Sex Differences in Cerebral Edema after Experimental Traumatic Brain Injury

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ABSTRACT

Traumatic brain injury (TBI) is one of the leading causes of death and disability worldwide. Cerebral edema following TBI is known to play a critical role in injury severity and prognosis. In the current study we used multimodal magnetic resonance imaging (MRI) to assess cerebral edema 24 hours after unilateral contusive TBI in male and female rats. We then directly quantified brain water content in the same subjects *ex vivo.* We found that in male rats, the injured cortex had higher brain water content and lower apparent diffusion coefficient (ADC) values compared with the contralateral side. Females did not show hemispheric differences for these measures. However, both males and females had similarly elevated T_2 values in the injured cortex compared with the contralateral side. A strong correlation was observed between brain water content and T_2 values in the injured cortex in male rats, but not in females. These findings raise questions about the clinical interpretation of radiological findings pertinent to edema in female TBI patients. A more mechanistic understanding of sex differences and similarities in TBI pathophysiology will help improve patient management and the development of effective treatment strategies for TBI in men and women.

INTRODUCTION

Traumatic brain injury (TBI) is a leading cause of death and disability in the United States [1, 2]. Post-traumatic edema is a critical element of TBI pathophysiology and is strongly related to patient outcomes [3-6]. Cerebral edema and subsequent increases in intracranial pressure post-TBI are well-established prognostic factors for morbidity and mortality [3, 6].

Although women incur a significant number of TBIs, much is still unknown regarding differences in pathophysiology between men and women [7, 8]. Historically, men have outnumbered women in clinical trails and most studies in animal models have been limited to male animals [8, 9]. In TBI patients, the severity, type, and duration of symptoms appear to differ between men and women, although prospective and retrospective studies have not supported a significant association between sex and mortality after TBI [10-14]. In rodent TBI models, sex differences in brain damage and neurobehavioral recovery have long been established [15]. Steroid hormones such as estrogen and progesterone are thought to play a protective role against brain edema development and may help explain signficant sex differences [16-18].

In animal research, water content in the brain can be directly quantified via the "wet-dry" method, where freshly dissected tissue is weighed, desiccated, and weighed again to calculate the percent tissue water. Previous studies have used this approach to characterize cerebral edema in diffuse and focal TBI models [19-22], but translation of findings requires alternative non-invasive methods. Magnetic resonance imaging (MRI) is routinely used for clinical management in stable TBI patients [23-25]. MRI is also increasingly being used in animal models to investigate TBI mechanisms including cerebral edema. However, MRI methods have not been used to examine possible sex differences in edema after TBI. The goal of this study was to assess cerebral edema in male and female rats after moderate contusive TBI using multimodal MRI followed by direct quantification of brain water content.

MATERIALS AND METHODS

Animals

Twenty-three male and 23 female rats (F344; Charles River, Wilmington, MA, USA) were used in the study. Rats were 67-83 days old and weighed 157-229 g (male) and 112-152 g (female) at the time of surgery. Animals were housed in same-sex pairs on a 12-hour light-dark cycle with free access to food and water. All protocols were approved by the KUMC Animal Care and Use Committee consistent with the standards of animal care in the US Public Health Service Policy on the Humane Care and Use of Laboratory Animals. Each surgery cohort contained an equal number of male and female rats, and each animal was randomized to TBI or sham groups using a random sequence generator.

Controlled Cortical Impact (CCI) and Sham surgeries

CCI and sham surgeries were carried out as we have previously described [26-28]. In brief, animals were anesthetized with isoflurane (4% induction, 2% maintenance in 2:1 Medical air:Oxygen). Body temperature was maintained with a heating pad. The head was immobilized in a stereotaxic frame, and the skin shaved and scrubbed with iodine and ethanol. Bupivacaine (0.25%) was administered subcutaneously at the surgical site. Utilizing aseptic technique, a midline incision was made and the skull exposed. The circular craniotomy was formed by a 6mm Michele trephine over the right sensorimotor cortex, lateral to bregma and centered between bregma and the temporal ridge. Moderate CCI was delivered with an impactor mounted to a stereotaxic manipulator (Leica; Saint Louis, MO; impactor tip diameter = 5mm; velocity = 5 m/s; depth = 2mm; contact time = 300 ms; angle = 5° from vertical). Sham rats received the same procedure without impact. Anesthesia time for sham surgeries was extended to match that of CCI surgeries. The incision was sutured closed and animals were transferred to a heated recovery cage. After recovery of locomotion animals were returned to their home cages.

Magnetic Resonance Imaging (MRI)

MRI scans were performed approximately 24 hours after TBI (average 23.4 ± 2.8 hours) on a 9.4 Tesla system with a Varian INOVA console (Agilent Technologies, Santa Clara, CA). The system is equipped with a 12 cm gradient coil (40 G/cm, 250 µs; Magnex Scientific, Abingdon, UK). A custom-made quadrature surface radiofrequency coil, consisting of two geometrically decoupled 18mm loops, was placed on the animal's head to transmit and receive at 400 MHz.

During imaging, anesthesia was delivered via nosecone (1.5 - 3% isoflurane in 2:1 Medical Air:Oxygen) to maintain a respiration rate of 40 - 80 cycles/minute. Respiration was monitored with a pressure pad (SA Instruments, Stony Brook, NY, USA). Animals were placed on a heating pad and body temperature was maintained at 37 ± 1 °C via feedback control (Cole Parmer, Veron Hills, IL, USA). Transverse and sagittal GEMS localization images were acquired to check the animal's positioning in the magnet (FOV_{ax} = 2.56 x 2.56 cm^2, FOV_{sag} = 5.12 x 2.56 cm^2, matrix = 128 x 128, TR/TE = 90 ms/2.8 ms, slices = 9, thickness = 1 mm). High-resolution T_2-weighted images were acquired using a RARE sequence (FOV = 2.56 × 2.56 cm^2, matrix = 256 × 256, TR/TE = 4000/72 ms, averages = 2, thickness = 1 mm, echo train length = 8, echo spacing = 18 ms, total acquisition time ~4 minutes).

Diffusion weighted imaging (DWI) was performed using a standard mono-polar diffusion weighted spin echo sequence (FOV = 2.56 × 2.56 cm^2, matrix = 128 × 128, TR/TE = 1500/26 ms, gradients applied along readout direction, b = 50, 500, 1000 s/mm^2, averages = 1, slices = 9, thickness = 0.8 mm with a 0.2 mm gap, total acquisition time 9 minutes 40 seconds). Apparent diffusion coefficient (ADC) values were calculated for each voxel in MATLAB (Mathworks Inc., Natick, MA, USA) from a 2-parameter exponential fit of the b values.

For mapping T_2 weighted images, a series of multi-slice spin echo acquisitions were performed (FOV = 2.56 × 2.56 cm^2, matrix = 128 × 128, TR = 3 s, TE = 20, 30, 50, 75, 100, 150 ms, averages = 2, slices = 9, thickness = 0.8 mm with a 0.2 mm gap, acquisition time = 6 minutes 25 seconds for each TE).

T_2 values were calculated for each voxel in MATLAB from a 2-parameter exponential fit of the TE values. The DWI and T_2 maps were acquired with identical slice positions, FOV, and spatial resolution.

Brain water content

Immediately following MRI, rats received an overdose of isoflurane and were euthanized via decapitation. Brain tissue was rapidly extracted from the skull, placed in a coronal slicing matrix, and a 10 mm block was cut beginning ~1 mm rostral to the cortical injury extending caudally (**Fig. 1A**). The block was removed, cut in half horizontally to separate dorsal from ventral structures, then cut in half vertically at the midline (**Fig. 1B**). The dorsal brain samples from the left (contralateral to TBI) and right (ipsilateral to TBI) hemispheres were used for wet-dry analyses. Each sample was immediately weighed (Mettler Toledo AE100 Scale, Columbus, OH, USA) and values were recorded as the wet weight. The samples were then placed in a laboratory oven (Grieve Laboratory Oven Model L R-271C, Round Lake, IL, USA) at 100 °C. Dry weights were recorded after 24, 48 and 72 hours and the lowest dry weight was used in water content calculation; for most subjects this was at 72 hours. Water content was calculated using the formula: [wet weight (g) - dry weight (g)] / [wet weight (g)] x 100.

MRI analysis

Regions of interest (ROIs) were drawn over the ipsilateral and contralateral cortex for each subject using MIPAV software. ROIs were drawn on three sequential diffusion weighted images (b = 50 s/mm^2) encompassing the center of the injury (centered at bregma). First a vertical line was drawn at the midline, and from a point marked at the base of the brain a second line was drawn at a 45-degree angle. The ROI was completed by connecting the points where these lines crossed the cortex, following the dorsal surface of the brain and the corpus callosum (**Fig. 2A**). After the ROI was drawn for the ipsilateral cortex it was flipped 180 degrees across the vertical midline and manually adjusted to fit the contralateral hemisphere. The same ROIs, generated by a single experimenter, were used for analysis of DWI and T_2 images.

Statistical analysis

For ipsilateral-to-contralateral comparisons to evaluate the effect of TBI or sham injury on tissue water, ADC, and T_2 values, we used the Wilcoxon signed rank test. To test for sex differences, we conducted a two-way analysis of covariance (ANCOVA) for ipsilateral tissue water, ADC, and T_2 values with sex and group as two factors using the contralateral side as a covariate. For each outcome measure, we removed interaction terms that were not significant from the model, then re-ran the simplified ANCOVA. Post-hoc tests were conducted if there was a significant interaction between sex and group, controlling for family-wise error at the 0.05 level. Correlations between MRI parameters and brain water content were analyzed using the Spearman test. Statistical significance was defined as $p < 0.05$. Data are reported at mean ± standard error. All tests were two-sided and performed with R software [29].

Subject exclusions and MRI quality screening

Fifteen male and 15 female rats received CCI; eight male and eight female rats received sham surgery. One female sham was excluded from the study due to surgical error. One female in the TBI group died during imaging and was excluded. Tissue water data was excluded from 4 rats (1 male TBI, 3 female TBI) due to data collection error. Quality assessments were performed for MRI by a blinded screener. For DWI, motion artifacts led to exclusion of 2 male TBI, 2 male sham, 2 female TBI and 1 female sham. For the T_2 images, 2 male TBI and 1 female TBI were excluded due to data corruption and 1 female sham was excluded due to motion artifact.

RESULTS

Brain water content

The male TBI group had significantly elevated brain water on the ipsilateral compared with the contralateral side (83.16 ± 1.09 vs. 82.92 ± 1.08, $p = 0.0031$; **Table 1**) while there was no difference between sides for the male sham group (84.16 ± 1.59 vs. 84.26 ± 1.60). In female rats, there was no difference between ipsilateral and contralateral brain water in the TBI group (83.82 ± 1.03 vs. 83.86 ± 1.07) or in the sham group (83.05 ± 1.56 vs. 82.67 ± 1.51). Brain water content showed a significant interaction between sex and injury group: $F(1, 35)= 8.384$, $p = 0.006$. Followup testing showed that in males, there was a significant difference between the TBI and sham groups ($t=-2.57$, $p = 0.0146$), but no difference in females.

Diffusion weighted imaging

In the male TBI group, ADC values ipsilateral to the injury were lower than those on the contralateral side (6.2E-04 ± 8.63E-06 vs. 6.60E-04 ± 1.25E-05, $p = 0.023$; **Fig. 2B**). In the male sham group ADC values were not different between the ipsilateral and contralateral sides (6.38E-04 ± 1.40E-05 vs. 6.00E-04 ± 1.12E-05). In female rats, there were no differences between ipsilateral and contralateral ADC values in the TBI group (6.36E-04 ± 1.98E-05 vs. 6.13E-04 ± 2.15E-05) or in the sham group (6.58E-04 ± 1.67E-05 vs. 6.52E-04 ± 1.74E-05). For ADC there was no significant interaction between sex and injury group and no difference between TBI and sham groups for either sex.

T_2 -weighted image mapping

In both the male and female TBI groups T_2 values ipsilateral to injury were higher than on the contralateral side (male TBI 41.85 ± 0.68 vs. 38.38 ± 0.16, $p = 0.0002$; female TBI 41.56 ± 0.47 vs. 38.36 ± 0.31, $p = 0.0002$ **Fig. 2C**). There were no differences between the hemispheres in the male or female sham groups (male sham 38.46 ± 0.32 vs. 38.6 ± 0.15; female sham 38.86 ± 0.38 vs. 38.16 ± 0.23). For T_2, the main effect of injury group was significant ($F(1,36)= 29.449$, $p < 0.0001$), but there was no main

effect of sex and no interaction between sex and injury group. Followup testing showed that in both males and females there was a significant difference between the TBI and sham groups (male TBI vs. sham t =-4.57, $p < 0.0001$; female TBI vs. sham, t=-2.98, $p = 0.005$).

Correlation between brain water content and imaging measures

In the ipsilateral hemisphere of male TBI rats, brain water content trended toward a negative correlation with ADC values but this did not reach statistical significance (r = - 0.44, $p = 0.15$; **Fig. 3**). In males after TBI there was a strong positive correlation between brain water and T_2 values (r = 0.73, $p = 0.007$). By contrast, in the ipsilateral hemisphere of female TBI rats, brain water content was not correlated with either ADC (r = 0.30, $p = 0.43$) or with T_2 values (r = 0.012, $p = 0.96$).

DISCUSSION

Sex differences in TBI damage and subsequent functional recovery have long been observed. However, the mechanisms underlying this observation have not been well characterized. This study used multimodal MRI and brain water measurements to assess cerebral edema 24 hours after contusive TBI in male and female rats. At this acute time point we found greater evidence of edema in males than in females. In male rats the injured cortex had higher brain water and lower ADC compared with the contralateral side. Females did not show hemispheric differences for these measures. However, both males and females had higher T_2 values in the injured cortex compared with the contralateral side.

Classically, cerebral edema has been categorized as either vasogenic or cytotoxic in origin. More recently it has been recognized that this distinction may be somewhat arbitrary since the molecular mechanisms of both processes are highly inter-related [30]. Vasogenic edema arises from direct disruption to the blood brain barrier and leaking of solute-laden fluid from the blood into the brain interstitial space. Cytotoxic edema results from disruptions to cell membrane ion channels causing ion-driven movement of water into cells and a relative shrinkage of the extracellular compartment. Quantitative DWI and T_2 mapping have each been used as imaging biomarkers of cerebral edema [31-35]. The ADC calculated from DWI is a measure of water diffusion within the target tissue, which is influenced by the relative balance of water in the intracellular versus the extracellular compartment. Thus, elevated intracellular water (i.e. cytotoxic edema) tends to lower the ADC, while elevated extracellular water (vasogenic edema) tends to increase ADC [35-39]. By contrast, T_2 mapping measures the transverse relaxation time of protons and is sensitive to the total water content in the tissue regardless of cellular compartment.

In male rats we found lower ADC and higher T_2 values in the injured cortex, confirming a predominantly intracellular edema as previously reported [35, 37, 40]. In female rats, we found no change in ADC but elevated T_2 values in the injured cortex after TBI. One possible interpretation is that female rats

had increased water in both the intracellular and extracellular compartments, leading to elevated T_2 values but no *net* change in ADC. Alternatively, T_2 mapping may be a more sensitive measure of cerebral edema than diffusion weighted imaging. Why female rats showed elevated T_2 after CCI but not elevated brain water content remains unclear, but could arise from the slightly different regions sampled or from differing sensitivities of the two methods.

We measured cerebral edema 24 hours post injury, a time point that corresponds to maximal edema after CCI in adult male rats [21, 22, 40, 41]. Since cerebral edema is a dynamic process, the timing of which is likely to be affected by the severity and type of brain injury, results from single time point studies should be interpreted with caution. One interpretation of our results is that females develop less edema than males after identical TBI procedures; an alternative explanation might be that the time course of edema development differs in males versus females. Indeed, in a time course study of diffuse TBI, O'Connor et al. reported that female rats had more severe edema than males at 24h, *and also* had less edema than males at earlier and later timepoints [42]. To date, sex differences in the time course of edema have not been investigated after focal TBI, and longitudinal studies that include both sexes remain an important area for future research.

In this initial study we did not control for hormonal status in female rats, since in the real world women may sustain TBI at any time in their cycle. A growing body of research indicates that estrogen and progesterone may attenuate several aspects of TBI pathophysiology, including edema [17, 18, 43-47]. It is possible that differing sex hormone levels associated with cycle stage contributed to the greater variability of ADC values we observed in female rats compared with males. However, the variability of brain water and T_2 values was similar in males and females. This underscores the growing recognition that hormonal effects on TBI outcome are complex and may depend on the particular outcome measure used, the time point, and characteristics of the injury [7, 8, 17, 48-51].

Translational neuroimaging studies in animals allow for direct comparison between non-invasive MRI measures and invasive assays to confirm pathophysiological mechanisms. We found that ADC and T_2 values—imaging measures that have traditionally been used to make inferences about cerebral edema—were associated with measured brain water content as predicted in male rats, but not in females. The reason for this is not currently clear, but raises important questions about clinical interpretation of radiological findings in female TBI patients. Due to a historic focus on males in TBI research, much remains unknown about sex differences in TBI pathophysiology. The lack of data currently available on the time course and molecular mechanisms of cerebral edema in females needs to be addressed. A better understanding of the differences and similarities between sexes will help with patient management and the development of effective treatment strategies.

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AUTHOR CONTRIBUTION STATEMENT

J Harris and HM conceptualized and designed the study; J Harris performed surgeries; HM, SC, and PK performed the MRI and brain water experiments; HM, SC, PK, J Hu completed data processing and analysis; J Harris, J Hu, HM, BA contributed to data interpretation and manuscript preparation.

AUTHOR DISCLOSURE STATEMENT

The authors have no competing financial interests.

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Legends

Figure 1. Brain injury location and regions dissected for brain water content analysis. A) dorsal view of the rat brain. The circle depicts the site of cortical impact (or craniotomy in sham rats). Using a coronal slicing matrix, a 10mm thick section of brain was dissected beginning ~1 mm anterior to the cortical injury and extending caudally. (B) The 10 mm section was further dissected with a horizontal slice at the bottom of the corpus callosum as shown, and a vertical slice to separate the left (contralateral to TBI) and right (ipsilateral to TBI) sides. The dorsal brain samples from each side were used for analysis of brain water content.

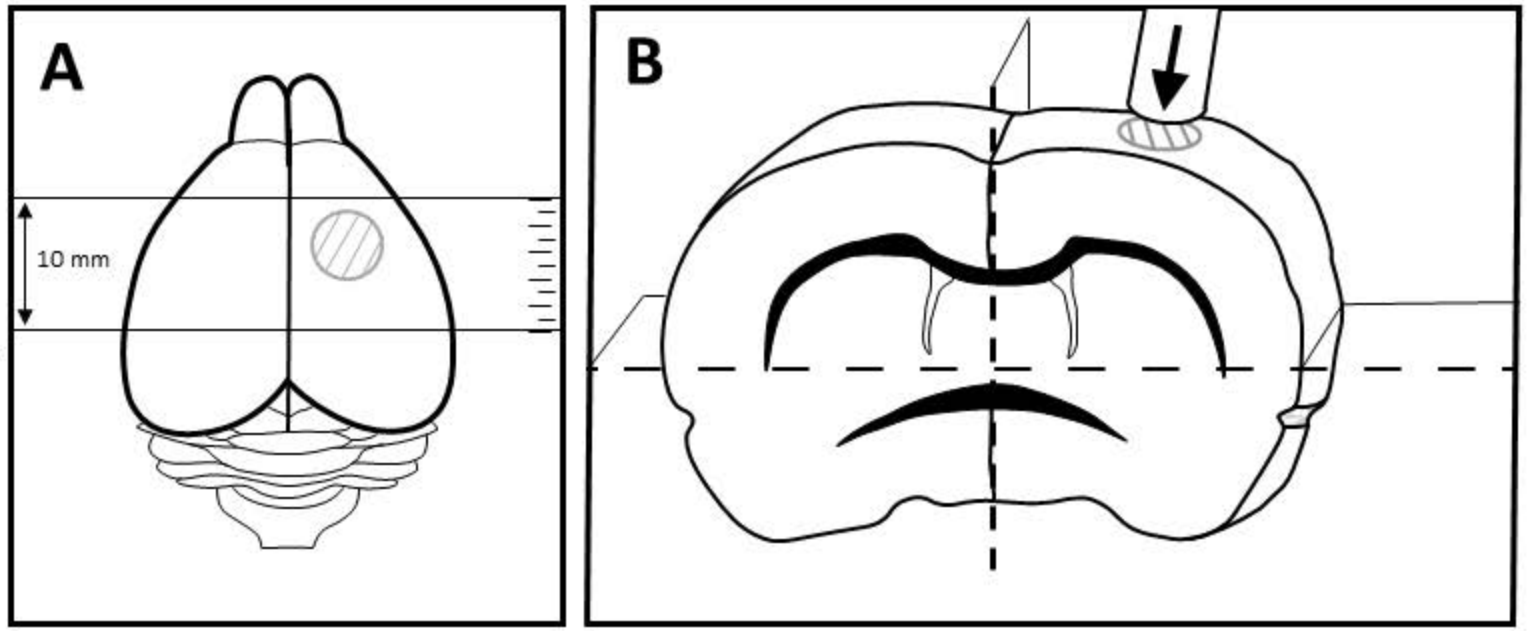
Figure 2. Magnetic resonance imaging measures of cerebral edema after TBI in male and female rats. **(A)** Regions of interest (ROI) for image analysis are shown for the ipsilateral (black outline) and contralateral (grey outline) cortex in MRIs from a male rat 24 hours after controlled cortical impact. **(B)** Apparent diffusion coefficient (ADC) values in male and female rats. In the male TBI group, ADC was lower ipsilateral to injury compared with the contralateral side (*p = 0.023). There was no significant difference between hemispheres in the female TBI group, nor in sham-injured rats of either sex. Plot shows ROI mean values for individual rats (male TBI n=13, male sham n=6, female TBI n=12, female sham n=6), black bars depict group means. **(C)** T_2 values in male and female rats. T_2 values were higher in the ipsilateral cortex compared with the contralateral side after TBI in both males (**p = 0.0002) and females (**p = 0.0002). There were no significant differences between hemispheres in sham-injured groups. Plot shows ROI mean values (male TBI n=13, male sham n=8, female TBI n=13, female sham n=6), black bars depict group means.

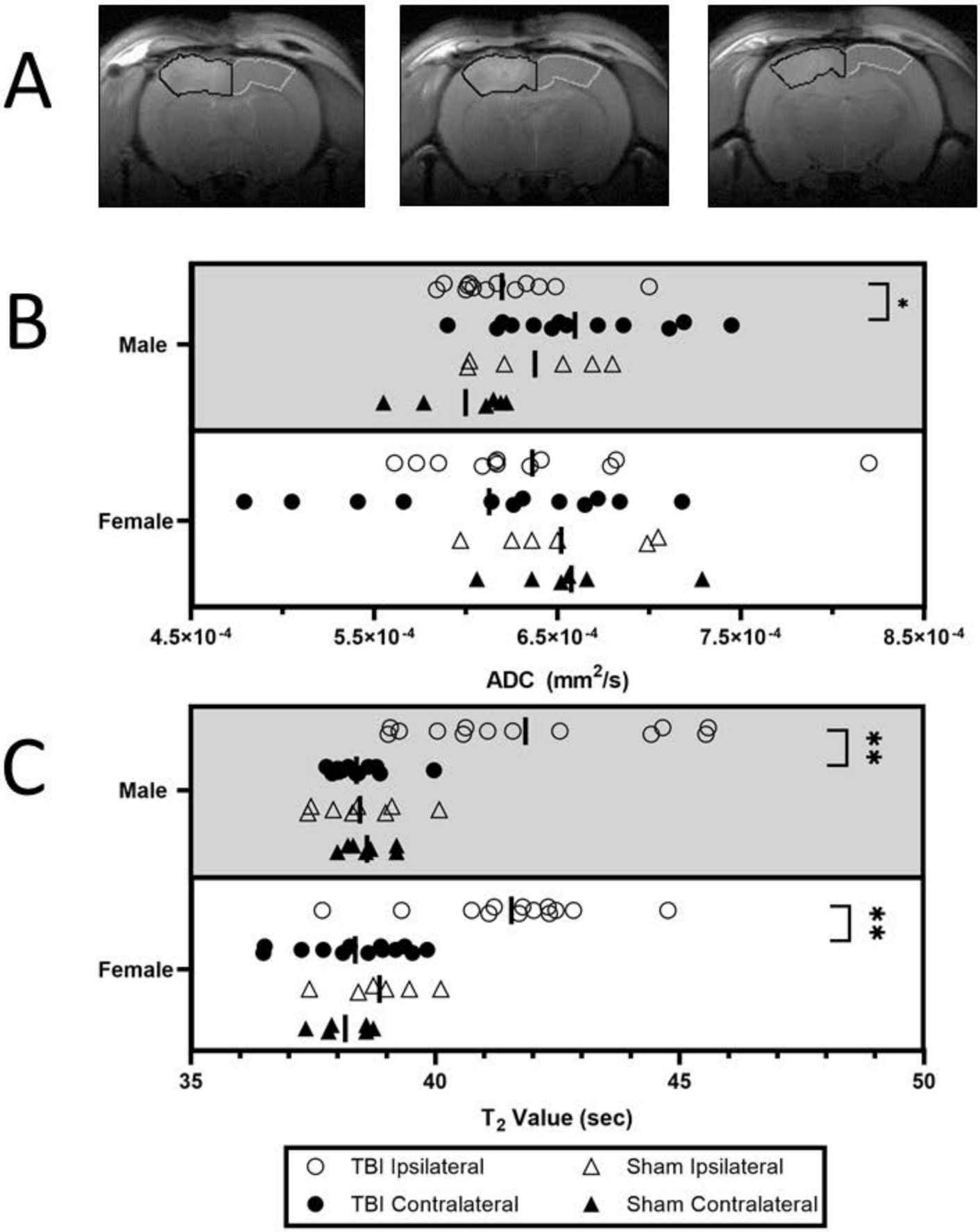
Figure 3. Relationship between brain water content and MRI measures of edema after TBI in male and female rats. In the injured hemisphere of male rats 24 hours after TBI, there was a non-significant trend toward negative association between brain water content and ADC values (r = -0.44, *p* = 0.15). In males, brain water content was strongly correlated with T_2 values in the cortex ipsilateral to injury (r = 0.73, *p* = 0.007). By contrast, water content in the injured hemisphere of female rats was not correlated with ADC (r = 0.30) or with T_2 values (r = 0.01).

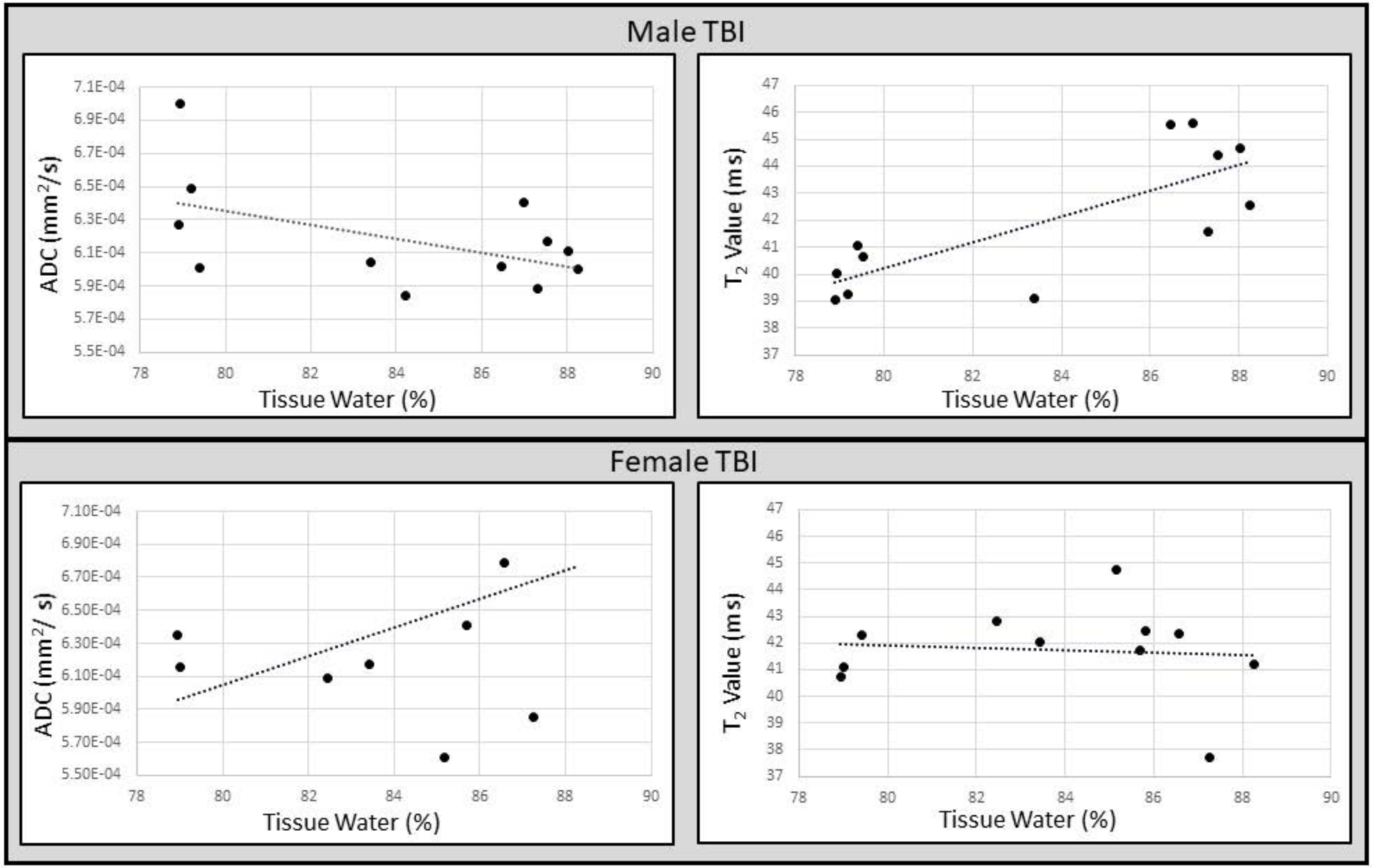
Table 1. Brain water content in male and female rats 24 hours after TBI or sham surgery.

	Ipsilateral (% Tissue water)	**Contralateral (% Tissue water)**	**P Value (Ipsilateral to Contralateral)**
Male TBI	83.16 ± 1.09	82.92 ± 1.08	0.0031*
Male sham	84.16 ± 1.59	84.26 ± 1.60	0.64
Female TBI	83.82 ± 1.03	83.86 ± 1.07	0.90
Female sham	83.05 ± 1.56	82.67 ± 1.51	0.16

Male TBI n=14, male sham n=8, female TBI n=11, female sham n=7. Data are represented as mean ± standard error.


A
10 mm
B


A
B
Male
Female
*
4.5×10-4
5.5×10-4
6.5×10-4
7.5×10-4
8.5×10-4
ADC (mm2/s)
C
Male
Female
**
**
35
40
45
50
T2 Value (sec)
TBI Ipsilateral
TBI Contralateral
Sham Ipsilateral
Sham Contralateral


Male TBI
ADC (mm²/s)
7.1E-04
6.9E-04
6.7E-04
6.5E-04
6.3E-04
6.1E-04
5.9E-04
5.7E-04
5.5E-04
78
80
82
84
86
88
90
Tissue Water (%)
T2 Value (ms)
47
46
45
44
43
42
41
40
39
38
37
78
80
82
84
86
88
90
Tissue Water (%)
Female TBI
ADC (mm²/ s)
7.10E-04
6.90E-04
6.70E-04
6.50E-04
6.30E-04
6.10E-04
5.90E-04
5.70E-04
5.50E-04
78
80
82
84
86
88
90
Tissue Water (%)
T2 Value (ms)
47
46
45
44
43
42
41
40
39
38
37
78
80
82
84
86
88
90
Tissue Water (%)