# Appendix B: MRI protocol and example of image segmentations

Prior to MRI imaging, the participant was advised to restrain from any strenuous physical activity during the 48 hours prior to the measurements to minimize possible transient changes in knee cartilage volume and composition. Participants were imaged at the same time of the day to avoid possible diurnal variation at the follow-up measurements.

The participants were imaged lying supine. The knee was positioned into the coil by adjusting inferior margin of patella according to the centre of line of the coil. The flexion angle and rotation of the knee was controlled by stabilising the ankle to a fixed position within the knee coil by using a leg holder and a custom made inflatable cushion. The cushion was specifically designed to stabilize the patella without causing any compression of the patellofemoral joint. The imaging session lasted 3 hours and included a standard clinical MRI series and T2 relaxation time followed by the dGEMRIC series.

T2 mapping was performed using a sagittal multi-slice multi-echo fast spin echo sequence (field of view (FOV) 140mm, acquisition matrix 256 x 256, repetition time (TR) 2090 ms, eight echo times (TE) between 13 and 104 ms, echo train length (ETL) 8, slice thickness 3 mm). The slices were positioned perpendicular to a line tangential to the posterior femoral condyles in the axial scout view. Two slices, each covering the central region of the medial and lateral condyles, were analysed.

For the dGEMRIC series, immediately after the clinical and T2 imaging, an intravenous injection of 0.4mL/kg (double dose) of Gd-DTPA2- (Magnevist, Schering, Berlin) was administered. The amount of contrast agent administered was corrected for body weight at each measurement point. This was appropriate because of the expected changes in body composition as a result of the intensive exercise intervention. In order to enhance the delivery of contrast agent into the knee cartilage, following administration of Gd-DTPA2-, the participants were instructed to perform 5 minutes of knee flexion-extension exercises in a sitting position without resistance, 5 minutes of walking on a flat surface and 10 gentle deep squats. Exactly ninety minutes after the injection, dGEMRIC mapping in the presence of Gd-DTPA2- was performed in the sagittal plane using a single slice inversion recovery fast-spin echo sequence (FOV = 14 cm, matrix 256 x 256, TR = 1800 ms, TE 13 ms, six inversion times (TI) between 50 and 1600 ms, slice thickness 3 mm). The slice positioning was copied from the T2 relaxation time mapping sequence, and the number of the slice in the correct orientation is reduced to one. The remaining slice was then positioned at the centre of the medial and lateral condyles as viewed on the axial scout image. The participants were positioned into an identical position as for the first MRI imaging. Knee with highest degree of OA, as measured by the radiographic Kellgren-Lawrence (K/L) scale, was imaged. In the cases where both knee had identical K/L score the right knee was imaged.

For quality assurance purposes, a set of phantom samples containing certain concentrations of agarose and nickel nitrate to modulate their dGEMRIC and T2 relaxation times were imaged following the study protocol prior to baseline and follow-up measurement sessions, and no evidence of scanner drift was observed during the intervention.

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**Figure 1.** Example image of T2 and dGEMRIC segmentation