INTRODUCTION:
Proteoglycan 4 (PRG4) is a mucinous glycoprotein present in synovial fluid (SF) that contributes to boundary lubrication of articular cartilage in a dose-dependent manner. PRG4 interacts with hyaluronan (HA), another dose-dependent cartilage boundary lubricant, to further decrease friction to levels near that of SF. Concentrations of PRG4 in human SF (hSF) have been reported to be decreased immediately after acute injury, returning to normal levels in 1 year, and increased in late stage osteoarthritis (OA). Concentrations of HA have been reported to remain normal in advanced OA, although molecular weight (MW) distribution has been shown to shift lower. The lubricating ability of SF void of PRG4, as well as that after injury, has been reported to be diminished. Conversely, SF lubricating ability appears to remain normal in advanced OA. Collectively, these studies suggest that SF deficit in PRG4 lacks a normal cartilage lubricating ability, yet it remains unclear if PRG4 levels remain normal in all chronic OA SF. The objectives of this study were to 1) quantify PRG4 and HA content in normal and chronic OA SF samples 2) assess the human cartilage boundary lubricating ability of OA hSF deficient in PRG4, with and without supplementation of physiological levels of PRG4+HA, compared to normal hSF.

METHODS:
Samples. Collection of all human tissues and fluids was approved by the University of Calgary Joint Health Ethics Board. OA SF, OA hSF was aspirated from patients with chronic knee OA undergoing vascosupplementation injection (hSF was collected prior to injection). Normal SF and cartilage. Normal hSF (N=10, age 56±4) and normal human distal femurs (2 donors, age 68±7) were obtained through the Southern Alberta Organ and Tissue Donation Program. SF Biochemical Characterization. PRG4 Concentration. PRG4 concentration in hSF was measured by sandwich ELISA, in triplicate, after digestion of hSF with neuraminidase (Prozyme) and S. Hyaluronidase (Seikagaku). An anti-peptide capture antibody (LPN) recognizing full length PRG4 was used, followed by detection with horseradish peroxidase labeled peptide agglutinin recognizing Galβ1–3GalNAc glycosylations in the mucin domain. PRG4 was purified from culture medium conditioned by bovine cartilage explants by anion exchange chromatography (DEAE), as described previously, followed by size exclusion chromatography (Superose 6), and was used as a standard. HA Concentration. HA concentration in hSF was measured, in triplicate, using a commercially available ELISA (R&D Systems). HA MW Distribution. HA MW distribution in hSF samples, treated with Proteinase K (Roche), was determined in duplicate with 1% agarose gel electrophoresis (AGE), as described previously. The migration of HA was assessed by densitometric analysis with Image J. Cartilage Lubricating Ability. Lubricating ability of hSF was tested in a cartilage-on-cartilage friction test in the boundary lubrication regime using normal human osteochondral cores, essentially as described previously. After compression by 18% and stress relaxation, an effective velocity of 0.3 mm/s and pre-spin durations, Tps, of 120-1.2 s were used. OA hSF samples found to be deficient in PRG4 were tested in the following sequence of lubricants (n=3 for each hSF): PBS, OA, OA+PRG4, OA+PRG4+HA, NL. OA SF was reconstituted with physiologically normal concentrations of PRG4 (450 µg/ml), obtained as described above, and 1.5 MDa HA (Lifecore Biomedical, 1 mg/ml), as measured by ELISA. Static, kinetic friction coefficients were calculated. Statistical Analysis. Data is presented as mean±SEM. Repeated measures ANOVA was used to assess effects of lubricant solution on & kinetic friction coefficients, with post-hoc testing. T-tests were used to assess changes in PRG4 and HA composition.

RESULTS:
Biochemical Characterization. PRG4 Concentration. PRG4 concentration in normal SF was 511±31 µg/ml. OA SF samples (N=10, age 60±7) deficient in PRG4 compared to NL SF (p<0.05) ranged from 184±11 to 443±20 µg/ml and averaged 328±64 µg/ml (Fig. 1A). HA Concentration. Overall HA concentration was similar between OA SF deficient in PRG4 and NL SF (1.6±0.3 vs. 1.0±0.2 mg/ml, p=0.11) (Fig. 1B). HA MW Distribution. HA MW distribution was shifted towards the low MW range in OA SF deficient in PRG4 compared to NL SF. Relative HA concentration (as a % of total concentration) in the 3.1-6.1 MDa range was significantly lower in OA SF (37% vs 54%, p<0.05), while concentrations in the 1.1-3.1 (33% vs 24%), and 0.5-1.1MDa (22% vs 12%) ranges were significantly higher (both p<0.05) (Fig. 1C). Cartilage Lubricating Ability. Lubricants and Tps modulated friction. increased with increasing Tps and varied with test lubricant, with an interaction (all p<0.001) (Fig. 2A). Values were highest in PBS at all Tps, and lower and similar for OA SF, OA+PRG4, OA+PRG4+HA, and NL. also varied with lubricant, only slightly with Tps (values at Tps=1.2 s were within 11±10% of those at Tps=120 s), with an interaction (all p<0.001) (Fig. 2A). OA SF (0.051±0.006) was significantly greater than NL SF (0.029±0.004, p<0.05), and less than PBS (0.152±0.043, p<0.001). Upon reconstitution with PRG4+HA, OA SF+PRG4 (0.029±0.003) was significantly less than OA SF (p<0.05), and OA SF+PRG4+HA tended to be lower (p=0.06). Neither OA SF+PRG4+HA were significantly different than NL SF (p=0.737, p=0.129) (Fig. 2B).

DISCUSSION:
This data provides insight into the molecular basis for altered cartilage lubricating function of OA SF. PRG4 deficient hSF OA samples were identified by a sandwich ELISA: these samples had normal HA concentration and altered HA MW composition, which is consistent with a previous study. These results suggest that normal PRG4 levels may not be present in all chronic OA SF. It remains to be determined if the composition of monomeric-multimeric PRG4 is altered in OA hSF. Friction tests using normal human cartilage demonstrated that OA hSF deficient in PRG4 lacked normal lubricating ability, and could be restored through PRG4 reconstitution. Collectively these results suggest that some chronic OA patients may benefit from PRG4 supplementation as a biotherapeutic treatment.

REFERENCES:

ACKNOWLEDGMENTS: AI, CAN, NSERC (CREATE), TAS.