INTRODUCTION:
Proteoglycan 4 (PRG4) proteins, also known as lubricin, and superficial zone protein (SGP), are encoded for by the PRG4 gene and are synthesized by cells that line joint surfaces. PRG4 is present in synovial fluid and at the surface of articular cartilage where it functions as a critical boundary lubricant necessary for joint health. A recent study, motivated by diminished PRG4 levels associated with early osteoarthritis (OA), demonstrated aldehyde modification of PRG4 significantly enhanced its binding to a depleted articular surface. Such an approach may improve biotherapeutic treatment of early OA, since local administration of PRG4 has been shown to be chondroprotective in an animal model of OA. However, it remains to be determined if aldehyde modification alters the friction-reducing ability of PRG4.

Therefore, the objective of this study was to assess the cartilage boundary lubricating ability of aldehyde modified PRG4 (PRG4-CHO).

METHODS:
PRG4 Preparation. PRG4 was prepared by purification from media conditioned cartilage explants from bovine stifte joints, as described previously. PRG4-CHO was prepared using succinimidyl 4-formylbenzamide (CHO) at a PRG4-CHO molar ratio of 1:100 in 100mM PO4 buffer, essentially as described before. A treatment control (PRG4-SHAM) was exposed to modification buffers and incubations in the absence of CHO. Confirmation of PRG4 purity and the CHO modification was assessed by gel electrophoresis, as described previously, followed by quantification by bicinchoninic acid assay (data not shown).

Untreated and SHAM/CHO PRG4 was prepared from mature (in Calgary) and immature (in Evanston) bovine stifte joints, respectively.

PRG4 Characterization. The size distribution of immunoreactive PRG4 was assayed by SDS-PAGE Western Blotting. Briefly, reduced (R) and non-reduced (NR) samples of PRG4, PRG4-SHAM and PRG4-CHO were subject to 3-8% SDS-PAGE followed by protein stain, and western blotting using anti-PRG4 antibodies LPN & J108N for NR and R respectively, using manufactures instructions and standard Western Blot procedures, as described previously.

Cartilage Boundary Lubrication Tests. Two sequential test sequences were used to assess the cartilage boundary lubricating ability of the PRG4 preparations at a physiological concentration of 450 μg/mL in phosphate buffered saline (PBS). PBS and bovine synovial fluid (SF) served as negative and positive controls, respectively. Test 1: PBS, PRG4-SHAM, PRG4-CHO, PRG4, SF. Test 2: PBS, PRG4-CHO, PRG4-SHAM, PRG4, SF. Each lubricant was assessed using the previously described in vitro cartilage-on-cartilage friction test under boundary lubricating conditions. Briefly, bovine osteochondral samples (n=6) were incubated with test lubricants over night, then compressed by 18%, allowed to stress relax to depressurize the lubricant present, and then tested under rotating at a constant velocity of 0.3 mm/s with pre-spin durations (Tps) of 1200, 120, 12, and 1.2. Static (μstatic,Neq) and kinetic (μkinetic,Neq) friction coefficients were then calculated. Statistical Analysis. Data are presented as mean±SEM.

RESULTS:
PRG4 Characterization. PRG4-SHAM and PRG4-CHO had similar MW and reactivity to anti-PRG4 Ab LPN and J108N compared to untreated PRG4. Specifically, protein staining of NR PRG4, PRG4-SHAM, and PRG4-CHO showed two high MW bands slightly above (*) and below (**) the 460kDa marker (Fig. 1A). Upon reduction, the single high MW band (*) was observed as well as a predominant lower MW band (x) between the 71kDa & 117kDa markers (Fig. 1B). Western blotting of NR samples showed two high MW (**, *) LPN-reactive bands (Fig. 1C) that co-migrated with those observed by protein stain, while R showed the high (**) and lower (x) J108N immunoreactive bands (Fig. 1D) that co-migrated with those observed by protein stain.

Cartilage Boundary Lubricating Ability. All PRG4 preparations functioned as effective friction-reducing cartilage boundary lubricants. No significant differences were observed between friction coefficient values obtained for PRG4-SHAM and PRG4-CHO from the two test sequences (p=0.54 and p=0.45, respectively), therefore data was pooled.

Lubricants and Tps modulated friction. μstatic,Neq varied with Tps and test lubricant (both p<0.001), with an interaction (p=0.01). Values increased with Tps and were consistently highest in PBS (ranging from 0.30 ± 0.04 to 0.57 ± 0.06) and lowest in SF (0.032 ± 0.003 to 0.26 ± 0.03). Values in PRG4, PRG4-SHAM, and PRG4-CHO were intermediate and similar to each other (Fig. 2A). μkinetic,Neq values exhibited similar trends, varying with lubricant and Tps (both p<0.001) increasing only slightly with Tps (values at Tps=1.2s were within 19 ± 12% of those at Tps=1200s). μkinetic,Neq values at Tps=1.2s were greatest in PBS (0.24 ± 0.04) and lowest in SF (0.024 ± 0.003). Values in PRG4 (0.11 ± 0.02), PRG4-SHAM (0.15±0.02), and PRG4-CHO (0.15±0.01) were again intermediate and significantly different than PBS and SF (both p<0.05), but not statistically different from each other (p=0.55-1.00, Fig 2B).

DISCUSSION:
This data indicates that CHO modified PRG4 maintains its ability to function as an effective friction reducing cartilage boundary lubricant. The lubrication test data coupled with the western blotting suggests that CHO modification does not significantly affect the protein structure or lubricating function of PRG4, which is consistent with a previous study examining CHO modification. This data also suggests PRG4 synthesized by, and purified from, immature bovine cartilage has similar MW and lubricating function as that from mature bovine cartilage, although future studies would provide additional insight into potential differences.

While the mechanism of molecular interactions of PRG4 with the cartilage surface remains to be elucidated, local administration of recombinant PRG4 has been shown to localize to joint tissues for up to 28 days following a single injection. As such, it remains to be determined if PRG4-CHO has an enhanced ability to adhere and reside on joint tissues, and potentially provide prolonged cartilage boundary lubricating function even in the absence of PRG4 in solution.

SIGNIFICANCE:
CHO modification may be a useful approach for molecular resurfacing of tissue surfaces, specifically articular cartilage, with lubricating molecules such as PRG4, potentially providing enhanced biotherapeutic treatment of early OA.


ACKNOWLEDGMENTS: ABHS, CAN, NIH, NSEC, TAS.