# Effect of In-ovo Immobilization on Development of Chick Hind Limb Articular Cartilage: An Evaluation Using Micro-MRI Measurement of Delayed Gadolinium Uptake

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**INTRODUCTION**: Immobilization causes a reduction of glycosaminoglycan (GAG) in articular cartilage [1, 2]. Further, in-ovo paralysis by decamethonium bromide in developing chick embryos interferes with the formation of normal joint anatomy similar to arthrogryposis [3, 4]. However, the effect of immobilization on the GAG content of articular cartilage in the developing chick embryo is unknown. MRI in conjunction with the gadolinium contrast agent, $Gd(DTPA)^{2-}$, has been demonstrated to measure local GAG concentration in articular cartilage non-destructively [5]. Using this MRI technique, the effect of decamethonium bromide paralysis in-ovo on the GAG concentration of articular cartilage in the developing knee joint of the embryonic chick was investigated.

**METHODS**: Fertilized eggs were divided into a paralyzed group and non-paralyzed control group. In the paralyzed group, decamethonium bromide (DMB) was injected into the eggs every other day starting at day 10 after fertilization. Hind limbs were harvested from each group at day 13 and day 16 after fertilization. The hind limbs were equilibrated in either charged gadolinium aqueous solution (1 mmol Gd/l $Gd(DTPA)^{2-}$) or neutral gadolinium aqueous solution (1 mmol Gd/l Gd(HPDO3A)) for 48 hours. Each hind limb was evaluated using an 8.45 Tesla microimaging system (DRX-350TM, Bruker Bio-spin, Karlsruhe, Germany) to measure the T1 relaxation time at the epiphyses of the knee joint. T1 measurements were then taken using a conventional spin-echo sequence with six different repetition times (TRs) ranging 0.05 to 3 seconds, 6.6 msec echo time, 0.5 mm slice thickness, 10 mm field of view, 128x128 matrix, and 4 (for day 13) or 2 (for day 16) number of excitation, affording (78 μm)$^2$ in-plane resolution. The T1 relaxation times were compared using student's t-test. Four of thespecimens in each group were stained with Toluidine Blue or Safranin O for histomorphometry.

**RESULTS**: On day 13 post fertilization the hind limbs equilibrated with $Gd(DTPA)^{2-}$, the femoral T1 relaxation time was shorter in the paralysis group than the control group ($p=0.02$) whereas no significant difference was found between the groups for the tibia. However for hind limbs equilibrated with Gd(HPDO3A), the T1 relaxation times were the same for both the tibia and femur for both groups. On day 16 post fertilization the hind limbs equilibrated with $Gd(DTPA)^{2-}$, the T1 relaxation time was longer for the paralyzed group for both the femur ($p<0.01$) and tibia ($p=0.04$). However for hindlimbs equilibrated with Gd(HPDO3A), the T1 relaxation time was shorter for the femur in the paralyzed group ($p<0.01$, Fig 1,2). No clear differences were found between control and paralysis groups at day 13 or day 16 evaluated by histology using Toluidine blue and Safranin O.

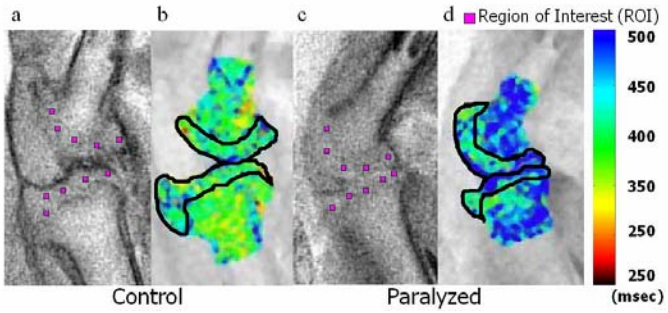


**Fig.1 T1 map of day 16**
T2 images (a,c) and T1maps (b,d) of 16 days hind limbs. T1 map shows difference of T1 relaxation time through the knee joints.

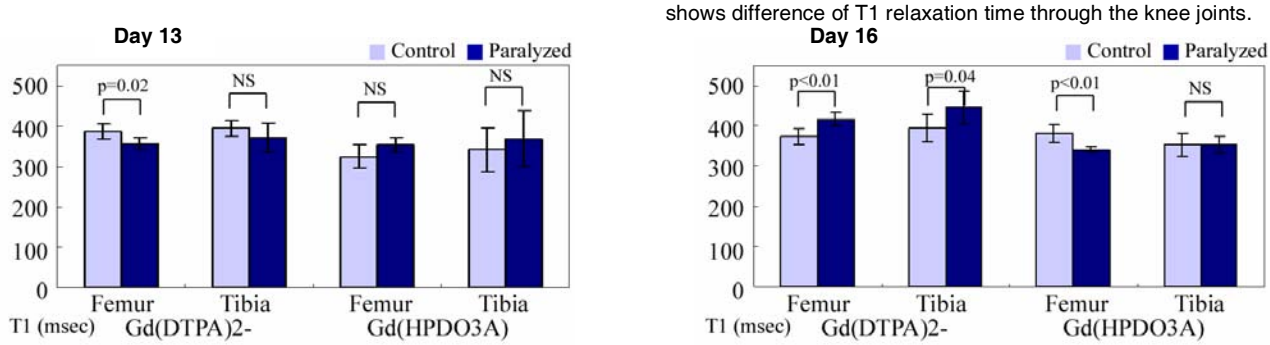


**Fig.2 T1 relaxation time at day 13 (left) and day 16 (right)**
Comparison of T1 relaxation time (Mean ± SD) between control and paralyzed groups in femoral and tibial epiphyses.

**DISCUSSION**: In the present study, we investigated the effect of decamethonium bromide paralysis on th appearance of GAG in the developing embryonic knee joint by MRI to measure the relative $Gd(DTPA)^{2-}$ concentration in the cartilage. At 13 days after fertilization, the T1 relaxation time was shorter in the paralyzed chick hind limbs equilibrated with $Gd(DTPA)^{2-}$ whereas the T1 relaxation times for both the femur and tibia equilibrated with Gd(HADO3A) showed no differences among the groups. This suggests that the development of articular cartilage (as reflected by the decreased GAG concentration in the matrix) was prohibited or delayed by decamethonium bromide paralysis. However at day 16 after fertilization the T1 relaxation times at the tibia and femur were longer in the paralyzed chick hind limbs equilibrated with $Gd(DTPA)^{2-}$ indicating that the GAG concentration was higher in both the tibial and femoral epiphyses on day 16 in the paralyzed chicks compared to the control group. This unexpected result might reflect the delayed maturation of the paralyzed chicks where the secondary ossification center at the epiphyses was less developed and therefore the volume of the cartilage anlage relatively larger at day 16 after fertilizaton in the paralyzed chicks compared to the control group.

It is important to note that none of the differences in GAG concentration at the chondral surfaces of the developing chick hind limb as a consequence of immobilization and time after fertilization were apparent using standard histologic analysis. Micro-MRI analysis of the embryonic chick hind limb allowed for non-destructive imaging of the chondral surface morphology and mapping of the distribution of $Gd(DTPA)^{2-}$ throughout the epiphyses thereby providing a functional assessment of the chemical composition of the developing joint.

**REFERENCES**: [1]Kiviranta, I. Arth Rheum, 1987. 30:801. [2]Videman, T. Orthop Scand, 1981. 52:11. [3]Drachman DB. Dev. Bio., 1966. 14:401. [4]Mitrovic, D. Acta Anat, 1982. 113:313. [5]Burstein, D. JBJS, 2003. 85-A S2:70.