisk (*).

A)	**Pain Characteristics [Scoring Range]**	**Controls (n = 19) Median [IQR]**	**KOA (Pre-TKA, n = 20) Median [IQR]**	**p-value**
	Pain Pressure Threshold (PPT, kPa) Trapezius [0 – 1110]	776.67 [651.7 – 1012.7]	576.7 [422.5 – 721.7]	**0.012***
	Conditioned Pain Modulation (CPM, kPa) Conditioned – baseline [-1110 – 1110]	100 [3.33 – 158.5]	28.33 [-52.17 – 230]	0.305
	Mechanical Temporal Summation (MTS) Finger WUR 256 mN [1 – 101]	1.1 [1 – 3.2]	3.3 [1.6 – 9.1]	**0.042***
B)	**Pain Characteristics**	**Pre-TKA (n = 14)**	**Post-TKA (n = 14)**	**p-value**
	Pain Pressure Threshold (PPT, kPa) Trapezius [0 – 1110]	571.7 [407.5 – 771.7]	544 [496.6 – 689.9]	0.890
	Conditioned Pain Modulation (CPM, kPa) Conditioned – baseline [-1110 – 1110]	20 [-45 – 230]	76.3 [30.4 – 122.8]	0.597
	Mechanical Temporal Summation (MTS) Finger WUR 256 mN [1–101]	3.1 [1.7 – 7.9]	2 [1.5 – 5.5]	0.462

than GABA+ and therefore has greater sensitivity to drift (Harris et al., 2014). Given differences in acquisitions resulting in differential components of the GABA signal, comparisons across studies should be interpreted cautiously.

While this work is exploratory, the observed reductions in ACC GABA align with other literature relating GABA in the anterior cingulate cortex with chronic pain levels in veterans (Legarreta et al., 2021) and in fibromyalgia (Foerster et al., 2012). As the ACC governs affective-motivational pain processing, reduced GABA in this region may reflect diminished inhibitory control, contributing to the amplified emotional experience of pain observed in chronic pain. Although direct evidence remains limited, psychological interventions have been shown to modulate prefrontal GABA levels measured with MRS (Namgung et al., 2021), supporting a potential neurochemical basis for centrally directed therapies. Together, these findings suggest that regional neurochemistry may reflect distinct components of pain processing, which could, in future, help inform therapeutic approaches.

Despite surgical intervention, GABA levels in the ACC did not significantly increase at the post-TKA visit. This may reflect the persistence of nociplastic pain mechanisms beyond the resolution of peripheral nociceptive input, consistent with models proposing that cortical disinhibition contributes to pain chronification (Cruz-Almeida et al., 2021; Peek et al., 2020) and the lack of changes in QST (see below). The increased interindividual variability in GABA observed postoperatively suggests heterogeneous recovery. It may be that some patients have restoration of inhibitory tone and others remaining centrally dysregulated despite surgical intervention (Weerasekera et al., 2024). Longer-term follow-up is required determine if GABA increases are delayed or if there are nonlinear recovery patterns in cortical neurochemistry. Group-level differences were not observed for Glx, Myo, or tCho, which may be due to the small sample size or may indicate these are not primary drivers of nociplastic pain in knee OA.

4.2. Differences in QST metrics

Despite substantial improvements in self-reported pain (seen on the WOMAC and PainDetect) following TKA, QST measures (PPT and MTS) did not significantly change at the follow-up timepoint, despite preoperative differences relative to controls. Self-reported questionnaires primarily reflect perceived pain and functional limitation, which may improve relatively rapidly following correction of peripheral joint pathology. In contrast, QST measures aim to probe sensory processing and pain modulation mechanisms e.g., as central sensitization and conditioned pain modulation. This dissociation between patient-reported pain

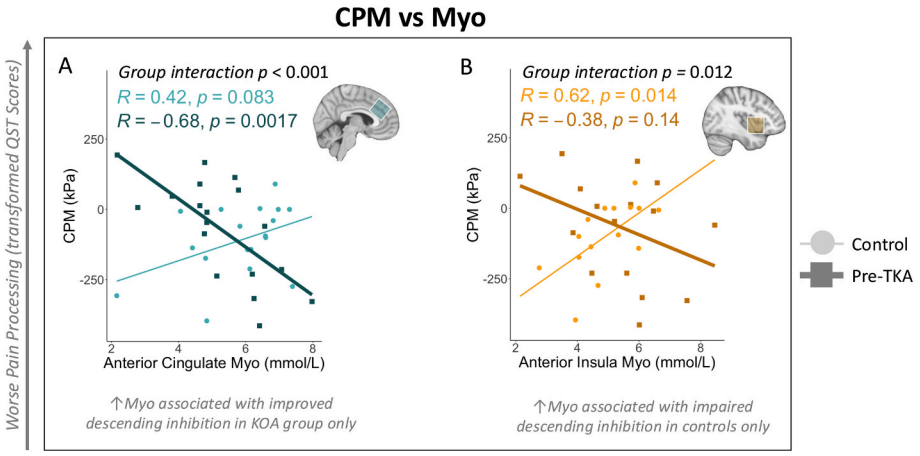

Fig. 4. Univariate analyses of the statistically significant multivariate regression group interactions (Multivariate regression p-values shown in black, see Table S2) between A) CPM and myoinositol levels (mmol/L) in the anterior cingulate cortex and B) CPM and myoinositol levels (mmol/L) in the anterior insular cortex for controls (circles, light colour, thin regression line) and KOA patients (squares, dark colour, thick regression line). QST metrics were adjusted so that higher values indicated higher pain sensitivity.

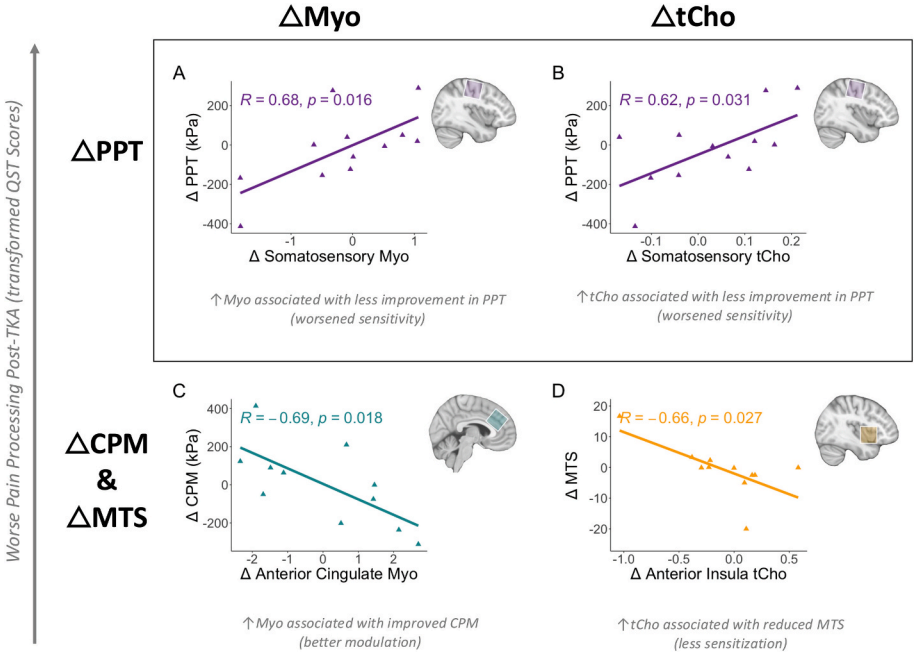

Fig. 5. Significant Pearson correlations coefficients between changes in metabolite levels (mmol/L) and changes in QST measures before and after TKA. (Change calculated as post – pre). All QST measures were adjusted so that lower values indicated improvement in QST post-TKA.

relief and persistent sensory abnormalities suggests that nociplastic alterations may persist even after resolution of joint-specific nociceptive input. Prior longitudinal studies have similarly reported delayed or incomplete normalization of QST measures following TKA (Wylde et al., 2013), and more recent evidence suggests that preoperative sensory dysfunction may be predictive of worse long-term pain outcomes (Vervullens et al., 2024). We selected the follow-up time point at 3 months as this is commonly used in TKA outcome studies (Paredes et al., 2022; Petersen et al., 2023; Sonobe et al., 2025), and pain persisting beyond 3 months is defined as chronic postsurgical pain (IASP, 2019). In this context, the investigation of metabolite-QST associations was intended to explore potential neurochemical correlates of sensory processing mechanisms rather than changes in symptom severity alone. Given our data, we suggest the normalization of all aspects of pain processing in the cortex may take longer than 3 months, or the absence of change in QST measures reflects the relatively early stage of postoperative recovery. Given the modest sample size, these findings should be interpreted cautiously.

Although patients with KOA are frequently characterized by impaired descending pain inhibition, as evidenced by impaired CPM in cross-sectional and meta-analytic studies (Edwards et al., 2016; Rodríguez-Lagos et al., 2025), we did not observe a statistically significant group difference in CPM between patients and controls. This may reflect the substantial interindividual variability in CPM responses observed across both groups. While median CPM values were lower in KOA patients, several individuals in the control group also showed minimal or even negative modulation, consistent with prior reports of high variability in conditioned pain modulation among healthy adults (Edwards et al., 2003; Julien et al., 2005; Kosek and Hansson, 1997; Maixner et al., 1995; Olesen, 1991; Pielsticker et al., 2005; Yarnitsky et al., 2008). The lack of significant difference may also stem from the small sample size and potential floor effects in CPM among controls. These findings,

together with the observed metabolite–QST associations, suggest that alterations in pain processing may not be fully captured by group-level QST comparisons alone.

4.3. Exploratory associations between metabolites and QST measures

These associations should be interpreted cautiously, as the analyses were exploratory and involved multiple potential associations. Nevertheless, the observed patterns may highlight candidate neurochemical mechanisms underlying pain processing and warrant further investigation in larger, hypothesis-driven studies.

4.3.1. Cross-Sectional associations

Differences in the direction of associations across groups or regions may reflect distinct functional roles of these regions within pain-processing and modulatory networks, rather than a single shared underlying mechanism. Although group-level differences in Myo were not observed, it showed significant associations with QST pain metrics in the KOA patients (pre-TKA). While Myo is often interpreted as a glial-related metabolite, MRS-derived metabolite concentrations cannot directly identify specific cellular processes. Higher Myo in the anterior cingulate cortex – and to a lesser extent the anterior insula – was associated with better descending pain inhibition, suggesting a role for glial-related processes in modulating endogenous pain control. This interpretation is supported by prior human MRS work linking myo-inositol alterations to chronic musculoskeletal pain and neuroinflammatory processes, while also acknowledging that the functional significance of these metabolite changes remains incompletely understood (Weerasekera et al., 2024). In addition, recent preclinical studies in KOA models support a role for microglial and astrocytic activation in central sensitization and pain modulation, including within somatosensory pathways (Liu et al., 2025; Wang et al., 2025). This interpretation is consistent with prior work linking glial-related processes to reduced descending pain inhibition (Bannister and Dickenson, 2017; Li et al., 2022). Increased Myo may reflect activation of glial cells in response to sustained nociceptive signaling within pain-related cortical regions. The absence of a significant Myo-CPM relationship in healthy controls may suggest that glial activity becomes important for pain modulation mainly when the pain system is disrupted, as in chronic pain states. Similar elevations in anterior cingulate Myo have been reported in patients with severe neuropathic pain following spinal cord injury, further supporting a link between glial-related neurochemical changes and nociplastic pain mechanisms across chronic pain conditions (Widerström-Noga et al., 2013; Wood, 2013).

4.3.2. Longitudinal associations

In the somatosensory cortex, increases in Myo and tCho were associated worsening pain thresholds from pre- to post-TKA (more positive changes in PPT; Fig. 5**A** and Fig. 5**B**). Myo is more abundant in glial cells than neurons and increases with glial activation and osmotic stress, hence it is a commonly used MRS marker of glial involvement (Patkee et al., 2021). tCho reflects phospholipid membrane turnover and increases with cellular membrane synthesis and breakdown, processes that often accompany glial activation and neuroinflammatory responses (Chang et al., 2013; de Graaf, 2019). Together, these findings suggest that persistent or increasing glial-related activity and inflammatory processes within the sensory cortex may be associated with poorer normalization of pain thresholds post-operatively.

In contrast to the somatosensory cortex, which primarily supports the sensory-discriminative aspects of pain (Sun et al., 2023), metabolite levels within affective pain-processing regions (Geuter et al., 2017; Gu et al., 2012; Hsieh et al., 1999; Sun et al., 2023) were associated with improved pain processing after TKA. Specifically, greater increases in Myo in the anterior cingulate cortex were linked to improved descending pain inhibition while increases in tCho in the anterior insula were associated with reduced central sensitization.

Together, these findings indicate that glial-related processes may have region-specific effects on pain recovery rather than uniform effects across the brain. In sensory-discriminative regions, increases in glial-associated metabolites were linked to poorer recovery of pain thresholds after surgery, whereas in affective pain-processing regions, similar metabolite changes were associated with improved pain modulation. This pattern is consistent with models suggesting that glial and neuroinflammatory processes can support recovery in some brain regions but contribute to persistent pain-related dysfunction in others, depending on their location and functional role (Weerasekera et al., 2021). These observations support the relevance of targeting nociplastic pain mechanisms in a subset of patients with persistent, nociplastic pain following TKA (Clauw and Hassett, 2017).

While MRS currently remains primarily a research tool due to limitations in cost, scan time, and specialized analysis requirements, QST offers a clinically accessible and feasible method for phenotyping pain mechanisms (Arendt-Nielsen et al., 2015; Yarnitsky, 2015). These findings should be considered preliminary and require confirmation in larger longitudinal and interventional studies.

4.4. Limitations

The exploratory nature of this study and modest sample size may limit the generalizability of findings regarding nociplastic pain mechanisms in females with KOA. Although overall spectral quality was high, exclusion of some region-sequence datasets following quality control reduced the sample size for certain analyses and may have introduced selection bias if participants with greater motion, discomfort, or more severe pain were more likely to yield unusable spectra. This limitation is likely most relevant for spectra from the anterior cingulate, which is susceptible to motion and magnetic field inhomogeneities. Additionally, only female participants were included in this study to reduce biological heterogeneity related to known sex differences in pain perception and pain processing (Fillingim et al., 2009; Mogil, 2012). While this approach decreases the sources of variance, it does limit the generalizability of the results and highlights the need for replication in larger cohorts.

Control participants were scanned at only one time point, so we cannot fully distinguish surgery-specific changes from time-related fluctuations. However, longitudinal MRS studies in healthy adults have shown that GABA levels remain relatively stable (CV $< 10\%$) over periods of up to seven months (Near et al., 2014; Wijtenburg et al., 2019), and that changes in key neurometabolites (e.g., GABA, Glx, Myo, and tCho) occur gradually over years rather than months (Haga et al., 2009; Salsano et al., 2025). Furthermore, test–retest reliability of the MM-suppressed GABA methods shows within participant CV on the order of 10% (varies by region; 8.6% in the occipital cortex, 12.6% in the anterior cingulate) (Mikkelsen et al., 2016). These reports support the interpretation that minimal biological change in controls would be expected over the $\sim$ 3 month interval examined here. While some studies suggest that pain-related brain alterations can begin to reverse as early as one month following TKA (Weerasekera et al., 2021), our findings indicate that three months may be too early to detect consistent or significant reversals in cortical neurochemistry, for example, GABA in the anterior cingulate, though this may vary between individuals and a study with a larger sample size would be better positioned to investigate this.

Participants using medications known to directly influence central neurotransmission were excluded (e.g., gabapentin, pregabalin, duloxetine, vigabatrin), as pharmacologic modulation of inhibitory pathways has been shown to alter brain GABA levels (Cai et al., 2012; Petroff et al., 1996). Common analgesics (e.g., NSAIDs, acetaminophen) were permitted given their widespread, routine use in patients with KOA, making exclusion of participants taking these medications impractical, and limiting recruitment and generalizability. Medication use therefore represents a potential confounder that could not be fully accounted for

due to the modest sample size and variability in medication type, dose, and timing. While these agents do not directly target central neurotransmitter systems measured with MRS and are therefore less likely to systematically influence GABA, Glx, Myo, or tCho concentrations (Ohashi and Kohno, 2020; Vane and Botting, 1998), they may have influenced pain perception or QST measures.

Although the group difference in ACC GABA was maintained after correction for multiple voxels, these results should be interpreted cautiously due to the exploratory nature of this study. The observed pattern aligns with prior work showing that anterior cingulate GABA levels are associated with pain severity (El-Najjar et al., 2020; Legarreta et al., 2021; Reckziegel et al., 2016).The lack of group-level metabolite changes with TKA and the presence of distinct metabolite-QST relationships suggests that KOA patients may not have altered brain chemistry but rather differ in how pain information is processed across sensory and affective circuits. These associations remain correlational, and questions of causality and directionality will require further investigation in larger, prospective samples.

We did not assess psychosocial or behavioral contributors that may influence postoperative pain outcomes, such as pain catastrophizing (Edwards et al., 2009) or sleep disturbance (Bartosiak et al., 2022). Although demographic characteristics were broadly comparable between groups and we aimed to limit the sample variability (e.g., restricting to just females), additional factors such as BMI or comorbidities may confound the results. Future studies incorporating these factors may offer more comprehensive insight into individual variability in pain persistence and recovery following TKA.

5. Conclusion

This study provides novel evidence linking regional neurochemical alterations and features of nociplastic pain in knee osteoarthritis. We examined neurochemicals in multiple regions and found lower GABA in the anterior cingulate cortex of KOA patients, reinforcing the role of disrupted inhibitory tone in chronic pain. Other metabolites did not differ at the group level. However, exploratory associations between Myo and tCho with QST measures suggest additional alterations in pain processing but should be interpreted cautiously. Overall, these findings highlight the importance of regional neurochemical alterations, particularly reduced ACC GABA, in the central processing of pain in KOA. These results provide a foundation for future studies investigating neurochemical contributions to pain mechanisms and their potential relevance for pain phenotyping.

CRediT authorship contribution statement

Samantha A Leech: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Marilena M DeMayo:** Writing – review & editing, Formal analysis, Data curation. **Tiffany K Bell:** Writing – review & editing, Formal analysis. **Mataea AL Armstrong:** Formal analysis, Visualization, Writing – review & editing. **Claudia M Campbell:** Writing – review & editing, Methodology, Conceptualization. **Chel Hee Lee:** Writing – review & editing, Methodology, Formal analysis. **Eldridge Batuyong:** Writing – review & editing, Investigation, Data curation. **Geoff Schneider:** Writing – review & editing, Methodology. **Neil J White:** Writing – review & editing, Investigation, Data curation. **Richard EA Walker:** Writing – review & editing, Methodology, Formal analysis. **Kayla Millar:** Writing – review & editing, Project administration, Investigation, Data curation. **Charley Hasselaar:** Writing – review & editing, Investigation, Data curation. **Richard Ng:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **Sarah L Manske:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Ashley D Harris:** Writing – review & editing, Writing – original draft, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research was supported by the Workers' Compensation Board – Alberta, Arthritis Society Canada (Strategic Operating Grant 23-0000000270) as well as the McCaig Institute for Bone and Joint Health, Canada and the Hotchkiss Brain Institute, University of Calgary. ADH holds a Canada Research Chair in MR Spectroscopy in Brain Injury and Pain. SAL (PhD Salary Award 22-0000000127) and SLM (Stars Career Development Award STAR-18-0189) were supported by the Arthritis Society Canada. SAL was also supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) Canada Graduate Scholarships – Doctoral (CGS D) program.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ynpai.2026.100215.

Data availability

Anonymized data may be made available to qualified investigators upon request.

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Glossary

ACC: anterior cingulate cortex
BDI: Beck Depression Inventory
CPM: conditioned pain modulation
CSF: cerebral spinal fluid
CSI: Central Sensitization Inventory
FWHM: full-width at half maximum
GABA: gamma-aminobutyric acid
Glx: glutamate + glutamine
GM: grey matter
KOA: knee osteoarthritis
MEGA-PRESS: MEshcher-GArwood Point RESolved Spectroscopy
MRS: magnetic resonance spectroscopy
MTS: mechanical temporal summation
Myo: myoinositol
PCS: Pain Catastrophizing Scale
PD-Q: painDETECT questionnaire
POMS: Profile of Mood States
PPT: pain pressure threshold
PRESS: Point RESolved Spectroscopy
QST: quantitative sensory testing
SF-36: 36-Item Short-Form Health Survey
SF-MPQ: Short Form McGill Pain Questionnaire
tCho: total choline
TKA: total knee arthroplasty
WM: white matter
WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index