SYNOVIAL FLUID FROM PATIENTS WITH CAMPTODACTYL-ARTROPATHY-COXA VARA-PERICARDITIS SYNDROME (CACP) LACKS BOUNDARY LUBRICATING ABILITY

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American Standard for Testing of Materials (ASTM) D2857-95. At least 4 data points for each sample were plotted graphically and back extrapolated to c = 0 in a linear regression model. The value of y-intercept was \( \eta \). Healon™ was chosen as a model hyaluronate (HA) solution as it is a pure with a well characterized polymer molecular weight of 1 x 10^6 daltons, similar to synovial hyaluronate. Bovine synovial fluid was used as a control as the Ubbelohde type semi-micro capillary viscometer requires a 7 ml sample volume.

Results:
Two patients with CACP syndrome were identified who agreed to undergo diagnostic arthrocentesis.

Lubricating Ability
Both aspirates of synovial fluid from patients with CACP syndrome failed to lubricate. In fact, the observed values of \( \mu \) were very similar to the Healon™ solution alone (Fig). Open circles correspond to respective NS control \( \mu \) values.

Lubricating Ability of CACP and Normal Synovial Fluid Compared to Hyaluronate

Intrinsic Viscosity
Both Healon and BSF demonstrated decreasing \( \eta \) as the concentration of hyaluronate lessened. This differed from the CACP sample which had an increasing \( \eta \). Hyaluronate concentrations for the CACP synovial fluid, bovine synovial fluid and Healon were 3.62, 2.15 and 3.62 mg/ml respectively. Values of \( \eta \) for Healon, CACP and BSF were 18.8 cS, 18.4 cS and 13.6 cS respectively. As the molecular mass of hyaluronate was similar across these samples at 1 x 10^6 daltons, it stands that CACP and Healon possessed similar \( \eta \).

Discussion
Articular cartilage surfaces in the joints of patients with CACP syndrome are more likely to come in mechanical contact. Resulting in mechanical wear, evidenced by early fibrillation of articular cartilage, and possibly apoptosis of the superficial zone chondrocytes which have been shown to be sensitive to increased mechanical load.

Hyaluronate and lubricin together in the form of BSF (in lieu of human synovial fluid) possessed significantly lower \( \eta/c \) than either CACP synovial fluid or Healon. Similar observations have been made for link protein and aggrecan. Theories of the frictional properties of polymer molecules in solution show that \( \eta/c \) is proportional to the effective hydrodynamic volume of a molecule divided by its molecular weight. Paradoxically, molecular weight is increasing in the present experiments and yet \( \eta/c \) is decreasing a significant amount in the case for BSF. One possible explanation lies in the way hyaluronate and lubricin interact and whether a smaller hydrodynamic volume is achieved. In place of a random coil, hyaluronate assumes a more confined structure. These data indicate that an interaction between hyaluronate and lubricin is very likely and can be interrupted by the C-terminal truncating mutations which are characteristic for CACP syndrome.

49th Annual Meeting of the Orthopaedic Research Society
Paper #0134

Camptodactyly-arthropathy-cova vara-pericarditis (CACP) syndrome is rare autosomal recessive trait which as the name implies gives rise to a complex phenotype. Characterized by phalangeal-inter-phalangeal joint flexion contractures, weight bearing joint arthropathy and variable occurrence of coxa vara and pericarditis. Up until recently the genetic basis and pathophysiology of this trait were unknown. The involved gene is megakaryocyte stimulating factor (GenBank U70136) producing a protein product with numerous names owing to different routes of discovery: CACP protein, superficial zone protein (SZP) and lubricin. Several truncating mutations have been discovered in the CACP protein product, most towards the 3’ (prime) end of the MSF gene from patients with phenotypic CACP. SZP is expressed by the superficial zone chondrocytes and thought to provide for chondroprotection. Lubricin is a classical protein expressed into synovial fluid by the synovium which also provides for lubrication of articular cartilage. These independent and distinct lines of pathophysiology, anatomical and biomechanical research appear to have merged. MSF expressed by chondrocytes and synovial fibroblasts appear very similar and both have lubricating ability.

CACP syndrome is a rare trait but may offer an opportunity to understand the importance of lubrication in joint homeostasis. Fibrillation of articular cartilage is an early hallmark of CACP syndrome but it is unknown whether this is a primary or secondary event. It is conceivable that loss of lubrication will lead to excessive wear and thus loss of the superficial zone. In this study, samples of synovial fluid were obtained from patients with CACP syndrome for measurement of lubricating ability and intrinsic viscosity. Lack of lubricating ability would strengthen the above hypothesis and for the first time implicate basic tribologic theories in understanding a type of arthritis.

Method
Collection of Human Synovial Fluids
Synovial fluid was obtained from patients with CACP syndrome undergoing diagnostic knee joint arthrocentesis. Synovial fluid was immediately centrifuged at 10,000g at 4°C for 1 hr to remove cellular debris. Samples were stored at –80°C. Aliquots with normal lubricating ability were pooled and stored at –20°C. Samples of post-mortem human synovial fluids from male and female subjects without gross degenerative joint disease was obtained in grants from Dr. Martin Lotz at Scripps Research Institute. These samples served as controls to identify normal lubricating ability. Synovial fluid collected in this manner was approved by respective Institutional Review Boards.

Collection of Bovine Synovial Fluid
Bovine synovial fluid, for a viscosity assay control was aspirated percutaneously from the lateral aspect of radiocarpal joints of freshly slaughtered cattle. Sterile 18-gauge needles were used after cleansing the skin with alcohol swabs. The cattle were 1 year old and of both sexes (PelFreeze Corp., Little Rock, AK).

Measurement of lubrication
Synovial fluids were assayed for lubricating ability in the friction apparatus which oscillated latex against polished glass under a pressure of 0.37 x 10^6 N/m². This was an improved version of McCutchen’s instrument. Coefficient of friction (\( \mu \)) was reported relative to the \( \mu \) for 0.9% NaCl (NS) which serves as a control for each measurement.

Hyaluronate Concentration Determination
Synovial fluid aspirates were assayed by the manual carbazole reaction for uronic acid in synovial fluid.

Viscosity Measurement
Intrinsic viscosity is independent of concentration by virtue of extrapolation to concentration \( c = 0 \). Undiluted samples of synovial fluid or hyaluronate were first measured at zero shear by an Ubbelohde type semi-micro capillary viscometer (Cannon Instruments, State College, PA). Samples were then progressively diluted 3 times with 0.9% NaCl and re-introduced into the viscometer each time. Measurements were conducted at 37°C. Calculation of \( \eta/c \) was performed in accordance to American Standard for Testing of Materials (ASTM) D2857-95.