# Articular cartilage $T_1$-relaxation time changes precede alterations in mechanical properties in a rat model of diet-induced obesity

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INTRODUCTION: Obesity is a recognized risk factor for the onset and progression of osteoarthritis (OA) [1]. High-fat and high-sucrose (HFHS) diets in rodents reproduce symptoms of human metabolic syndrome, including insulin resistance and dyslipidemia [2]. These changes may modulate cartilage degeneration [3], yet the alterations in articular cartilage mechanics and composition remain to be fully elucidated. Moreover, the mechanisms driving cartilage degradation may differ depending on the age at which obesity develops. In this study, we investigated the effect of diet-induced obesity in weanling and adult rats on the mechanical properties and $T_1$-relaxation time in patellar articular cartilage.

METHODS: Animal model: new analyses were performed on the patellae from contralateral knee joints of rat knees previously studied by MacDonald et al. and Rios et al. [2,4]. Male Sprague-Dawley rats were assigned to either a lean chow diet (CHOW, Lab Diet 5001) or a high-fat/high-sucrose diet (HFHS, custom Diet #102412, Dyets). Two age cohorts included 3-week-old weanlings and 12-week-old adults (Fig. 1A). The weanling cohort was maintained on the diets for 14 weeks ($WEAN_{14}$: $N_{HFHS} = 5$, $N_{CHOW} = 6$), while the adult cohort was maintained for 24 weeks ($ADULT_{24}$: $N_{HFHS} = 6$, $N_{CHOW} = 6$). For the first seven weeks, the HFHS-diet weanlings were maintained on a diet with additional protein (custom Diet #103915, Dyets). A male Sprague Dawley rat model of diet-induced obesity was used, as it is well established [2]. Following the diet intervention period, animals underwent assessments of e.g., body composition, and the animals were sacrificed. The knee joints were dissected, and the patellae were harvested and frozen. Biomechanical testing: the patellae were thawed, and the articular cartilage thickness was measured using optical coherence tomography. Afterwards, a four-step stress relaxation (a step size of 5% cartilage thickness, ramp rate 20 %/s, 200 s relaxation time) was conducted in indentation geometry with a flat-top cylindrical indenter (diameter: 0.5 mm; Mach-1 tester, Biomomentum Inc; Fig. 1B). Dynamic modulus and phase differences were measured with sinusoidal testing (1 Hz frequency, 2 % amplitude, 4 cycles) after stress-relaxation. Micro-computed tomography (μCT) imaging: the patellae were scanned using μCT (Nikon XT H 225, Nikon Metrology Europe) at a voxel size of 4 $\mu m^3$ (tube voltage = 50 kVp). Micro-magnetic resonance (μMRI) imaging: following μCT, patellae were placed in glass tubes (diameter: 5 mm) filled with perfluoropolyether and imaged at room temperature using an 11.7 T vertical magnet (500 MHz Ultrashield, Bruker Biospin GmbH) with a 5 mm $^1$H RF coil. Zero echo-time (ZTE) sequences with flip angles of 2°, 5°, and 13° [5] were acquired at a voxel size of 25 $\mu m^3$ (repetition time = 4.44 ms). Analysis: the equilibrium modulus was calculated as the slope between the strain and equilibrium stress points obtained at the end of each relaxation step. The MRI volumes were Gaussian-filtered, and $T_1$-relaxation time was estimated with non-linear least squares fitting. The $T_1$ maps were co-registered to the μCT volumes using the Python package *itk-elastix*. A 1 × 1 mm² region was selected from the volumes at the geometric center of the patellar cartilage (Fig. 1C) from which depth-wise $T_1$-relaxation time profiles extending from articular cartilage surface to the subchondral bone were calculated. The $T_1$-relaxation time profiles were interpolated to 10 evenly spaced points, representing 0–100% of cartilage depth. Statistical analysis: The equilibrium modulus was compared between HFHS and CHOW groups with the Mann–Whitney U test. $T_1$-relaxation was analyzed with a linear mixed model, with depth set as a fixed variable and animals as the random subject to account for the repeated measures. Significance was based on 95% confidence intervals of group means and those of depth-wise $T_1$-relaxation time profiles.

RESULTS: The cartilage equilibrium modulus, dynamic modulus, and phase difference were not statistically different between the CHOW and HFHS diet animals in either the $WEAN_{14}$ group or the $ADULT_{24}$ group (Fig. 1D). Interestingly, the cartilage $T_1$-relaxation time was shorter in the HFHS diet animals for both the $WEAN_{14}$ and $ADULT_{24}$ cohort (Fig. 1E).

DISCUSSION: Diet-induced obesity did not alter cartilage mechanics, indicating a preserved mechanical integrity. However, reduced $T_1$-relaxation times in HFHS-fed rats suggest compositional changes in the cartilage matrix, potentially reflecting decreased proteoglycan content or increased protein (collagen) crosslinking [6]. The shorter $T_1$-relaxation time in the HFHS-fed rats of the $WEAN_{14}$ group may indicate disrupted cartilage maturation [7], whereas in the $ADULT_{24}$ group the shorter $T_1$-relaxation time could reflect cartilage remodelling – unlike in generic OA with longer $T_1$-relaxation time.

SIGNIFICANCE/CLINICAL RELEVANCE: $T_1$-relaxation mapping may serve as an early biomarker of obesity-related OA, and metabolic changes in cartilage composition may precede alterations in mechanical properties.

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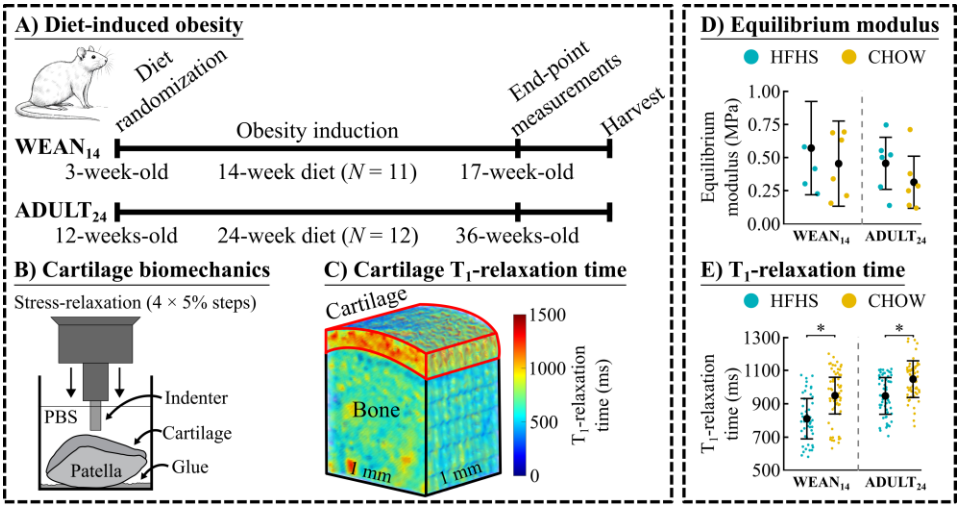


**Figure 1.** A) a high-fat/high-sucrose (HFHS) diet or a normal chow (CHOW) diet was maintained on 3-week-old weanling ($WEAN_{14}$) and 12-week-old adult ($ADULT_{24}$) male Sprague-Dawley rats. B) Articular cartilage equilibrium modulus was measured in via stress-relaxation testing in an indentation setting. C) Cartilage $T_1$-relaxation time was measured using micro-magnetic resonance imaging. D) Cartilage equilibrium modulus for the $WEAN_{14}$ and $ADULT_{24}$ groups. E) $T_1$-relaxation time for the $WEAN_{14}$ and $ADULT_{24}$ groups. The data is represented as mean ± 95 % confidence intervals.