Compressive Properties of Normal Human Articular Cartilage are Enhanced Between Infant, Child, and Adult and Related to Distinctive Timing of Collagen and Glycosaminoglycan Deposition

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INTRODUCTION: Healthy adult articular cartilage (AC) function and composition results from growth and maturation of epiphyseal cartilage. In bovine knees, AC compressive modulus (H_{A0}) achieves its adult levels by increasing 3-fold from late fetus to early calf, but not changing substantially between calf and adult; this increase is in association with an elevation in collagen (COL) content, while sulfated glycosaminoglycan (GAG) is steady at adult levels.[1,2] During human development, AC compressive properties, and the basis for age-related variations, are unknown. Infants, children, and adults exhibit distinctive stages of growth, with height velocity being rapid, decelerating, and none (completed), respectively.[3] The hypothesis of this study was that AC compressive properties improve between infants, children, and adults, and that such functional AC changes are related to increases in COL and GAG contents.

METHODS: ***Sample Preparation.*** AC discs (3 mm diameter, 1 mm thick) with intact articular surface were isolated from grossly normal distal femurs of previously frozen cadaveric knees of anonymized donors (n=19), from infants (0-2 yr, 2M+3F, 0.6±0.2 yr), children (2-10 yr, 6M+2F, 6.1±1.0 yr), and adults (18+ yr, 5M+1F, 22.3±1.4 yr). ***Biomechanics.*** Discs were tested in a radially confined configuration, with static compression between two porous platens to 15, 30, and 45% and superimposed oscillations of 1.0–0.3% at 0.01–0.5 Hz to determine H_{A0} [MPa] and strain-dependent hydraulic permeability, k_p [m^2/(Pa·s)]. ***Biochemistry.*** Discs were weighed wet (WW), lyophilized, weighed dry (DW), solubilized, and analyzed for GAG, COL as 7.1×hydroxyproline, and cells as 7.3 pg DNA/chondrocyte. Residual (RES) DW was computed as the DW not accounted for by GAG, COL, or cells (assuming 0.1 ng DW/cell). ***Statistics.*** Effects of growth stage were analyzed by ANOVA and Tukey *post-hoc* test. The dependencies of mechanical properties on composition were analyzed by multivariate linear regression with backward elimination. Data are expressed as mean±sem, with significance at $p<0.05$.

RESULTS: ***Biochemistry.*** AC biochemical properties varied with growth stage (**Fig 1**). Water content (**Fig 1A**) diminished from infant (88%) to child (81%) to adult (76%). COL content (**Fig 1B**) was elevated from infant (51 mg/g WW) to child and adult (103 and 127 mg/g WW). GAG content (**Fig 1C**) was elevated from infant and child (18 and 19 mg/g WW) to adult (42 mg/g WW). Cellularity (**Fig 1D**) decreased from infant (46×10^6 cells/g WW) to child and adult (11 and 16×10^6 cells/g WW). ***Biomechanics.*** AC biomechanical properties varied with growth stage (**Fig 2**). H_{A0} (**Fig 2A**) was elevated from infant and children (0.07 and 0.11 MPa) to adult (0.33 MPa). k_p (**Fig 2B**) was lower with compression and decreased from infant to child. ***Function-Composition Correlates.*** H_{A0} increased (r^2=0.90) with COL (**Fig 2C**) and GAG (**Fig 2D**) according to H_{A0} = 0.0012*COL + 0.0089*GAG - 0.1614. k_p varied (r^2=0.56) with matrix constituents according to $\log(k_p)$ = -0.0053*COL + 0.0111*GAG - 0.0796*CELL - 0.0155*RES - 13.30.

DISCUSSION: The relative softness (low H_{A0}) of human AC at infant and child stages, relative to adult, may reflect its conforming and remodeling functions during growth, similar to animal joints. However, the distinctive time course of matrix changes in human AC (marked increase in COL from infant to child, then a marked increase in GAG between child and adult), appear different than the timing of matrix changes in bovine knees, where GAG is already present at high, mature levels in the antenatal period.[1] In humans, the increase in GAG between child and adult stages is likely responsible for the corresponding elevation in H_{A0}, whereas the combined increase of COL and GAG, in children compared to infants, are likely responsible for the lesser permeability k_p in children. The maturation-related differences between animals and humans in COL vs GAG contents, and thus attainment of mature compressive functionality, may be related to differences in mechanical demands, e.g. onset of locomotion and extent of load-bearing, during the antenatal and postnatal growth periods.

SIGNIFICANCE/CLINICAL RELEVANCE: These mechanical and biochemical properties serve as benchmarks for the post-natal maturation of normal human femoral condyle articular cartilage. The growth sequence of increasing COL, then GAG, may be targeted as biomimetic cartilage regeneration strategies.

REFERENCES: [1] WilliamsonAK+. *J Orthop Res* 2001. [2] HanEH+. *Biophys J* 2011. [3] TannerJM+ *Arch Dis Child* 1966.
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FIGURES:

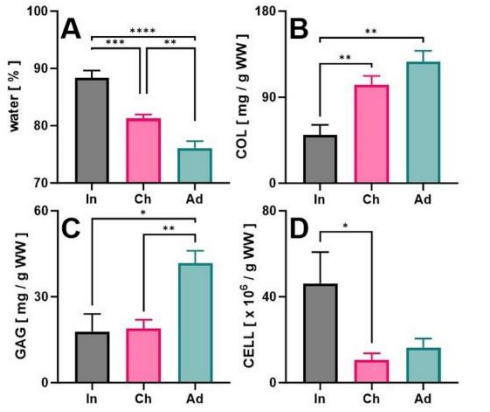

Figure 1. Effect of developmental stage on (**A**) water, (**B**) COL, (**C**) GAG, and (D) CELL content. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

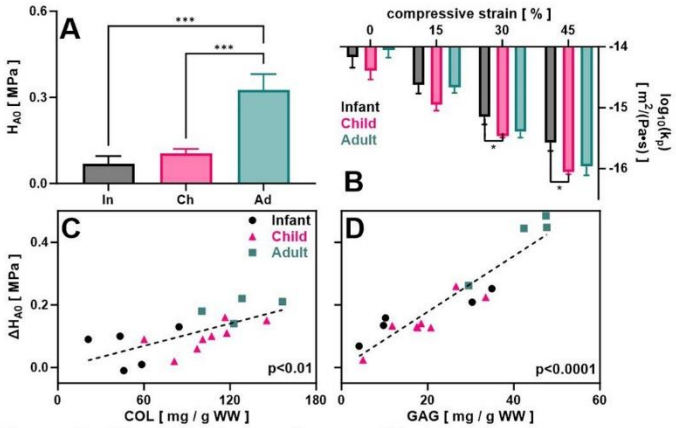

Figure 2. Effect of developmental stage and biochemical content on biomechanics. (**A**) H_A and (**B**) k_p dependence on developmental stage and the change in H_{A0} (ΔH_{A0}) with (**C**) COL and (**D**) GAG. *p<0.05, ***p<0.001