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Thalamic neurometabolite alterations in patients with knee osteoarthritis before and after total knee replacement

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Abstract

The weak association between disability levels and "peripheral" (ie, knee) findings suggests that central nervous system alterations may contribute to the pathophysiology of knee osteoarthritis (KOA). Here, we evaluated brain metabolite alterations in patients with KOA, before and after total knee arthroplasty (TKA), using 1H-magnetic resonance spectroscopy (MRS). Thirty-four presurgical patients with KOA and 13 healthy controls were scanned using a PRESS sequence (TE = 30 ms, TR = 1.7 seconds, voxel size = 15 × 15 × 15 mm). In addition, 13 patients were rescanned 4.1 ± 1.6 (mean ± SD) weeks post-TKA. When using creatine (Cr)-normalized levels, presurgical KOA patients demonstrated lower N-acetylaspartate (NAA) ($P < 0.001$), higher myoinositol (mIns) ($P < 0.001$), and lower Choline (Cho) ($P < 0.05$) than healthy controls. The mIns levels were positively correlated with pain severity scores ($r = 0.37$, $P < 0.05$). These effects reached statistical significance also using water-referenced concentrations, except for the Cho group differences ($P \geq 0.067$). Post-TKA patients demonstrated an increase in NAA ($P < 0.01$), which returned to the levels of healthy controls ($P > 0.05$), irrespective of metric. In addition, patients demonstrated postsurgical increases in Cr-normalized ($P < 0.001$), but not water-referenced mIns, which were proportional to the NAA/Cr increases ($r = 0.61$, $P < 0.05$). Because mIns is commonly regarded as a glial marker, our results are suggestive of a possible dual role for neuroinflammation in KOA pain and post-TKA recovery. Moreover, the apparent postsurgical normalization of NAA, a putative marker of neuronal integrity, might implicate mitochondrial dysfunction, rather than neurodegenerative processes, as a plausible pathophysiological mechanism in KOA. More broadly, our results add to a growing body of literature suggesting that some pain-related brain alterations can be reversed after peripheral surgical treatment.

Keywords: Magnetic resonance spectroscopy, Knee osteoarthritis, Neuroinflammation

1. Introduction

As in other chronic pain conditions, knee osteoarthritis (KOA) studies have consistently found tenuous relationships between physical pathology (eg, the Kellgren Lawrence grade) and subjective pain, which are at best modestly correlated.[12,35] Among individuals experiencing knee pain, only approximately 15% have radiographic changes compatible with stage 2 to 4 osteoarthritis.[36] Even when studies report a statistically significant association between radiographic findings and pain severity, they note only modest relationships, with broad individual differences in reported pain and function among individuals with any particular stage of KOA.[27] The tenuous relationship between peripheral pathology and pain/disability is further illustrated by the fact that approximately 20% of patients continue to experience significant pain and functional limitations months or years after a successful TKA, that is, after the pathology in the joint has presumably been resolved.[15,92] Altogether, the weak association between disability levels and "peripheral" findings suggests that central nervous system alterations may contribute to the pathophysiology of KOA pain. However, our knowledge of the central nervous system mechanisms underlying KOA pain remains limited.

Recently, several studies from our group and others have demonstrated the presence of increased levels of the 18 kDa translocator protein (TSPO) in the brains of patients with chronic low back pain,[49] fibromyalgia,[4] and migraine[6] as well as in the spinal cord and neuroforamina of patients with lumbar radiculopathy.[3] Because TSPO is a marker of glial activation,[1,11,22,23,26,41,52,74,85,86] these studies are in line with those reporting elevated levels of brain metabolites linked to

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neuroinflammation using magnetic resonance spectroscopy (MRS)[43,61,91] or elevated levels of proinflammatory cytokines in the cerebrospinal fluid,[10,44,46] and suggest that neuroinflammation might be a pervasive phenomenon that can be observed across multiple, etiologically heterogeneous human pain disorders. Because glial cells (microglia and astrocytes, mainly) play a key role in the establishment and maintenance of persistent pain,[20,42,84,88] we hypothesized that neuroinflammation, and specifically in the thalamus, is implicated in KOA pain. We focused on the thalamus because this region was characterized by significant neuroinflammation in our first TSPO back pain study,[49] an observation that was later replicated in an independent cohort.[82] In this study, we evaluated thalamic alterations in KOA patients, and their response to TKA, using MRS, a noninvasive method to assess the brain chemical and cellular processes through the quantification of several metabolites.[66] Because we were interested in evaluating neuroinflammation, we focused on myoinositol (mIns), a metabolite which is believed to be a glial marker because it is more abundant in glial cells rather than other cell types.[18,31,39] Moreover, we have evaluated choline (Cho), a cell membrane metabolism and cellular turnover marker that is also often linked to neuroinflammatory processes.[43,50] Finally, because in (hip) osteoarthritis patients, the thalamus demonstrated reduced grey matter volume, which was reversed after arthroplasty, alongside with a decrease in pain and increase in function;[34] we also evaluated N-acetylaspartate (NAA), which is commonly interpreted as an in vivo marker for neuronal integrity.[89]

2. Materials and methods

2.1. Study design

The study was conducted at the Athinoula A. Martinos Center for Biomedical Imaging at Massachusetts General Hospital. The study was approved by the Partners Institutional Review Board and the Radioactive Drug Research Committee at Massachusetts General Hospital, Boston, MA. All participants provided written informed consent for study participation according to the Declaration of Helsinki.

2.2. Study participants

Thirty-four KOA patients scheduled to receive a TKA were recruited and enrolled in the study, after providing informed consent. All patients were scanned before the surgery. In addition, a subset (n = 13) were also scanned within 6 weeks post-TKA. All patients were recruited from outpatient clinics in the Boston area, through advertisements posted on various social media platforms, or through the Partners Clinical Trials website. See **Table 1** for demographic characteristics. Although we had initially planned to also enroll 25 healthy participants demographically well matched to the patient cohort, data acquisition was prematurely interrupted in early March 2020 due to the COVID-19 pandemic. As a result, our healthy control cohort consisted of a smaller group (n = 13) which, despite being matched in terms of sex, happened to be significantly younger. To address this limitation, all comparisons between patients and controls were performed both 1) including all available data sets (controlling for age statistically) and 2) by including only a subset of KOA patients (n = 11) and controls (n = 11) who were age-matched and sex- matched (See 3.1 demographic and clinical variables and **Table 1**).

2.3. Inclusion criteria

Patients between the ages of 40 and 85 were deemed eligible if they had been diagnosed with KOA, were scheduled to undergo primary unilateral TKA, and had facility with the English language. In this study, we excluded patients or healthy volunteers with current infection or cognitive impairment, current or histories of major neurological disorders, major cancers, significant head traumas, or severe psychiatric illness, as well as those who routinely used steroids or unstable doses of anti-inflammatory medications. Patients incompatible with the scanning procedures, such as those with contraindications to fMRI and positron emission tomography (PET) scanning, were also excluded. In addition, the presence of any pain, systemic inflammatory, or autoimmune disorders was an exclusion criterion for healthy controls. Because participants were simultaneously scanned with the PET radioligand [^{11}C]PBR28, which binds to the 18 kDa TSPO,[4,5,49] formerly known as the peripheral benzodiazepine receptor, we also excluded for the use of benzodiazepines whose affinity for TSPO was either known to be high or unknown.[59] However, the PET results are beyond the scope of this investigation, which focuses solely on MRS, and will not be discussed further here.

2.4. Outcomes assessment: pain and function

On the day of the presurgical and postsurgical scans, pain and physical function were assessed by multidimensional self-administered Western Ontario McMaster Osteoarthritis index (WOMAC) questionnaire,[13] which assesses pain (score: 0-20), stiffness (0-8), and disability (0-68).

2.5. Brain imaging data acquisition and processing

Brain imaging was performed with a 3T Siemens Biograph mMR integrated PET/MRI scanner equipped with a 12-channel head coil. In all participants, a high-resolution multiecho MPRAGE (T1-weighted structural MRI) volume was also acquired (TR/TE1/TE2/TE3/TE4 = 2530/1.69/3.55/5.41/7.27 ms, flip angle = 7°, voxel size = 1 mm isotropic), for the purpose of anatomical localization, MRS voxel placement, and the correction for partial volume effects of cerebrospinal fluid (CSF). In a subset of participants (n = 41), a second, lower-resolution multiecho MPRAGE (TR/TE1/TE2/TE3/TE4 = 2530/1.34/3.04/4.74/6.44 ms, flip angle = 7°, voxel size = 2.1 × 2.1 × 1.5 mm) was acquired just before the MRS scan to account for any motion that may have occurred between high-resolution MPRAGE and MRS data acquisition, thus increasing precision in MRS voxel placement.

2.5.1. ^{1}H-magnetic resonance spectroscopy protocol

Single voxel MRS was acquired using a conventional PRESS sequence (echo time [TE] = 30ms, TR = 1.7 seconds, bandwidth = 1.2 kHz, and 128 averages, 1024 sample points). A 15 × 15 × 15 mm voxel was placed in the left thalamus because this was the region showing the largest effect size in our previous PET study of neuroinflammation in chronic lower back patients (cLBP).[49] Of note, we elected to collect spectra from the left thalamus in every subject, irrespective of which knee was scheduled to be replaced (and likely to be the most affected by KOA), because (1) only rarely symptomatic KOA is unilateral[17] and, even in those rare cases, both knees are usually affected (because of changes in load bearing, postural changes, etc., which can lead to greater wear-and-tear and eventually to osteoarthritis in the initially unaffected knee); (2) consistently imaging the same side allowed us to evaluate whether any neurometabolite changes potentially observed might be more pronounced in the thalamus contralateral to the knee to be replaced or the ipsilateral one; and (3) in both

Table 1

Subject characteristics.

	Controls	KOA patients	
		Pre-TKA	Post-TKA*
All subjects			
N	13	34	13
Sex (male: female)	7:6	16:18	7:6
Age (y: mean ± SD)	49.4 ± 17.0	66.1 ± 8.2†	67.1 ± 7.9
TKA site (left: right)	—	16:18 (scheduled)	6:7 (performed)
WOMAC pain (0-20)	—	9.4 ± 3.9	8.5 ± 3.5
WOMAC stiffness (0-8)	—	3.8 ± 1.7	3.9 ± 1.5
WOMAC physical disability (0-68)	—	25.0 ± 12.4	23.2 ± 10.3
Scan, wk from surgery	—	1.8 ± 1.5	4.1 ± 1.6
Matching subgroups			
N	11	11	
Sex (male: female)	6:5	6:5	
Age (y: mean ± SD)	53.4 ± 15	59.2 ± 9.2	
Scheduled TKA (left/right)		5/6	

* Post-surgical assessment was on average 1 month after surgery.
† KOA vs controls, $P < 0.0001$.
TKA, total knee arthroplasty; WOMAC, Western Ontario McMaster Osteoarthritis index.

our prior study of cLBP,[49] and a subsequent replication study in an independent cohort,[82] the left thalamus appeared to demonstrate a slightly stronger neuroinflammatory signal, with no clear link to pathology lateralization.

Magnetic resonance spectroscopy data were analyzed using java-based magnetic resonance user interface (jMRUI) v6.0.[80] Spectra were phase-corrected. Hankel–Lanczos Singular Values Decomposition filter was applied to remove the residual water signal. Signal-to-noise ratios were determined by jMRUI QUantum ESTimation (QUEST)[69] in time-domain (maximum of free induction decay (FID) SD of FID tail), and full-width half-maximum of unsuppressed water signals were measured using jMRUI-AMARES algorithm. Metabolites were quantified with the QUEST algorithm (combined with "Subtract" for background modelling) in jMRUI. QUEST metabolite basis set was quantum mechanically simulated at 3T using a press protocol (TE = 30 ms, 1024 data points, spectral width [SW] = 1200 Hz) in nuclear magnetic resonance (NMR) Scope-B. Spectral signals of 9 metabolites (total Cr, total NAA, mIns, total Cho, glutamate, glutamine, taurine, lactate, and scyllo-Inositol) were simulated, and a 2 Hz hard apodization was applied.

Water-referenced metabolite concentrations were reported relative to the water unsuppressed spectra, and concentrations were corrected for partial volume effects as follows. Magnetic resonance spectroscopy voxel was first registered to the T1-anatomical space and segmented (gray matter, white matter, and CSF) using Gannet toolkit v3.1[37] and SPM12.[63] The segmented tissue fractions were corrected for metabolite concentrations quantified using jMRUI-QUEST to account for CSF content according to the literature.[30,56] T1 and T2 values for grey matter, white matter, and CSF used in this study were 1331, 832, and 3817 ms and 110, 79, and 503 ms, respectively. Metabolite relaxation times used for calculating the final corrected metabolite concentrations were taken from previous studies.[83,87] In addition to the water-referenced concentrations, metabolite levels were also expressed relative to creatine.

The correct placement for each voxel was visually confirmed for each participant. In addition, to further evaluate the consistency of thalamic coverage across participants, we created a probabilistic map of voxel placement in standard space (**Fig. 1**). To this end, we used FSL FNIRT[40] to calculate a nonlinear transformation between each subject's MPRAGE volume and the MNI152 template and then applied the resulting transformation to the MRS voxel. In addition, MRS masks in MNI space were used to calculate the voxel centroid for each participant using Python scripts (https://github.com/nwd2918/MRS-voxel-plot).

2.6. Statistical analyses

Statistical analysis was performed using STATISTICA v12.0. Neurometabolite levels and clinical variables (WOMAC pain, WOMAC stiffness, and WOMAC physical function) were compared across groups. As previously mentioned, because age was significantly different across groups, these group comparisons were performed using a 2-fold strategy. First, we compared all patients and controls adjusting for age using an analysis of covariance. Next, in a sensitivity analysis, we compared age-matched and sex-matched subjects (**Table 1**) using an unpaired *t* test. In addition, because the MRS voxel was placed on the left thalamus irrespective of the knee that was scheduled to be replaced, and, test for the presence of lateralized effects related to the TKA site, we used an unpaired *t* test to compare the neurometabolite and clinical variables between patients scheduled to receive right TKA (ie, contralateral to the imaged thalamus) vs those scheduled to receive left TKA.

In addition to the group comparisons, we performed paired *t* test analyses to compare neurometabolite levels and clinical variables in all patients scanned before and after TKA (n = 13). The effect size for the group comparisons and the pre-TKA vs post-TKA comparisons were computed using Cohen's *d*. Pearson's correlation coefficient (Pearson's r) was calculated to assess relationship between various study variables (neurometabolite concentrations, clinical ,and demographic parameters) adjusting for age. Note that the WOMAC scores were unavailable for one patient, and therefore, the correlations with these clinical variables were performed with 33, instead of 34, patients. The association between changes in (unadjusted) pre-TKA and post-TKA levels of mIns and NAA were also evaluated using Pearson's correlation coefficient.

Sex differences were compared using χ^2 test. Continuous data were expressed as the mean ± SD, and categorical data were expressed as percentage. An alpha value = 0.05 was considered the threshold for statistical significance.

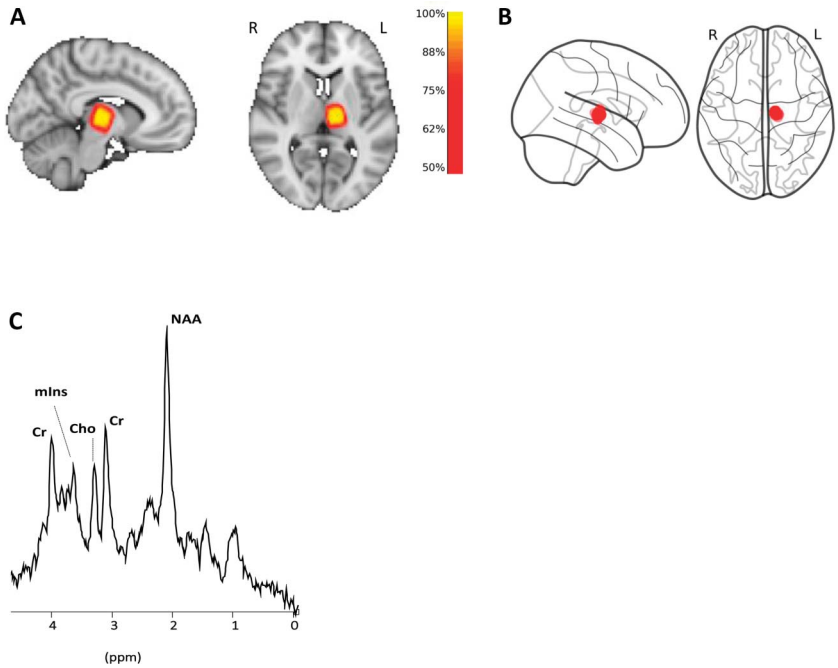

Figure 1. Voxel placement and representative spectrum. (A) Voxel overlap density map for all participants. Individual MRS voxels are converted to MNI space and then combined to show the overlap between participants. (B) Voxel centroids of all participants showing how tightly clustered MRS voxels are around a target anatomical location. (C) Representative MRS spectrum from the left thalamus. MRS, magnetic resonance spectroscopy.

3. Results

3.1. Demographics and clinical variables

The KOA patient cohort (n = 34) and the smaller (n = 13) healthy control cohort were matched in terms of sex ($P = 0.93$) but, as previously mentioned, not with age ($P < 0.0001$). To evaluate whether this age difference might have an impact on our group comparisons, subgroups of KOA patients (n = 11) and controls (n = 11) who were age-matched and sex-matched (P's = 0.31 and 1, respectively) were identified for follow-up analyses (**Table 1**).

On average patients demonstrated moderate pain, stiffness, and slight physical disability scores, as assessed using the WOMAC scale (**Table 1**). About 62% patients had bilateral OA diagnosis. Fourteen of 34 had prior surgery for the opposite knee. Three of 34 had scheduled TKA for the opposite knee.

At baseline, we observed a negative significant correlation between patient age and KOA severity of KOA assessed by WOMAC scores (pain, $r = -0.42$, $P < 0.05$; stiffness, $r = -0.42$, $P < 0.05$; and physical disability, $r = -0.47$, $P < 0.01$), indicating that patients scheduled to receive a TKA at a younger age were more likely to have more severe disease (Supplementary Fig. S1, available at http://links.lww.com/PAIN/B267).

Thirteen patients were followed up after TKA (4.1 ± 1.6 weeks post-surgery). One of the 13 subjects who returned for the perisurgical scan experienced complications in the hospital after their TKA surgery. This subject experienced vasovagal syncope with a brief loss of consciousness, elevated blood pressure, and hypokalemia which resulted in a prolonged hospitalization after surgery. None of these 13 subjects experienced apparent infection or other complications in the time between their TKA and the perisurgical scan. In their post-TKA assessment, neither of the WOMAC scores were significantly changed compared with pre-TKA levels (pain: $P = 0.91$; physical disability: $P = 0.67$; and stiffness: $P = 0.58$), indicating that patients continued experiencing pain and disability for several weeks after their TKA (in this case, likely because of the surgery itself).

3.2. Magnetic resonance spectroscopy quality parameters

No significant differences in spectral quality or amount of CSF in the voxel were apparent between groups or between pre-TKA and post-TKA timepoints ($P > 0.5$). The heat map in **Figure 1A** shows the percentage of overlap across all MRS voxel masks at each voxel in the MNI standard space. The overlap between the preoperative and postoperative voxel placement for each subject was 86% ± 7% (SD). mIns, NAA, Cr, and Cho were well within the standard Cramér-Rao lower bound (<20%) for controls and at both time points (pre- and post-TKA) for the patients (**Table 2**). However, about 30% of the Glx (glu + gln) measurements had standard Cramér-Rao lower bound >20%. Therefore, all Glx data were excluded from the analyses and will not be reported further in this article. Cr levels were not statistically different across groups, both when including all subjects, correcting for age, or when including only subsets of matching participants ($P \geq 0.46$), as well as between pre-TKA and post-TKA time points in patients ($P = 0.11$), supporting the appropriateness of using Cr as a normalizing factor.

3.3. Knee osteoarthritis–related alterations in neurometabolite levels

First, neurometabolite levels from the left thalamus of all KOA patients (n = 34) and all healthy controls (n = 13) were compared, adjusting for age (**Fig. 2**). This comparison showed elevated mIns (Cr ratio: $P < 0.001$, Cohen's $d = 1.36$; water-referenced: $P < 0.0001$, Cohen's $d = 2.29$) and lower NAA (Cr ratio: $P < 0.001$, Cohen's $d = 1.34$; water-referenced: $P < 0.01$, Cohen's $d = 0.86$) in KOA patients compared with healthy controls. In addition, lower Cho/Cr ($P < 0.05$, Cohen's $d = 0.95$) was observed in KOA

Table 2

^{1}H-MRS data quality characteristics.

	KOA patients (N = 34)	Controls (N = 13)	*P*
All subjects			
SNR	12.1 ± 1.7	12.2 ± 2.5	0.8675
$FWHM_{H2O}$(Hz)	7.7 ± 0.8	7.9 ± 1.1	0.7592
CRLB% [Cr]	4.9 ± 1.1	5.6 ± 1.6	0.1677
CRLB% [NAA]	5.3 ± 1.0	6.8 ± 2.0	0.2415
CRLB% [mIns]	12.3 ± 4.5	9.1 ± 4.5	0.1951
%CSF	3.1 ± 2.1	5.1 ± 2.2	0.3231
	KOA patients (N = 11)	**Controls (N = 11)**	***P***
Matching subgroups			
SNR	11.8 ± 1.3	12.0 ± 1.1	0.8834
$FWHM_{H2O}$(Hz)	7.8 ± 0.3	7.7 ± 1.2	0.9124
CRLB% [Cr]	5.1 ± 1.4	5.5 ± 1.3	0.8423
CRLB% [NAA]	5.6 ± 1.6	6.4 ± 1.2	0.7987
CRLB% [mIns]	11.2 ± 1.7	9.3 ± 2.2	0.3458
%CSF	4.4 ± 1.0	4.1 ± 2.5	0.7456
	Pre-TKA (N = 13)	**Post-TKA (N = 13)**	***P***
Pre–post TKA group			
SNR	11.3 ± 2.0	10.8 ± 1.4	0.7845
$FWHM_{H2O}$(Hz)	7.3 ± 0.4	7.1 ± 0.7	0.8412
CRLB% [Cr]	5.3 ± 1.2	5.6 ± 1.4	0.8654
CRLB% [NAA]	5.5 ± 1.1	5.1 ± 1.6	0.7232
CRLB% [mIns]	10.5 ± 2.1	11.3 ± 1.1	0.6545
%CSF	4.1 ± 1.1	4.3 ± 2.1	0.7544

CSF, cerebrospinal fluid; CRLB, Cramer-Rao lower bound; FWHM, full-width half-maximum; MRS, magnetic resonance spectroscopy; SNR, signal-to-noise ratio.

patients; however, this was not confirmed with water-referenced Cho ($P = 0.11$) (Supplementary Table S1, available at http://links.lww.com/PAIN/B267). Comparable group differences were observed in the analyses including only a subset of demographically well-matched KOA patients (n = 11) and controls (n = 11). These analyses confirmed higher mIns levels (Cr ratio: $P < 0.001$, Cohen's $d = 1.50$; water-referenced: $P < 0.0001$, Cohen's $d = 2.73$), lower NAA (Cr ratio: $P < 0.01$, Cohen's $d = 0.59$; water-referenced: $P = 0.07$, Cohen's $d = 0.86$) and, when using Cr-normalized ($P < 0.05$, Cohen's $d = 1.21$) but not water-referenced concentrations ($P = 0.07$), also lower Cho levels, in patients compared with controls (Supplementary Fig. S2 and Supplementary Table S2, available at http://links.lww.com/PAIN/B267).

To investigate whether the neurometabolite changes observed in KOA patients might be more pronounced contralaterally to the knee to be replaced (typically the most affected knee), we compared thalamic metabolite levels between patients who were scheduled to undergo left TKA (n = 16) vs those scheduled to undergo a right TKA (n = 18). No significant differences were found between the groups for either creatine ratios (mIns/Cr, $P = 0.62$; NAA/Cr, $P = 0.24$; Cho/Cr, $P = 0.35$) or water-referenced values (mIns, $P = 0.52$, NAA, $P = 0.76$; Cho, $P = 0.37$).

Across patients (n = 33), pre-TKA age-adjusted mIns/Cr showed significant positive correlation with the WOMAC pain scores ($r = 0.37$, $P < 0.05$; **Fig. 3A**). The correlations with stiffness and physical disability were also positive but did not reach statistical significance (r's ≤ 0.3; *P*'s ≥ 0.1). When using water-referenced concentrations, mIns revealed significant positive correlations with all WOMAC scores (pain, $r = 0.52$, $P < 0.01$; stiffness, $r = 0.39$, $P < 0.05$; and physical disability, $r = 0.48$, $P < 0.01$) scores (**Fig. 3B–D**). No other correlations between metabolites (NAA or Cho, whether water-referenced or creatine-referenced) and WOMAC scores (pain, stiffness, or disability) were statistically significant (r ≤ 0.3; $P \geq 0.09$). When evaluating the relationship across presurgical metabolite levels in patients, the only statistically significant association detected was a negative correlation between water-referenced mIns and Cho ($r = -0.46$, $P < 0.01$). To further evaluate whether age had a meaningful impact on our results, we have also correlated metabolite levels and age. No significant correlations were observed between the levels of any metabolite (whether water-referenced or creatine-referenced) and age, for either patients or controls (*P*'s between 0.08 and 0.95).

3.4. Postsurgical alterations in neurometabolite levels

Our within-subject assessment of TKA-related changes in neurometabolite levels (n = 13) revealed significant increases in NAA, both when using Cr-normalized ($P = 0.01$, Cohen's $d = 1.53$) and water-referenced NAA ($P < 0.01$, Cohen's $d = 1.44$), in post-TKA scans compared with pre-TKA scans (**Fig. 4A and B**). The post-surgical NAA levels (whether water-referenced or Cr-normalized), unlike those measured presurgically, were not statistically different from those in healthy controls (whether compared with all controls, correcting for age, or to the subset of age-matching controls). In addition, patients demonstrated a statistically significant post-TKA increase in mIns/Cr ($P < 0.05$, Cohen's $d = 0.82$), although this was not confirmed with the water-referenced mIns levels ($P = 0.20$). Interestingly, the amount of change between pre-TKA and post-TKA timepoints for mIns/Cr and NAA/Cr ratio was positively correlated and significant ($r = 0.61$, $P < 0.05$) (Supplementary Fig. S3, available at http://links.lww.com/PAIN/B267). However, this was not confirmed by the pre–post changes of the water-referenced concentrations ($P = 0.90$). Finally, the assessment of the association of pre–post changes in WOMAC scores with changes in pre–post water-referenced metabolite levels or ratios revealed no significant correlations ($P \geq 0.17$).

4. Discussion

In this study, we noninvasively assessed thalamic neurometabolic alterations in KOA patients, and their response to TKA, using ^{1}H-magnetic resonance spectroscopy. In presurgical KOA patients, we found the levels of mIns, a putative marker of neuroinflammation, to be significantly increased, whereas the levels of NAA, a neurometabolite traditionally interpreted as a marker of neuronal integrity, were reduced compared with healthy controls. Furthermore, age-adjusted presurgical mIns levels showed positive correlations with WOMAC pain scores (using both water-referenced and Cr-referenced concentrations), and stiffness and disability (using water-referenced concentrations alone).

Because myoinositol is found primarily in glial cells,[21] the heightened levels of this neurometabolite in patients might reflect neuroinflammation/glial activation. In fact, our group has previously showed that mIns/Cr levels were abnormally elevated in the motor cortex of patients with amyotrophic lateral sclerosis (a condition known to be characterized by glial activation). Furthermore, the mIns/Cr levels were positively correlated with the levels of the 18 kDa TSPO,[68] another putative imaging marker of glial activation.[19,47,48,76] Using [^{11}C]PBR28 PET imaging, our group showed elevations in TSPO levels, in the brains of patients with cLBP[49] fibromyalgia,[4] migraine[6] veterans suffering from Gulf War Illness,[7] and in the spinal cord of patients with lumbar radiculopathy.[3] Collectively, these TSPO studies suggest that neuroinflammation may be a general feature of chronic pain,

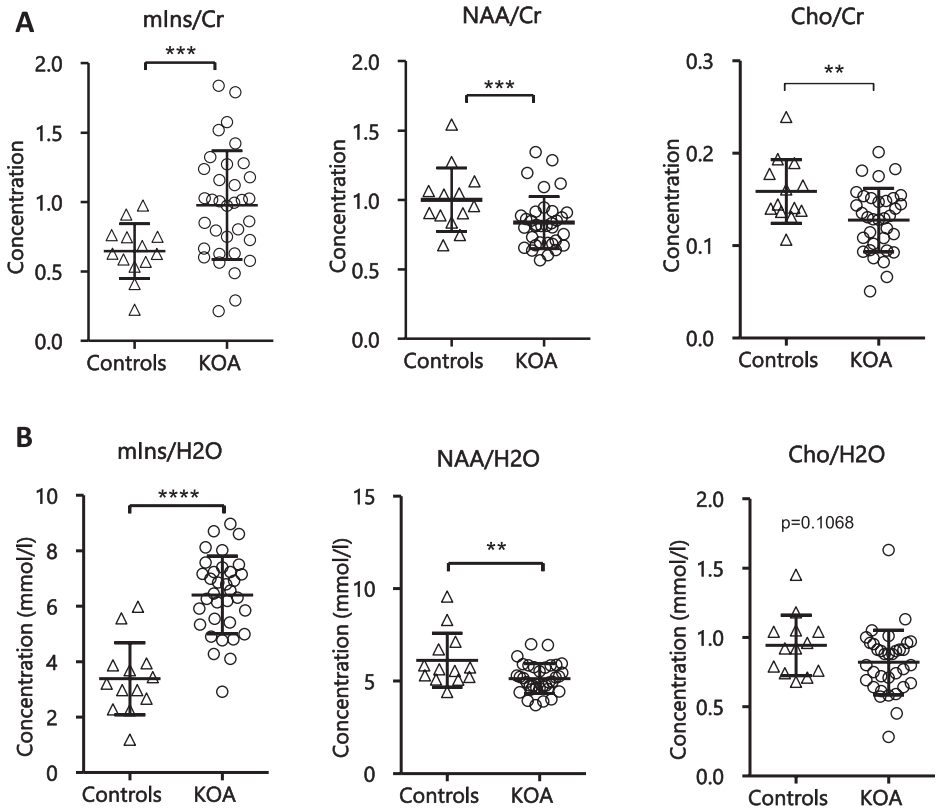

Figure 2. Neurometabolic levels in the left thalamus of KOA and healthy controls. (A) Mean neurometabolic ratios of all KOA (n = 34) and healthy controls (n = 13) corrected for age. (B) Mean water-referenced neurometabolic concentrations of all KOA (n = 34) and healthy controls (n = 13). Statistical significances between the 2 groups and mean concentrations within each group are shown. Concentration values are given as mean ± SD; ***$P < 0.001$, ****$P < 0.0001$. cr, creatine; KOA, knee osteoarthritis; mIns, myoinositol; NAA, N-acetylaspartate.

potentially observable across etiologically heterogeneous pain disorders, thus lending support to the interpretation that the mIns elevations observed in KOA patients may indeed reflect glial activation. After TKA, the patients' levels of mIns/Cr were found to be elevated further, suggesting a possible inflammatory response to surgery. Interestingly, postsurgical increases in patients' mIns/Cr were accompanied by a proportional increase in NAA/Cr levels (which presurgically were lower than in the controls). This observation raises the intriguing possibility that surgery-induced neuroinflammation might have a beneficial role in promoting the

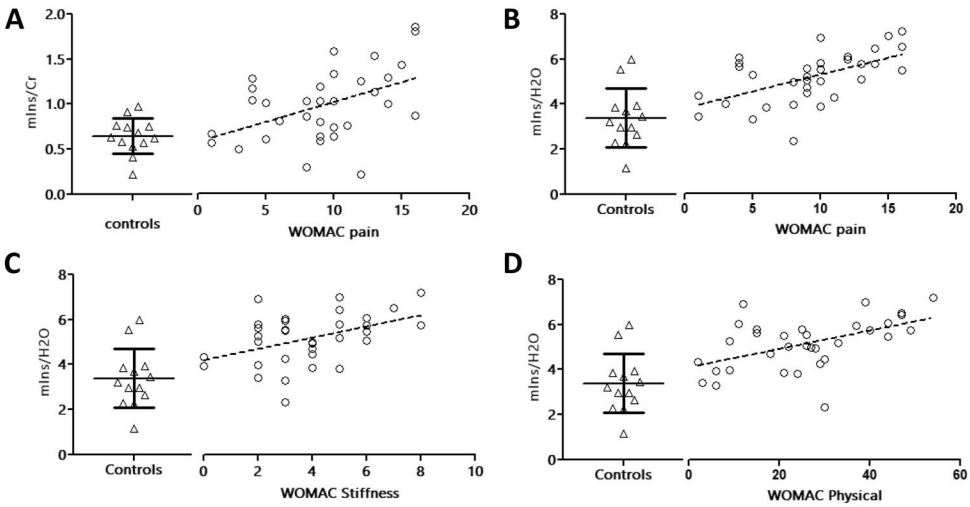

Figure 3. Correlations of neurometabolites and WOMAC pain scores. Correlation between neurometabolite concentrations and the KOA disease severity measured with the WOMAC scale, in KOA patients (n = 33). (A) Correlation of thalamic mIns/Cr and WOMAC pain scale after adjusting for age ($r = 0.37$, $P = 0.038$). Water-referenced concentrations of thalamic mIns with (B) pain ($r = 0.52$, $P = 0.002$), (C) stiffness ($r = 0.39$, $P = 0.026$), and (D) physical ($r = 0.48$, $P = 0.005$) after adjusting for age. The resulting slope in linear fit is represented by the dashed-black line. See Figure 1 caption for abbreviations. KOA, knee osteoarthritis; WOMAC, Western Ontario McMaster Osteoarthritis index.

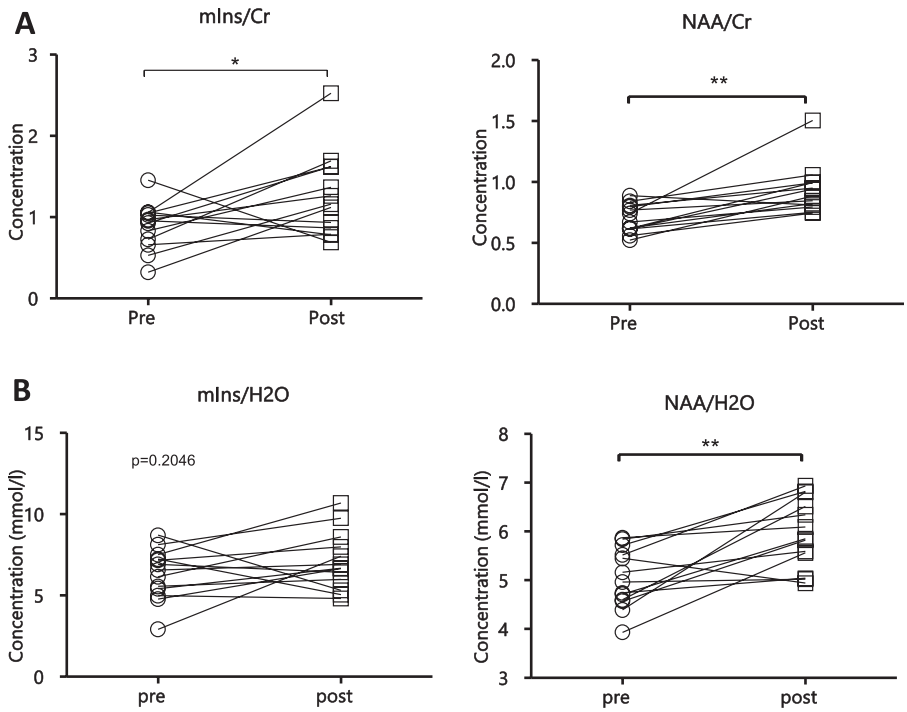

Figure 4. Presurgical vs postsurgical thalamic neurometabolic levels of KOA patients. Pre and post TKA (A) ratios of mIns/Cr ($P = 0.0297$) and NAA/Cr ($P = 0.01$) of KOA patients (n = 13) and (B) water-referenced mIns ($P = 0.2046$) and NAA ($P = 0.0018$). Statistical significances between the 2 groups and mean concentrations within each group are shown. Concentration values are given as mean ± SD; $^{*}P < 0.05$, $^{**}P < 0.01$. Slope in linear fit is represented by the solid black line. see Figure 1 caption for other abbreviations. KOA, knee osteoarthritis; NAA, N-acetylaspartate; TKA, total knee arthroplasty; WOMAC, Western Ontario McMaster Osteoarthritis index.

restoration of neuronal metabolism and/or viability, possibly supporting a dual role of neuroinflammation: adaptive in the acute/subacute context, such as in response to surgery, but pathogenic and maladaptive when dysregulated or in the chronic context.[62] However, when water-referenced concentrations were used, the post-surgical increases in patients' mIns levels were neither statistically significant nor significantly correlated with changes in NAA levels. As such, the significance and reliability of surgery-related changes in mIns remains to be further evaluated. Because NAA is usually considered a neuronal marker, the observed lower presurgical levels of this metabolite in patients would in principle be compatible with a potential reduction in neuronal integrity or viability, perhaps resulting from mechanisms analogous to the pain-induced apoptosis that has been reported in spinal cord neurons in animal models of neuropathic pain.[55,58,90] These neurodegenerative processes might be caused by toxic inflammatory mediators released by activated glia[24,67] or, alternatively, might represent the trigger for a neuroinflammatory response (eg, because of the accumulation of cellular debris).[32,45,53,64] After the surgery, on the other hand, NAA levels returned to levels comparable with those observed in healthy, pain-free controls, an observation that arguably would render less likely the possibility that presurgical reductions in NAA might be due to irreversible neurodegenerative processes. For instance, because some studies showed that NAA is produced in neuronal and oligodendrocytic mitochondria,[9,51,57] the decrease in NAA observed before surgery, and its subsequent reversal after surgery, may be reflective of changes in mitochondrial function,[25] perhaps caused by reactive oxygen species and reactive nitrogen species produced by activation of glial cells.[24,54,71,75] Indeed, mitotoxicity has been described in animal pain models.[14]

Mitochondrial dysfunction may also explain the observed lower Cho/Cr in KOA patients (an observation which, however, was not replicated when using water-referenced Cho concentration). Cho is a cell membrane metabolism and cellular turnover marker that is also often linked to neuroinflammatory processes.[50] The MRS detected choline signal is shown to originate from the water-soluble choline pool, which is one of the precursors of myelin phospholipids synthesis.[2] Because the regulatory processes of membrane homeostasis are sensitive to impairments in energy production,[81] Cho reductions may be affected by deficits in mitochondrial function. In fact, studies have documented significant reductions in both Cho/Cr (as well as NAA/Cr) in patients with mitochondrial diseases.[16] We therefore believe that the Cho/Cr reduction, particularly when viewed in light of the NAA results, might implicate mitochondrial dysfunction, rather than changes in neuroinflammatory processes. However, biological interpretation of MRS detected changes in metabolites will need to be aided by additional work because they are involved in multiple cellular functions.

Our observation of increased NAA levels after surgery is in line with results from previous studies suggesting that a variety of pain-related brain anatomical and functional alterations may be reversed by successful treatment. For instance, in patients with hip osteoarthritis, total joint replacement led to the reversal of at least some of the cortical and subcortical morphological measures found to be altered before treatment, to levels measured in healthy controls.[34,72,73] Similarly, in chronic low back patients, successful surgical treatment was found to reverse both anatomical and functional alterations in dorsolateral prefrontal cortex.[77] Altogether, a growing number of studies suggests that at least some of the microstructural or

macrostructural alterations that have been reported in chronic pain might not reflect irreversible neurodegeneration.

In this study, both water-referenced concentration and relative metabolite levels are reported as complementary results. Although these 2 quantification methods yielded similar results for the most part (ie, lower NAA and higher mIns in presurgical KOA patients, correlations between mIns and clinical pain, postsurgical NAA increase in patients, back to the levels of the healthy controls), some differences were noted (eg, postsurgical elevation in mIns and Cho results). Since both creatine-referenced and water-referenced concentrations are, in fact, ratios (the latter being computed using the unsuppressed water signal as a normalizing factor), random physiological variations inherent in either Cr or water signal may contribute to explaining discrepancies across methods.

To the best of our knowledge, this is the first MRS study to investigate the thalamic metabolic profile in knee osteoarthritic patients. In future studies, it would be interesting to assess whether similar neurometabolic alterations can be observed in other brain regions in the same patient population. Potential targets could be the anterior middle cingulate cortex (where others have reported increases in mIns/Glx ratio,[28] and an association between γ-aminobutyric acid (GABA) levels and ongoing clinical pain intensity, in KOA patients[70]), prefrontal–limbic regions (which were found to be engaged during the processing of ongoing osteoarthritis pain[60]), and the insula (a region demonstrating multiple neurometabolic alterations in various chronic pain disorders; eg, fibromyalgia[38]).

Similar to our findings, previous studies have also demonstrated elevations in thalamic mIns and reductions in NAA concentrations in other chronic pain conditions.[8,29,33,61,78,79] Thus, alterations in NAA and mIns might be a pervasive phenomenon observed across chronic pain patients of different etiologies.

There are several limitations in our study. First, the healthy control cohort, despite being matched in terms of sex, happened to be significantly younger because a disruption in the data collection due to the COVID-19 pandemic. However, our group differences in NAA and mIns were statistically significant both when including all available data sets (controlling for age statistically), as well as when including only a subset of well age-matched KOA patients and healthy controls. In addition, age was not statistically associated with neurometabolic levels in either group (irrespective of the quantification method). Thus, we do not believe that the age imbalance in the full cohorts represents a factor significantly confounding the interpretability of our results. Second, the post-surgical cohort consisted of a relatively small sample of patients, and the lack of longitudinal data in healthy controls limit our ability to interpret the significance of the postsurgical changes observed in the patients. It is also important to stress that, as mentioned before, the specific cellular source of each the MRS-visible metabolites cannot be determined with certainty. For instance, although mIns is commonly referred to as a "glial marker," it is worth noting that this metabolite is involved in a variety of cell functions, including cell signaling and water regulation.[65] As such, whether mIns elevations reflect neuroinflammatory processes remains to be further evaluated (eg, using other purported markers of glial activation or in postmortem evaluations). Finally, during the postsurgical scan, most patients were still taking over-the-counter analgesics/nonsteroidal anti-inflammatory drugs and/or opioids, for the management of their surgical pain. Because the patients were for the most part not taking the same medications during their presurgical scan, whether medications had an impact on the thalamic neurometabolic changes observed postsurgically remains to be evaluated.

In conclusion, our results support a role for glial activation in KOA pain and possibly postsurgical pain. These observations are in line with a growing body of the literature implicating neuroinflammation in pain states and provide the rationale for exploring neuroimmune activation as a potential therapeutic target for pain. Furthermore, by showing that KOA-related reduction in NAA can be normalized after surgery, our results add to a growing literature suggesting that some of the pain-related brain alterations can be reversed after treatment. Finally, when taken together, our results are suggestive of a possible role for brain mitochondrial dysfunction in KOA, although additional work is needed to further evaluate this interpretation.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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Appendix A. Supplemental digital content

Supplemental digital content associated with this article can be found online at http://links.lww.com/PAIN/B267.

Supplemental video content

A video abstract associated with this article can be found at http://links.lww.com/PAIN/B268.

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