Micro-CT Visualization and Indentation Properties of Whole Meniscus Following Mercury Exposure

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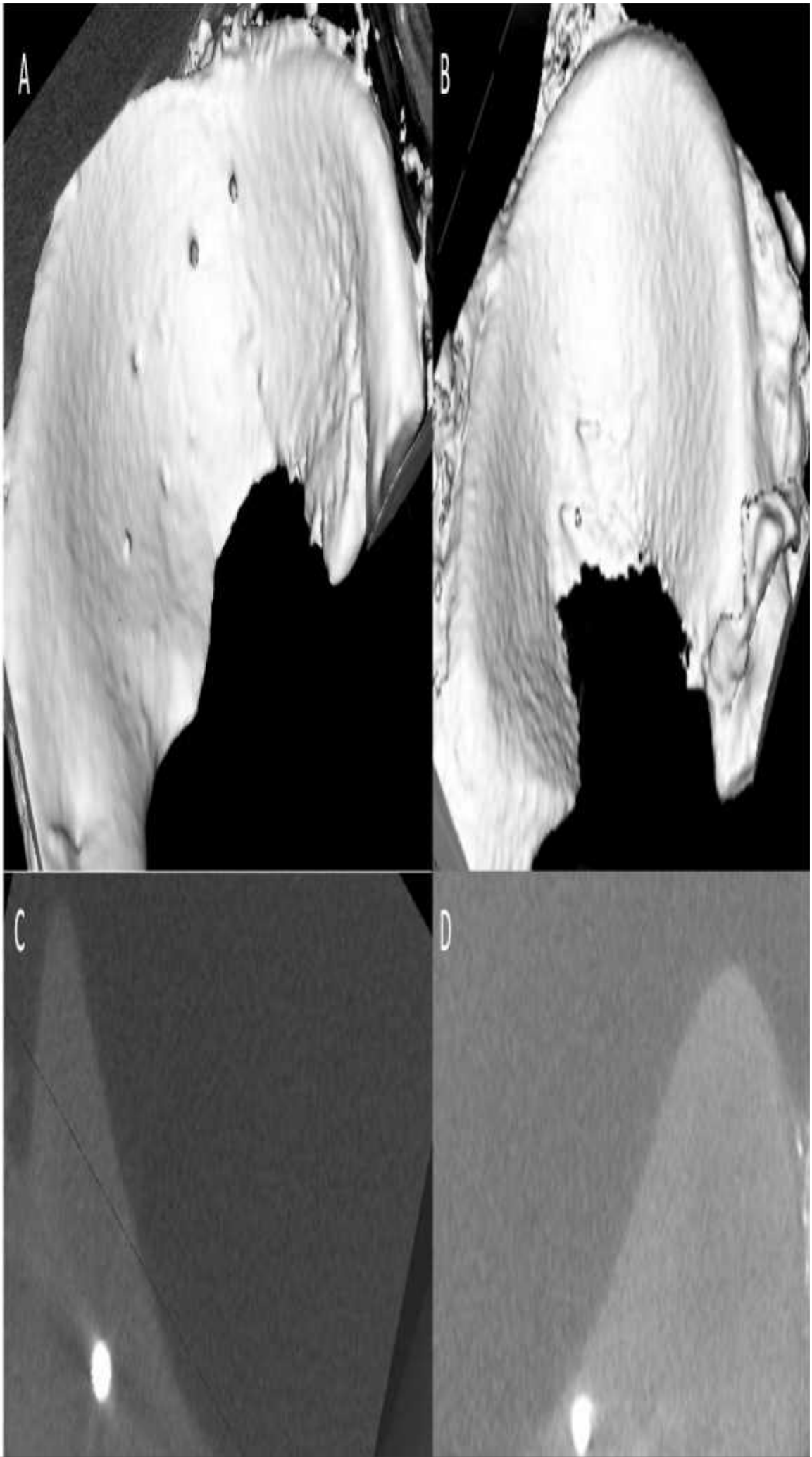
Introduction: A major risk factor for knee osteoarthritis (OA) is meniscal injury or excision [1]. Surgeons now advocate for meniscal repair or allografts rather than excision with this knowledge in hand [2]. Clinical assessment of the meniscal load sharing is limited to meniscus position by MRI, so the efficacy of replacement and repair must be done in cadaveric knees. In the past, pressure sensitive film and mechanical transducers have been used [3, 4] but our laboratory has measured regional strain in menisci using CT imaging and small implanted beads. One problem is that determining the volume of the meniscus requires segmentation in MRI or CT which is not accurate, particularly near capsular attachments. We hypothesized that contrast enhancement using heavy metals may allow automated segmentation of the volume on a CT image, allowing assessment of meniscal repair strategies from CT scans. The objectives of this study were to investigate whether indentation properties of the meniscus change after exposure to mercury, and to determine whether mercury exposure improves visualization of the entire meniscus in micro-computed tomography (micro-CT).
Methods: A pair of menisci were dissected from a porcine knee joint. The meniscotibial ligaments and abaxial capsular attachments were secured to a rigid (wooden) base with metal pins for mechanical testing. Automated indentation was performed at multiple sites (n=16) on the surface of the intact meniscus using a Mach-1 Mechanical Testing System (Biomomentum Inc. Laval, QC) with a six degree of freedom load cell. The contour of the meniscus was determined at each indentation site from surface detection measurements made at the central indentation site and at four surrounding sites placed at 0.5 mm intervals. These data were used to calculate the normal direction for the indentation. Indentations were 25.4% to 39.5% strain applied at a rate of 1 mm/s. Menisci were immersed in a PBS bath throughout testing to ensure the tissue was hydrated. Structural stiffness was calculated by selecting the force at specified displacements of 0.5 mm and 0.75 mm of indentation.
Both menisci were incubated in a mercury solution of 75 mg/250 mL water for two hours at room temperature. After incubation, the indentation was repeated at the same sites to determine any changes. The thickness at each indentation site was obtained using both the MACH-1 and CT scans. For the MACH-1 thickness measurements, a 27 gauge needle was attached to the indenter tip and was driven through the meniscal tissue at a rate of 0.5 mm/s until a force of 4 N was detected. The thickness was determined by the displacement corresponding to the changes in force [5]. For the microCT (GE eXplore Locus 45 micron resolution) thickness measurements, titanium beads of 0.79 mm diameter were inserted at the indentation sites. The thickness could then be measured on the resulting CT scan on a single slice using MicroView 3D Image Viewer & Analysis Tool by a line measurement perpendicular to the base. A paired t-test was used to compare structural stiffness pre and post mercury incubation (Statistica v.9).
Results: The structural stiffness pre and post mercury incubation is shown in Fig. 2 for the medial meniscus and Fig. 3 for the lateral meniscus. There are notable increases in the structural stiffness post mercury incubation in the medial meniscus at 0.5 mm displacement ($p=0.0038$) and 0.75 mm displacement ($p=0.0015$). The lateral meniscus shows a trend towards an increase in structural stiffness at 0.5 mm ($p=0.603$) but not at 0.75 mm displacement ($p=0.3519$). The CT scan of the medial meniscus post mercury incubation is shown in Fig. 4 and the lateral meniscus shown in Fig. 5.
Discussion: Mercury incubation allows for visualization of the meniscus using CT scanning as shown in Fig. 4, 5 but causes changes in the stiffness of the tissue. The visualization of the meniscus on CT can lead to analysis of the meniscus in a CT image including potential automated segmentation methods or volume measurements. Further work is required to determine if shorter incubation times would lead to decreased changes in properties while still enabling visualization on the resulting CT images. To allow for evenly distributed absorption of the mercury, the indentation was performed on a full intact meniscus. Previous studies investigating meniscal indentation have only looked at indenting segments of the tissue [6-9]. The meniscal thickness measurement method using the Mach-1 showed close agreement with the thickness measurement method using CT. This demonstrates the accuracy of using the Mach-1 to determine thickness for meniscal indentation. This study is limited by the small number of menisci tested. As well, the menisci must be removed from the base to incubate in mercury. Care is taken to ensure the menisci is secured exactly as it was previously by taking an outline of the tissue and using the same holes for the pins

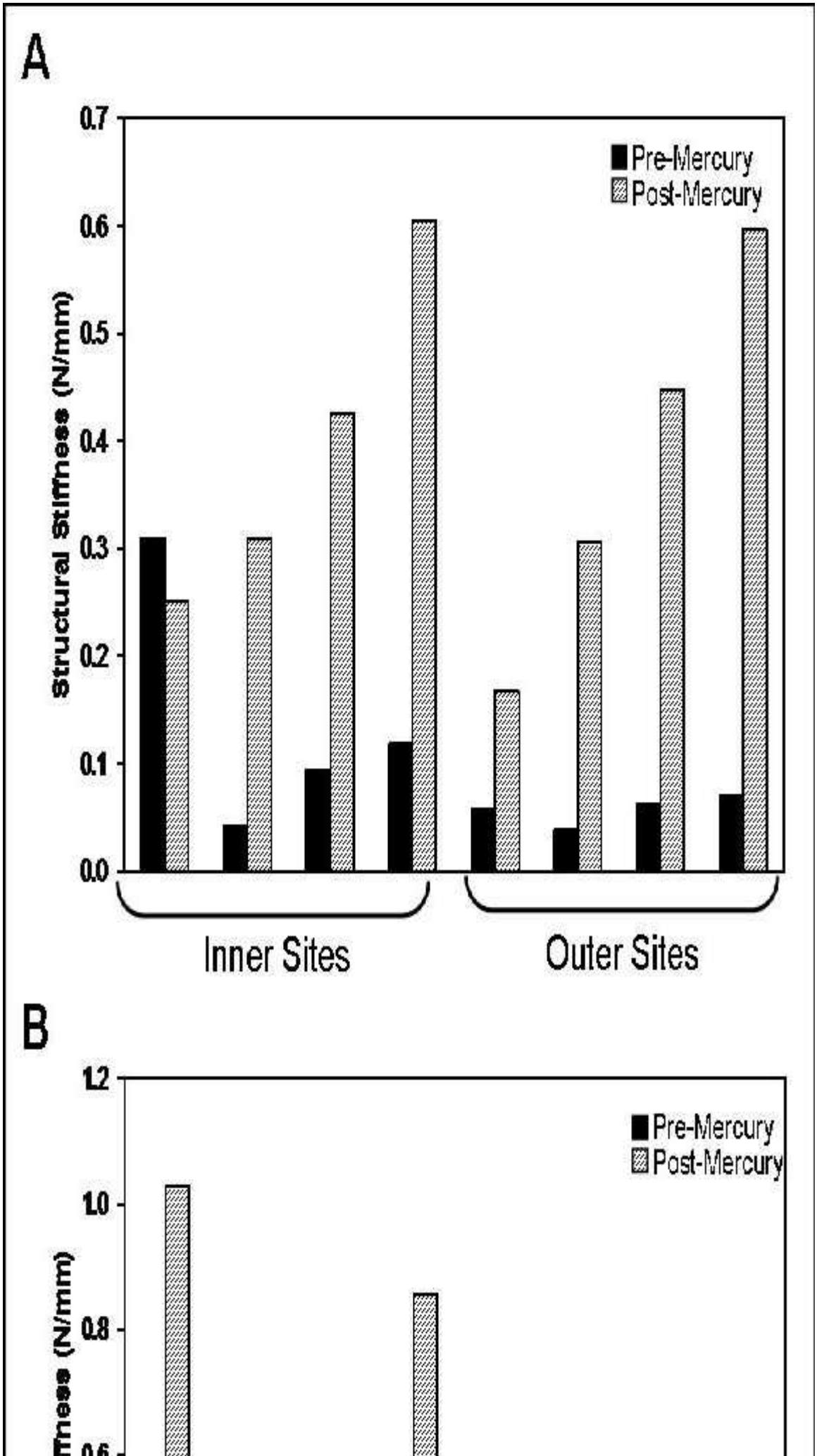
but this could potentially cause a slight shift in the location of the indentation sites.

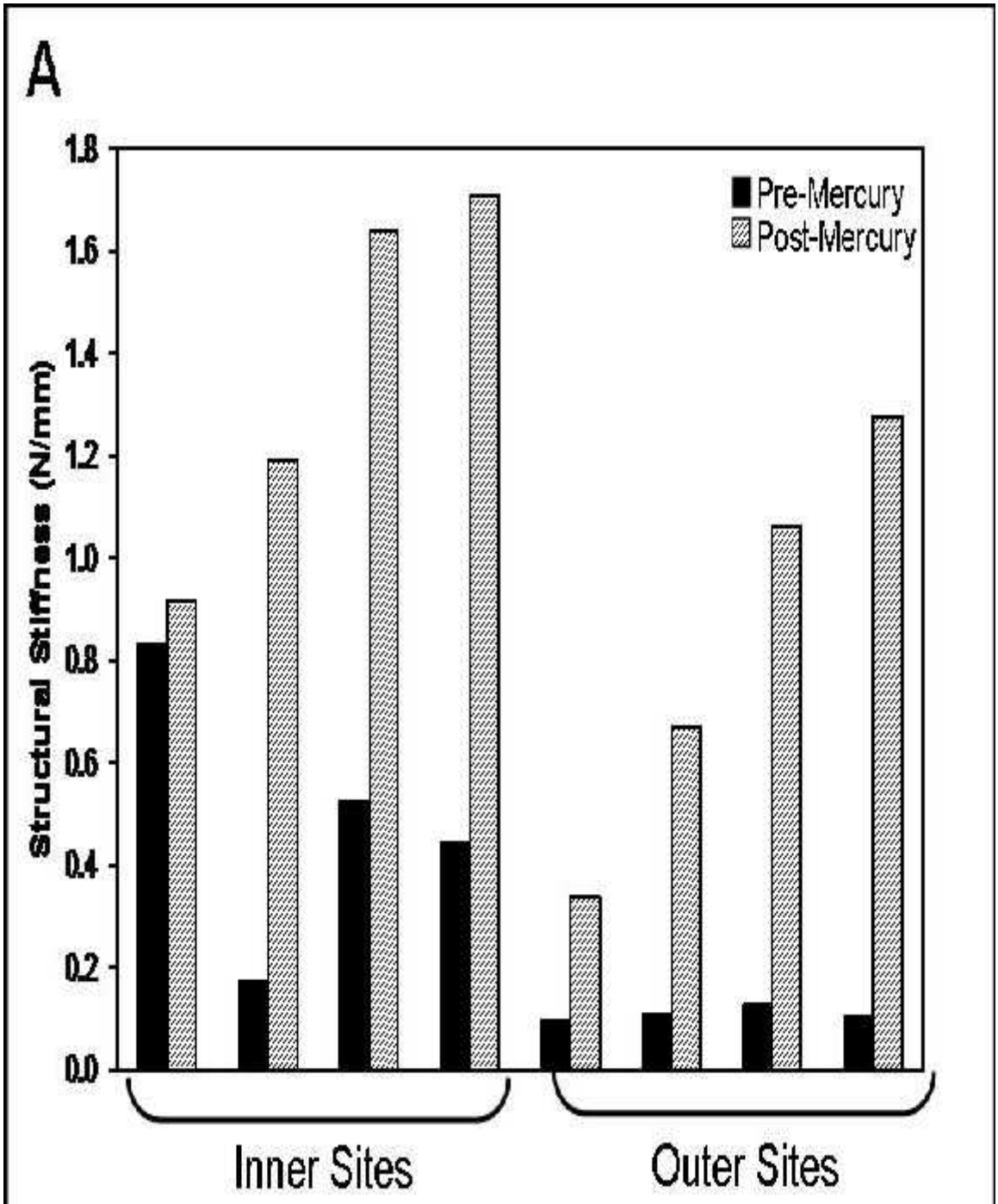
Significance: This is the first study to investigate indentation of a full intact meniscus and to evaluate the use of mercury to allow for visualization of the meniscus in micro-CT. Improved contrast from mercury exposure will allow automated segmentation of the meniscal volume, which would add a valuable in-vitro tool to assess the efficacy of meniscal repair and allograft transplant. Measurements of meniscus thickness using a needle probe agreed with measurements from CT scans, confirming the use of the Mach-1 for thickness measurements through the full meniscus.

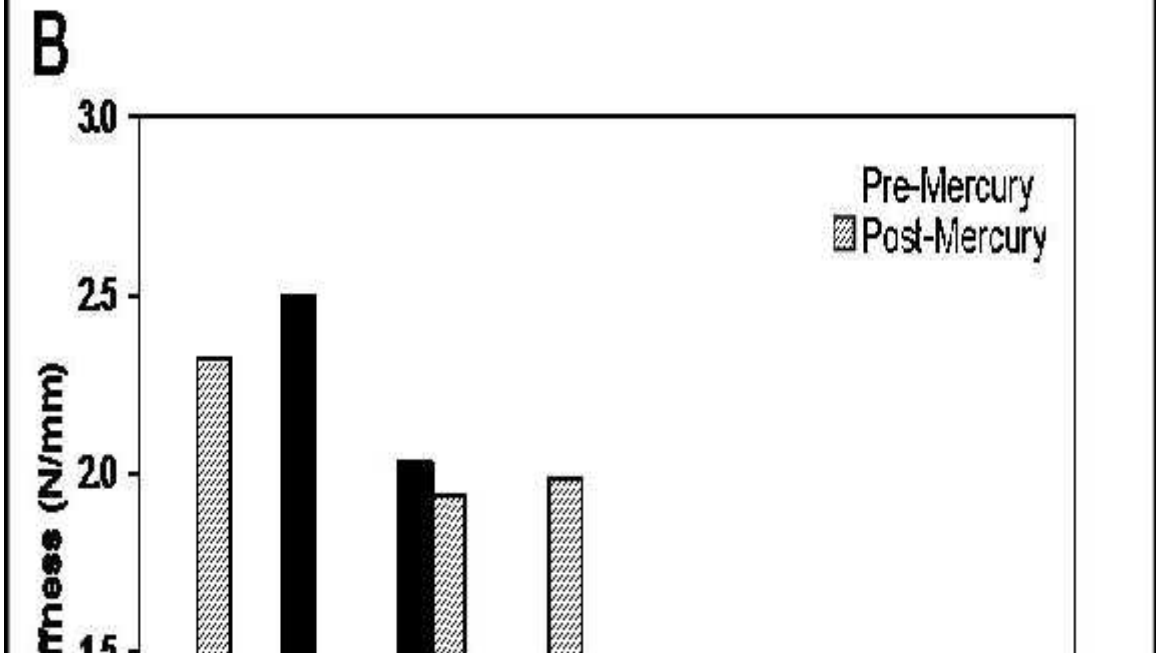
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A
B
C
D


A
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Structural Stiffness (N/mm)
Pre-Mercury
Post-Mercury
Inner Sites
Outer Sites
B
1.2
1.0
0.8
0.6
Pre-Mercury
Post-Mercury


A
Structural Stiffness (N/mm)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Pre-Mercury
Post-Mercury
Inner Sites
Outer Sites


B
3.0
2.5
2.0
Pre-Mercury
Post-Mercury

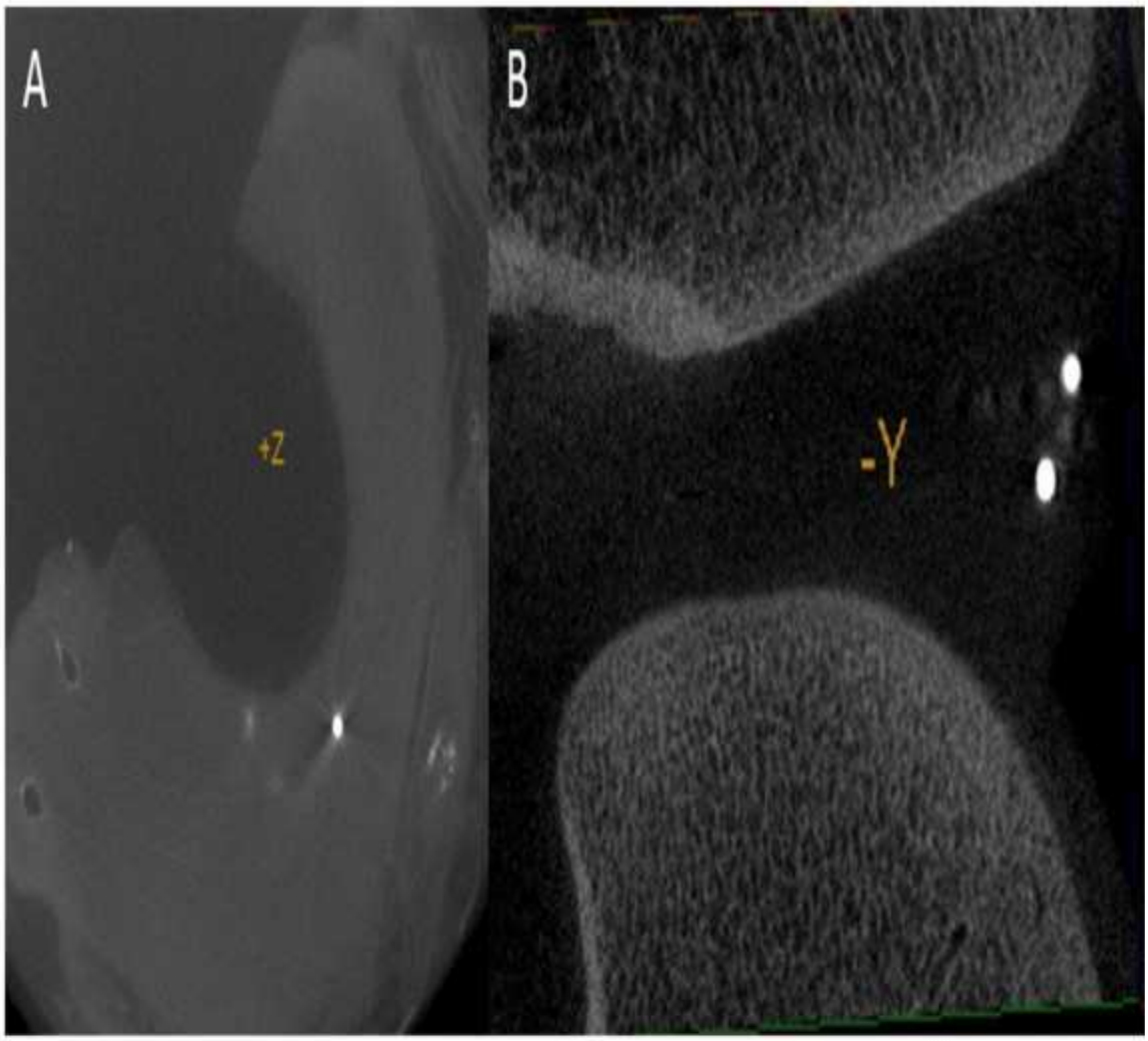


Fig. 4: CT scan of medial meniscus A) Post mercury incubation B) Pre mercury incubation

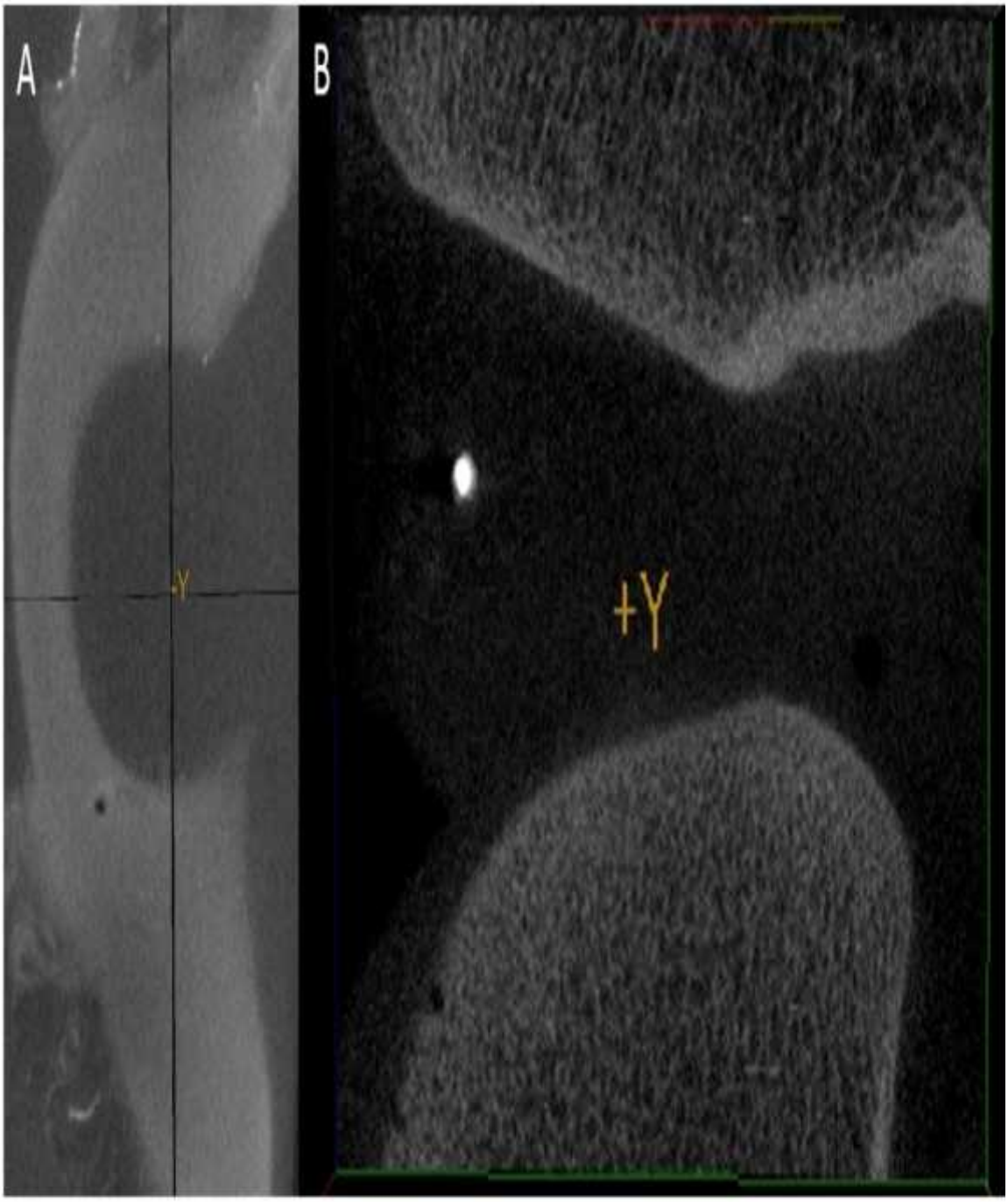


Fig. 5: CT scan of lateral meniscus A) Post mercury incubation B) Pre mercury incubation