Title: Magnetic Resonance measures of small bowel wall T2 are associated with increased permeability

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Magnetic Resonance measures of small bowel wall T2 are associated with increased permeability

Abstract:

Background: Increased small bowel permeability leads to bacterial translocation, associated with significant morbidity & mortality. Biomarkers are needed to evaluate these changes in vivo, stratify an individual's risk, and evaluate the efficacy of interventions. MRI is an established biomarker of small bowel inflammation.

Purpose: To characterize changes in the small bowel with quantitative MRI measures associated with increased permeability induced by indomethacin.

Study Type: Prospective single-center, double-blind, 2-way crossover provocation study.

Subjects: A provocation cohort (22 healthy volunteers) and intra-subject reproducibility cohort (8 healthy volunteers).

Field Strength/Sequence: 2D Balanced turbo field echo sequences to measure small bowel wall thickness, T2 and motility acquired at 3 T.

Assessment: Participants were randomized to receive Indomethacin or placebo prior to assessment. After minimum two week washout, measures were repeated with the alternative allocation. MR measures (wall thickness, T2, motility) at each study visit were compared to reference standard 2 hour Lactulose/Mannitol urinary excretion ratio (LMR) test performed by a lab technician. All analysis was performed blind.

Statistical tests: Normality was tested (Shapiro-Wilk's test). Paired testing (Student's t-test or Wilcoxon) determined significance of paired differences with indomethacin provocation. Pearson's Correlation Coefficient compared significant measures with

indomethacin provocation to LMR. Intra-subject (intra-class correlation) and inter-rater variability (Bland-Altman) were assessed.

Results: Indomethacin provocation induced a significant increase in LMR compared to placebo ($p<0.05$) and a significant increase in small bowel T2 (0.12 s compared to placebo 0.07 s, $p<0.05$). Small bowel wall thickness ($p=0.17$) and motility ($p=0.149$) showed no significant change. T2 and LMR positively correlated ($r=0.68$, $p<0.05$). T2 measurements were robust to inter-observer (intra-class correlation 0.89) & intra-subject variability (Bland-Altman bias of 0.005s, 95%CI -0.04s to +0.05s, and 0.0006s, 95%CI -0.05 to +0.06s).

Data Conclusion: MR measures of small bowel wall T2 were significantly increased following indomethacin provocation and correlated with 2-hour LMR test results.

Introduction

The bowel wall is a dynamic, porous barrier between the host organism and the environment (1). Gut wall permeability is a general term that refers to the phenomenon of material passing through the wall via paracellular transport and transcellular permeability. Increased gut permeability is implicated in the pathophysiology of a number of gastrointestinal diseases characterized by gut wall inflammation such as coeliac disease and inflammatory bowel disease, but also in disorders without overt gut inflammation such as liver cirrhosis, irritable bowel syndrome, obesity, diabetes, HIV and those who go on to develop or have inflammatory bowel disease in remission (2, 3).

Passage of nutrients through the gut wall is a normal physiological process, but an increase in gut wall permeability can result in bacterial translocation (BT) (4) which is defined as passage of bacteria and/or bacterial products across the apparently intact gut wall (5) either via the mesenteric lymph nodes or directly through the portal circulation. There is evidence that the small bowel is the principle focus of pathological BT (6, 7). BT is common in cirrhosis (8, 9) and leads to a systemic inflammatory response which exacerbates the hyperdynamic circulation resulting in increased portal pressure (4, 10, 11). BT is also implicated in complications of cirrhosis including variceal bleeding, spontaneous bacterial peritonitis and hepatic encephalopathy (10). In Crohn’s disease, increased bowel permeability has been reported before macro- and microscopic manifestation of the disease (12, 13) and is reversible with biological therapy (14). It has been suggested that ankylosing spondylitis and multiple sclerosis can be triggered by BT (15, 16).

Increased gut permeability has also been associated with hyperglycemia, which in mouse models has been shown to drive intestinal barrier permeability by altering tight and adherence junction integrity (3). The concept that increased permeability is directly associated with leakage through the gap-junctions of the mucosal barrier has been demonstrated using direct visualisation of bowel wall function in vivo using confocal endomicroscopy and a peripherally injected contrast agent (17). However, visualisation is limited to small regions of the bowel, and the cost and sedation required limit this technology to highly selected patients in specialist centers. Alternatively, measurement of intestinal permeability involves monitoring differential urinary excretion of sugars or sugar alcohols that are absorbed in the bowel and poorly metabolised (e.g., lactulose, mannitol, rhamnose, sucralose) (18). The lactulose to mannitol excretion ratio (LMR) is the most widely used and validated marker (3, 19) as evidenced by its inclusion as a treatment efficacy endpoint in clinical trials (20, 21). The LMR test has the benefit of using the ratio of two molecules rather than the measured amount of a single molecule, which is thought to correct for inter-individual differences in processing of the molecules (e.g., bowel transit, renal function and tissue distribution) that are unrelated to permeability (18). Administration of oral indomethacin is a well-validated, safe provocation that increases small bowel permeability (22), with a two week washout period being demonstrated to be adequate in preventing cross-contamination (19). Animal models have also demonstrated that indomethacin causes an acute stimulation of gut motility (23, 24).

There is a pressing need for standardized, widely available, non-invasive markers of gut wall changes related to increased permeability and BT. Such measures would allow study of the underlying mechanisms of altered gut permeability and the effects of

interventions and could improve management of therapies in key patient groups in a personalized medicine approach. Various aspects of bowel structure and function can be measured with MRI.

Several publications have identified subjective T2-weighted measures as being important in the MR assessment of the bowel wall, particularly in relation to Crohn's disease (25). Aside from gut wall enhancement with contrast, a meta-analysis of MR enterography showed the parameters with consistently highest sensitivity and specificity for bowel wall inflammation were wall thickness and motility (25). Terminal ileal motility score showed good agreement with endoscopic and histopathologic activity in Crohn disease, suggesting that it is sensitive to gut inflammation (26).

We hypothesized that quantitative MRI measures of small bowel wall thickness, T2 and motility would relate to increased small bowel permeability in healthy volunteers exposed to an indomethacin challenge (22). Our aim was to undertake a single institution, validation of these quantitative MR small bowel measures as a test of intestinal permeability compared to LMR as a reference standard in an indomethacin-challenged healthy volunteer model of increased intestinal permeability. Due to the semi-automated analysis of the quantitative MR measures we hypothesised there would be an excellent intra-class correlation with minimal intra-subject variability.

Materials and Methods

The study protocol was approved by the University of Nottingham School of Medicine ethics board (Ref. B10112015) and ran from April 2016 until December 2016. An additional study cohort was performed without the use of indomethacin to test the inter observer reproducibility and intra-subject test-retest variability of T2 measurements in healthy volunteers (approved by the University of Nottingham School of Medicine ethics board Ref. J/3/2007). Written informed consent was taken for all subjects as specified by Good Clinical Practice.

Design

This was a single-center study with two healthy volunteer cohorts. The provocation cohort underwent a double-blind, 2-way crossover provocation study administering two doses of 75 mg slow-release indomethacin or placebo. Participants returned after a minimum two week washout period for a repeat study day on the alternative allocation. The order of indomethacin or placebo administration was randomised and blinded with both pills manufactured to appear identical. The second cohort underwent exactly the same study visits, again a minimum of two weeks apart, but without the indomethacin/placebo administration. The study ran from April 2016 until December 2016. The second cohort was performed without the use of indomethacin to test the inter-observer agreement and intra-subject test-retest variability of T2 measurements.

Participants

Participants in the provocation cohort were screened for eligibility and consented prior to randomisation. To be eligible, participants had to have no exclusion factors known to increase small bowel permeability. Exclusion criteria included: pregnancy, chronic gastrointestinal disorders or symptoms, diabetes mellitus (type 1 or 2), smoking, psychiatric disease, coeliac disease, food allergy, history of atopy, allergy or intolerance to non-steroidal anti-inflammatory drugs (NSAIDs), first degree relative with inflammatory bowel disease, coeliac disease or type 1 diabetes mellitus, alcohol dependency, estimated glomerular filtration rate <45mL/min or any contraindications to MRI. For two weeks prior to a study visit, volunteers were instructed not to take any regular medications other than oral contraceptives. Participants were informed not to smoke, drink alcohol or ingest any artificial sweeteners for 72 hours prior to either study visit. In addition, all NSAIDs were prohibited throughout the study. Height and wight were recorded at the screening visit to calculate the Body Mass Index (BMI) of each participant prior to both study visits.

Randomization and blinding

All participants in the provocation cohort were randomised to receive either indomethacin or placebo administration first. After a minimum two week washout period they returned for a second study visit for repeat measures on the alternative treatment arm to act as their own controls. The indomethacin and placebo tablets were manufactured to appear identical. All analyses were performed blind to the treatment allocation and other biomarker results.

Interventions and procedures

Provocation cohort

The order of the procedures for each study day (two study days in total for each participant) is shown in Supplementary Figures 1 and Supplementary Figure 2. Participants were fasted on the day of the study. They took time-stamped digital photographs of themselves consuming the treatment tablet (placebo or indomethacin) 16 hours and 4 hours before the planned midway point of a two-hour Lactulose/Mannitol urinary excretion test (usually at 10:30am). Upon arrival at the test center, a cannula was inserted, and blood samples were taken prior to the test. Subjects emptied their bladders and within 5 minutes ingested 5 g of lactulose and 2 g of mannitol dissolved in 100 ml of water, in a 1-minute time window in the presence of an investigator. Thirty minutes after sugar administration, 500 ml of water was given to aid in the collection of urine. Water was allowed *ad libitum* thereafter. The LMR in the urine collected in the first two hours after ingestion was used to quantify the small bowel permeability.

Prior to the MRI scan, participants were given an oral contrast solution (consisting 1 L of water, 25 g/2.5% Mannitol and 2.0 g/0.2% locust bean gum). Forty-five minutes prior to the start of the MRI scan 0.5 L of the solution was given. The remaining 0.5 L was ingested equally over the 15 minutes prior to the start of the MRI data acquisition to obtain optimal distension of the small bowel and terminal ileum.

MRI acquisition

All images were acquired using a whole-body Philips 3T Achieva (N=46) with a 16 channel XL Torso coil or Philips 3T Ingenia (N=2) with a 32 channel dStream Torso coil (Philips Healthcare, Netherlands). Participants lay in the prone position with their arms by their head scanned feet first. After acquisition of the anatomical scans to locate the regions of interest, small bowel motility scans were acquired. Subjects were then given two doses of 20 mg intravenous Buscopan™ (hyoscine N-butylbromide) separated by a minimum of 10 minutes followed by the T2 and bowel wall thickness scans.

To provide images to measure bowel wall thickness, a 2D balanced Turbo Field Echo (bTFE) sequence was acquired covering the entire small bowel in two 16 second breath-holds. Twenty coronal slices were acquired at resolution 1.2 × 1.2 × 3 mm^3 with an in-plane reconstruction of 0.78 × 0.78 mm^2. Additional parameters included the following: echo time (TE)/repetition time (TR) of 1.79/3.59 ms, flip angle of 50^o, half Fourier acquisition (0.7), sensitivity encoding (SENSE) factor of 1.5 , field of view (FOV) of 340 x 352 mm^2, and 2 acquisitions were averaged.

For the T2 measurement a single slice, spin echo prepared, 2D bTFE (TE/TR = 1.68/3.4 ms; flip angle = 50°, half Fourier acquisition (0.625)) was acquired at echo times of 20, 50, 80, 120, 180, and 300 ms (35). Each spin echo was acquired in a separate breath-hold with a minimum wait time of 15 s between scans to ensure full recovery of the magnetization before the next acquisition. Therefore, the number of echo times was limited by the time that Buscopan remains effective (~7 min (36)). The images were acquired at 1.3 x 1.5 mm^2 in-plane resolution and reconstructed to 1 x 1 mm^2 over a FOV of 340 x 350 mm^2. A 5 mm thick coronal imaging slice was placed in the plane where the

terminal ilium enters the cecum. This limited the amount of small bowel in the imaging plane but ensured consistency across the two study days.

We used a slightly altered version of a previously published protocol for measuring small bowel wall motility (27, 28). 2D bTFE images were acquired free breathing at a rate of 1 acquisition per second for 60 seconds. A flip angle of 50° was used with a TE/TR of 1.16/2.32 ms, half Fourier acquisition (0.7) and a SENSE factor of 1.5. Images were acquired at 1.5 x 1.5 mm^2 in plane resolution reconstructed to 1 x 1 mm^2. The number of slices was set to cover the entire small bowel wall and ranged from 7 to 10 depending on the participant.

MRI Analysis: Small bowel wall thickness

Software developed in-house with IDL (Research Systems Inc, Boulder, CO, USA) was used to calculate the mean small bowel wall thickness on the higher resolution bTFE images. Regions of interest (ROIs) were manually selected as freeform shapes in the right lower quadrant around the terminal ileum at sites where loops of small bowel lay adjacent to each other (red line on Figure 1). RS (3 years of experience) performed the manual ROI segmentation and saved electronic copies of all the drawn profiles which were then reviewed by HW (2 years of experience). Both readers were supervised and ROIs checked by CH (>10 years of experience).The right lower quadrant of the abdomen was chosen to ensure that similar regions of the wall were being sampled at both visits. The program then automatically measured the small bowel wall thickness by generating a profile of the intensity values perpendicular to the wall at adjacent points along the ROI. As profiles were drawn across adjacent loops of bowel, the thickness measured was twice

the wall thickness. A minimum of 200 profiles were used to calculate the mean small bowel wall thickness for each individual at each study visit.

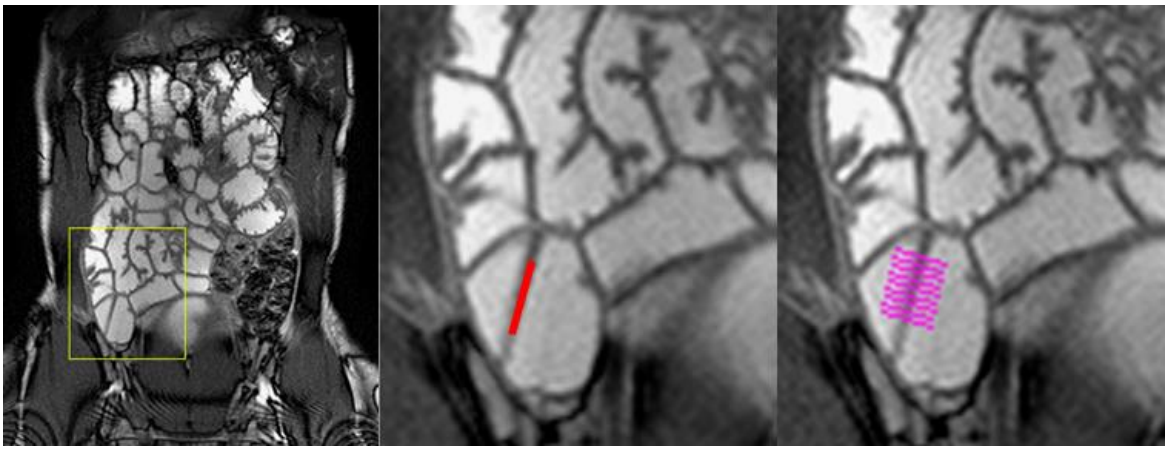


Figure 1 - Overview of method used to measure the mean small bowel wall thickness, on the high resolution balanced Turbo Field Echo (bTFE) images. The yellow box indicates the region where the data was analyzed (left). The red line (middle) indicates a section of wall where perpendicular profiles (pink lines, right) have been drawn across loops of the small bowel wall. Multiple sections were selected so that >200 profiles were used to provide a final average estimate.

MRI Analysis: Small bowel T2

The single slice spin-echo prepared bTFE images were used to measure T2 of the small bowel wall. We developed in-house software using Matlab (The MathWorks, Inc., Natick, Massachusetts, United States) to identify the bowel wall from T2-weighted images. A semi-automated analysis pipeline was set up to isolate the bowel wall and extract the signal from it (UK patent application 2002582.1).

In brief, the images were first registered to the first echo time image via non-linear registration using Matlab's image registration function imregister, an intensity-based image registration process. A second motion correction step was applied using Matlab's function for estimating displacement fields aimed at correcting local image distortions. The motion correction was run as a bulk process for all data sets, taking approximatley 3-4 minutes for each data set. Three freeform ROIs from different locations were then manually drawn in the content of the bowel to measure the signal intensity of the content at each echo time, which was used in subsequent thresholding and partial volume correction. This was performed by HW (with 2 years of experience) under the supervision of PG (with >10 years of experience) with saved copies of the electronic masks reviewed by CH (with >10 years of experience).

Following this, a series of automated steps isolated the bowel wall. A single mask of the area containing the bowel was created using thresholding to remove subcutaneous fat, muscle and visceral fat (determined by histogram analysis of the different tissues). Only the areas inside this mask were used for further analysis (Figure 2b). Images were normalized to allow consistent threshold values to be used throughout the analysis. Next, a binary mask of the bowel wall was created using edge detection and thresholding for each echo time. These masks were then combined to produce a mask which only contained voxels which were identified as wall at every echo time (Figure 2c).

Following this, a manual quality control step was used to ensure that only small bowel wall was included in the final mask (performed by HW, under supervision by PG, and CH). The removal process was done by visually inspecting the mask overlaid on all 6 images and then drawing around areas that did not cover the bowel wall, including the

wall of the colon, stomach, uterus and bladder (Figure 2d). These areas were removed from the mask.

The final mask was automatically split into smaller sub-ROIs to allow for the heterogeneity along the bowel wall to be investigated. The signal for each sub-ROI at each echo time was extracted. Data sets which contained three or less sub-ROIs were excluded from analysis as these either had a lack of bowel in the imaging plane or through plane motion which could not be corrected for the image registration step. After the initial batched image registration, the analysis for each data set took approximately 1-2 min, including the two manual steps (i.e., drawing three ROIs in the content and the manual removal of misidentified sections of bowel).

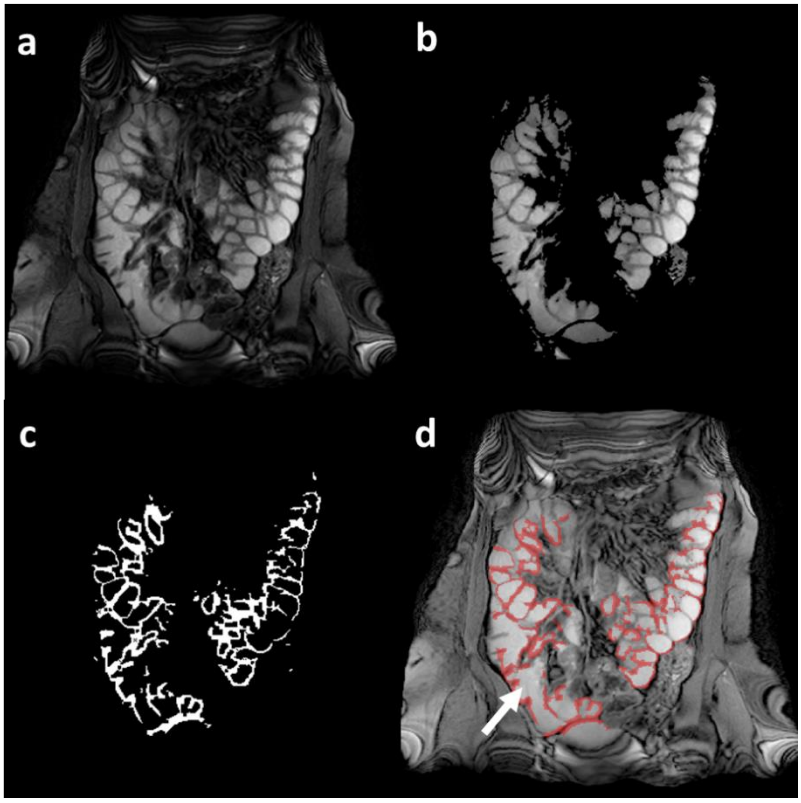


Figure 2 – Bowel wall segmentation. a) T2 image at echo time TE=20 ms. b) T2 image after a series of thresholds were applied to remove subcutaneous fat, muscle and visceral fat. c) Binary masks of the bowel wall overlayed with image from echo time TE = 20ms#. d) Mask of the bowel wall overlaid the T2 image at TE=20 ms. Note that the colon walls were identified and manually removed (white arrow).

The T2 fit took the full bTFE readout into account (35). To overcome partial volume effects, the signal from each ROI was fit to a two-compartment model (small bowel and content). The T2 of the content taken as the average T2 measured from three ROIs located within the lumen of the bowel. Any ROI which produced an R-squared value of less than 0.9 for the T2 fit was removed from further analysis. The median and

interquartile range of T2 across all sub-ROIs were calculated and used as the data values for subsequent statistical analysis.

MRI Analysis: Small bowel motility

Analysis of MR measurement of small bowel motility has been described previously in detail (27, 28). In brief, initially Robust Data Decomposition Registration (RRDR) was used to remove the effects of respiratory motion. The global motility index was determined using GI-Quant (Motilent, London, UK) applied to an ROI (performed by RS with 3 years of experience saved maps reviewed by CH with > 10 years of experience) encompassing the entire visible small bowel region across all slices acquired according to published protocols (27, 28). This index was generated from non-linear registration parameters generated over the entire image dataset (27).

Analysis of LMR data for small bowel permeability

The in vivo permeability test is a standard differential urinary sugar excretion test using hydrophilic interactions liquid chromatography (HILIC) with electrospray ionization tandem mass spectrometry (ESI-MS/MS) (29, 30). After collection, the total urine volume was noted and 1.5 mL sample aliquots were filtered with 450 nm filters (Merck Millipore, Billerica, Massachusetts, USA) and stored at −20°C until batch analysis was performed. All the samples were coded without reference to the test condition. The measurements were performed by a lab technician (CO) blinded to the test condition.

To precipitate any excess salt, 20 µl aliquots were diluted with 980 µl 90% acetonitrile to which internal standards xylitol and raffinose were premixed at 0.5 µg/ml final concentration. These were vortexed, incubated at -20ºC overnight, and centrifuged, and the supernatant was decanted into amber HPLC vials. Calibration standards were made as a dilution series from 2.5 to 500 ug/ml of mannitol and lactulose from stocks made in water. The method was validated by creating six independently prepared dilutions of 5, 50 and 500 µg/ml. To accurately identify lactulose, sucrose standards were also prepared.

For convenience, two liquid chromatography columns, a Sequant ZIC-pHILIC (5 µm) 100 x 2.1 mm and a ZIC-HILIC (5 µm) 150 x 2.1 mm from Merck KgaA (Darmstadt, Germany), were used in series and kept at 15ºC. The mobile phase was acetonitrile and 5 mM ammonium acetate adjusted dropwise to pH 6.85 with 0.05% ammonium hydroxide solution. The flow rate was 0.3 ml/min. The detector was a Sciex 4000 QTrap (Framingham Massachusetts, United States) operating in –ve ion electrospray mode with the source at 350ºC with curtain, nebuliser and auxiliary gases were set to 10, 40 and 20 respectively. The ion-spray voltage was -4200V. As the two analytes had very different ranges of concentrations, samples were quantified against the appropriate region of the line. A minimum of 5 points were used for each analyte.

Inter-observer and intra-subject reproducibility

The second cohort of 10 healthy volunteers was scanned twice a minimum of two weeks apart to look at the intra-subject reproducibility of T2 in the small bowel wall in healthy volunteers. The only difference to the provocation protocol cohort was that no

indomethacin or placebo was given. Furthermore, the T2 measurement slice was not constrained to be over the terminal ileum but was chosen to maximise the amount of small bowel imaged. The analysis was performed by two observers to allow inter-observer agreement to be tested (performed by HW with 2 years of experience and AA with 2 years of experience, supervised by CH and PG, both with >10 years of experience).

Statistical Analysis

A previous study measured mean, healthy, small bowel thickness to be 1.5 mm with a standard deviation (SD) of 0.5 mm (31). Assuming a 66% increase in bowel thickness as a result of indomethacin provocation (22) would be comparable to active Crohn's disease (25, 32, 33), we anticipated that 24 participants in the provocation cohort would give us more than 90% power to reject the null hypothesis with alpha of 0.05 and between group correlation of 0.5.

The data was tested for normality using Shapiro-Wilk's test. Paired testing was then carried out to determine whether paired differences between measures with placebo and indomethacin provocation were significant. Data that was found to be normally distributed were tested using a paired t-test, otherwise data were compared using a Wilcoxon signed rank test. The relationship between T2 and LMR was investigated using the Pearson's Correlation Coefficient. In this exploratory study a covariate of interest was to describe the variability of T2 data across ROIs between subjects (interquartile range across sub-ROIs for each participant).

Inter-rater variability between two independent observers for the second cohort of healthy volunteers who had repeat measures on two study days a minimum of two weeks apart was reported by Bland-Altman (34). The inter-observer variability was assessed by calculating the intra-class correlation with a two-way random model of absolute agreement and interpreted as follows: 0.81–1: almost perfect correlation; 0.61–0.8: good correlation; 0.4-0.6: moderate correlation; 0.21–0.4: fair correlation; 0.0–0.2: poor correlation (34).

The repeatability was defined as poor when the coefficient of variation (CoV) was >30%, acceptable when CoV was between 20–30%, good when CoV was between 10–20% and excellent when CoV ≤10% (35).

Statistical analyses were performed using SPSS version 22 (IBM Armonk, NY) or GraphPad Prism version 8.0 for Windows (GraphPad Software, La Jolla California USA).

Results

Provocation cohort

Twenty-four healthy volunteers consented to the provocation study (Supplementary Figure 3). All participants attended both study days with placebo and indomethacin administration, the order of which was randomly and blindly allocated. Two participants were excluded (one male, one female) from the per-protocol final analyses as one was non-compliant with the study protocol and one had an incidental finding of an asymptomatic thickened terminal ileum prior to the intervention on review of the MRI data (Supplementary Figure 3). This participant was subsequently diagnosed with

inflammatory terminal ileal Crohn's disease on colonoscopy, confirmed by histology. No participants suffered any adverse events caused by administration of indomethacin Fifteen of the volunteers were female (63.6%).. Median age was 23 years (inter-quartile range (IQR) 22 – 25), and median body mass index was 23.9 (IQR 21.6 – 28.0) kg/m^2. The median interval between study visits was 21 (IQR 18 – 27) days.

MRI measures associated with indomethacin provocation

Small bowel wall thickness

There was no significant measurable difference (p=0.17) between small bowel wall thickness around the terminal ileum between placebo (1.28 mm, IQR 1.21 – 1.36 mm) and provocation with indomethacin (1.29 mm, IQR 1.25 – 1.36 mm).

Small bowel wall T2

For the T2 measurements, six data sets were not used because the number of final sub-ROIs was too small (3 or less) due to significant respiratory or bowel motion that could not be corrected. Figure 3 shows that indomethacin provocation induced a statistically significant increase in small bowel wall T2 compared to placebo (mean T2 ± standard deviation: 0.115±0.063 s vs. 0.070±0.036 s respectively, $p<0.05$. There was also a non-significant trend toward increased variation in T2 along the bowel wall after administration of indomethacin compared to placebo (0.16 s vs. 0.10 s, p = 0.065, Figure 3c).

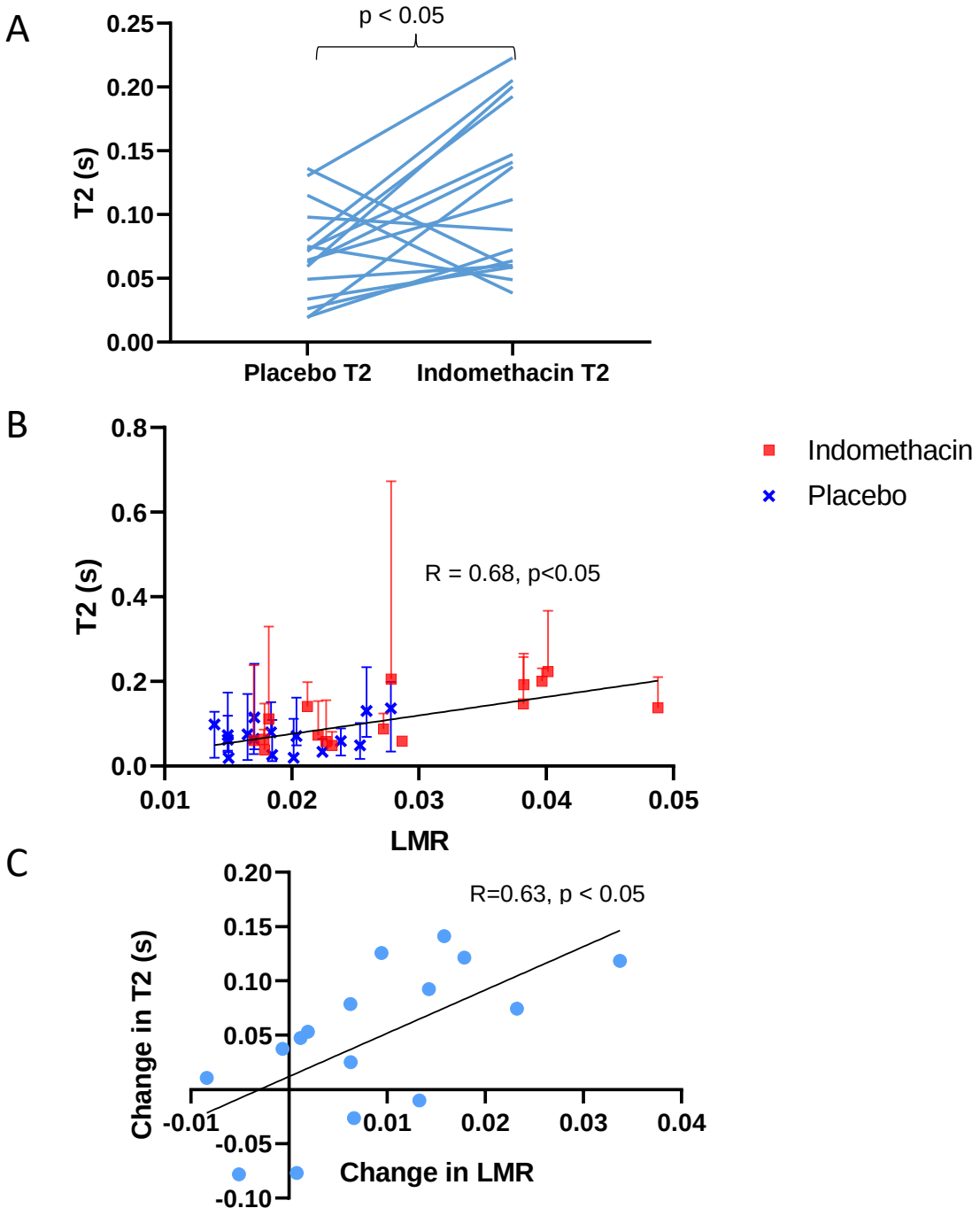


Figure 3 – T2 and LMR values**.** A: Median T2 for each participant. B: Median and IQR T2 for each participant and the corresponding LMR. C – Change in LMR for each participant on indomethacin compared to placebo vs. the corresponding change in T2.

Motility

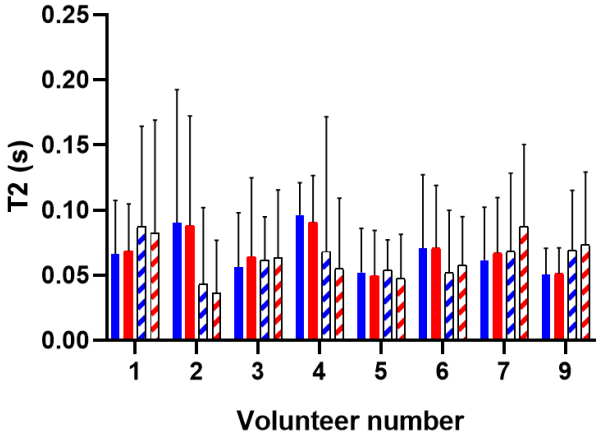
Global small bowel (SB) motility showed no change 0.30 (0.25-0.35) a.u. with placebo compared to 0.27 (0.25-0.31) a.u. with Indomethacin provocation ($p = 0.149$).

LMR test quantification of bowel permeability

Indomethacin induced significantly increased LMR from 0.019 (IQR 0.016-0.026) on placebo to 0.025 (IQR 0.021-0.039) on indomethacin provocation ($p<0.05$). There was a significant positive correlation ($r=0.68$, $p<0.05$) between LMR and SB wall T2 (Figure 3b). There was also a significant positive correlation ($r = 0.63$, $p=0.05$) between the change in LMR and the change in T2 induced by the indomethacin challenge for each subject (Figure 3c).

Inter-observer agreement and intra-subject reproducibility

Two subjects' data were excluded from the analysis due to having less than three sub-regions due to significant peristaltic movements during the acquisition. Figure 4 shows the results obtained from the remaining 8 healthy volunteers with two study visits per participant, separated by a minimum of two weeks (16 study days in total). Inter-observer agreement was excellent for T2 measurements ($N=16$, intra-class correlation = 0.89, $p<0.05$). Bland-Altman estimated bias was 0.005s for observer A (95% CI limit of agreement -0.04s to +0.05s) and 0.0006 for observer B (95% CI limit of agreement -0.05s to +0.06s). The coefficient of variation was 22% for observer A and 34% for observer B. The results are summarized in Figure 4.



	Small bowel wall T2	
	Observer A	Observer B
Visit 1 – mean and std	0.07 ±0.02s	0.07 ±0.01s
Visit 2 – mean and std	0.06 ±0.01s	0.06 ±0.02s
CoV	22%	34%
Bland-Altman Bias	0.005s	0.0006s
CI(95% limits of agreement)	-0.04s to + 0.05s	-0.05s to +0.06s

Figure 4 - Median and interquartile range of T2, intra-observer and intra-subject reproducibility.

Discussion

We have shown that small bowel wall T2 increased following indomethacin provocation and correlated with increased permeability as demonstrated by a 2 hour Lactulose/Mannitol urinary excretion ratio (LMR) test and that MRI measures of small bowel wall thickness and motility were unchanged by indomethacin provocation. We also showed that the test-retest repeatability of small bowel wall T2 measurement was acceptable with the variation in values lower than the difference seen from the indomethacin provocation. In addition, the inter-observer reproducibility was excellent.

The prospective double-blind cross-over study design minimises confounding factors and increases the power of the study. All the participants included in the per

protocol analysis were well-phenotyped, healthy volunteers. All analysis was performed blind to treatment allocation and compared to small bowel permeability as defined by 2-hour LMR, the current standard measure of small bowel permeability (2, 29). The MRI measures obtained are quantitative, in contrast to qualitative MRI measures that are commonly used to assess the small bowel (25), which may improve the power of studies involving repeated measurement within and between subjects. These MRI techniques do not require administration of intravenous contrast and are, therefore, safe and appropriate for repeated measurements (36). The protocols are based on widely available scan sequences and, as such, can be rapidly adopted into clinical and research protocols. In order for quantitative T2 to become a viable clinical measure, the analysis must be as fast and automated as possible. The T2 analysis method developed here can be applied to any images in which the small bowel content and wall have a different signal intensity, not just for T2 mapping.

While two doses of 75mg indomethacin is a known positive control and increased small bowel permeability as expected (19, 22), the dose was relatively low and the intervention was only transient. Nonetheless, it was sufficient to induce a change in small bowel wall T2. Larger or more frequent doses of oral NSAIDs are known to cause variable patchy small bowel erosions (37, 38), which may explain the heterogeneity and increased intra-subject range of the small bowel wall T2 measurements calculated here. NSAID enteropathy increases permeability by direct injury to the intestinal mucosa with inflammation and oedema but also disrupts the tight junctions between cells which permits the passage of ions and water (2, 3). The changes in small bowel wall T2 could

reflect direct inflammation and/or shifts in water through the tight junctions defects associated with increased permeability.

Although indomethacin is known to cause an acute stimulation of motility in animal models, a feature thought to be important in the secondary bacterial penetration of the mucosal barrier (23), the longer term effect is more dominated by the inhibitory effect of mucosal inflammation, as is seen in humans with Crohn's disease (26). At the doses used here the mucosal changes would be predicted to be much less severe than is seen in the animal models, where haemorrhage and marked ulceration is common (24). This may account for the lack of any effect of indomethacin on small bowel motility (increase or decrease) observed in this study. It is intriguing to note that motility is a sensitive marker of inflammation when compared to endoscopy or histology (26). Indomethacin provocation did not cause a change in motility but did cause a significant change in bowel wall T2. Hence, bowel wall T2 may either be a more sensitive marker of mucosal inflammation than motility or measuring more subtle change within the bowel wall (e.g. oedema) that correlates with gut permeability.

Bowel gas is mainly located in the large intestine. With the subjects lying prone gas was pushed away from the small bowel region (as it lies at the posterior edges of the large colon) minimising its influence on the images. bTFE sequences are prone to artefacts from poor shimming due to field distortions and these would have been eliminated using the thresholding techniques.

The wall thickness measurements may not be sensitive enough to detect the subtle changes caused by this indomethacin intervention. Changes to these measurements are

seen in Crohn's disease where the damage to the bowel wall due to inflammation is much more extensive and prolonged (25).

The inter-observer reproducibility of the T2 measurement was found to be robust. Intra-subject variability was high in some cases, which was probably due to the fact that the imaging slice was placed to cover a large area of bowel rather than being restricted to the plane containing the terminal ilium as was a requirement in the initial study. Defining the imaging plane based on a fixed anatomical location would be likely to reduce the intra-subject variability. This could be overcome by using multi-slice imaging (39). Two out of the 10 subjects were removed due to the presence of peristalsis during the imaging, which prevented the T2 of the bowel wall from being measured.

Our study suggests non-contrast enhanced quantitative MR measurement of the small bowel wall T2 could provide a sensitive biomarker of permeability. This has far-reaching implications if validated in a wider range of patient groups where increased small bowel permeability and bacterial translocation contribute significantly to pathogenic process and are associated with clinical manifestations or outcomes. This method may have impact in non-GI diseases where increased permeability of the gastrointestinal tract has been considered a putative pathogenic mechanism, for example in Ankylosing Spondylitis, diabetes and multiple sclerosis. Arguably, the lack of robust, accessible and affordable biomarkers of these potentially pathophysiological changes has hampered research in this area. A widely available, non-invasive, in-vivo measure of small bowel structure and integrity would be an important tool for long-term, non-invasive mechanistic studies, and to evaluate the efficacy of specific interventions.

Limitations

The associated T2 analysis tool requires manual inspection of bowel wall maps and removal of misidentified regions of wall, which is inherently subjective and only partly addressed by averaging several regions of interest in each subject. This is an inherent weakness; however, the inter-observer reproducibility suggests that this has minimal impact on the measurement of T2.

Although LMR is the most validated measure of small bowel permeability, it does have known shortcomings (3, 40). First, up to 30% of participants have detectable urinary mannitol at baseline (prior to administration of test sugars) or disproportionate excretion relative to the mass of mannitol administered for the test. This is hypothesised to be a result of inadvertent ingestion of mannitol in diet or medications (40). Secondly, the measurement made at 0-2 hours mostly reflects small bowel permeability but may also reflect colonic permeability.

In this pilot exploratory study, six out of the 22 subjects were removed from analysis due to motion in the T2 measurement images. This is likely to have statistically underpowered our study based upon the original sample size calculation informed by Crohn's data but suggests quantitative T2 is a sensitive imaging biomarker. This motion was largely due to respiratory motion resulting in the imaging slice moving between acquisitions. This is a weakness of single slice imaging which could be overcome by using simultaneous multi-slice methods (39). A further shortcoming of the test-retest of the T2 measurements was the small sample size.

Conclusion

We implemented a non-contrast MRI technique to measure T2 of the bowel wall in-vivo and showed that changes in bowel wall T2 are related to changes in small bowel wall permeability following indomethacin provocation. Sensitive MR measures of bowel structure and function, including quantitative T2, could be used to characterize relevant patient populations where increased gut permeability is thought to be a key event in the pathophysiology towards clinical outcomes and measure the effect of interventions.

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