Synovial Fluid Nutrient Delivery in the Diathrial Joint: An Analysis of Rabbit Knee Ligaments

David Amiel, Mark F. Abel, Jeffrey B. Kleiner, Richard L. Lieber, and Wayne H. Akeson

Division of Orthopaedics and Rehabilitation, University of California, San Diego, La Jolla, California, U.S.A.

Summary: The role of synovial fluid in providing nutrition to rabbit knee ligaments and menisci was evaluated by intraarticular injection of a labeled collagen precursor, tritiated proline. Incorporation of this substrate as tritiated hydroxyproline was measured in collateral and cruciate ligaments and menisci. The injectate volume (0.35 ml) did not appreciably change the overall joint pressure as measured by a wick catheter; therefore, no alteration of synovial membrane diffusion characteristics resulted. The concentration of the injected proline (0.52 mg%) was well below that normally present in serum (2.65 mg%). Therefore, incorporation of this substrate was not driven by a concentration gradient and represented normal uptake of synovial fluid and physiological incorporation of label as measured by the presence of tritiated hydroxyproline. Autoradiography was performed on all ligaments and menisci, and demonstrated concentration of the isotope and its metabolite (tritiated proline and tritiated hydroxyproline, respectively) in and around fibroblasts. This study indicates that rabbit knee ligaments and menisci can derive nutrition from a synovial fluid source. Key Words: Cruciate ligaments—Menisci—Collateral ligaments—Nutrition—Synovial fluid.

The early investigations of Hunter (11) showed hyaline articular cartilage to be avascular. However, synovial fluid was not identified as its sole nutrient source until the twentieth century (3,8,23). Recent advances in the understanding of tendon (10,17–22,24) and ligament (25,31) physiology have shown that tracers from synovial fluid enter these structures. Lundborg (17,19) inserted partially lacerated tendons into the suprapatellar pouch of rabbits and noted cellular proliferation. Renzoni et al. (25), using tritiated proline administered intraarticularly, have shown incorporation as tritiated hydroxyproline in the anterior cruciate ligament. However, when a lavage system was used to prevent synovial fluid contact, deliver was significantly decreased. This suggests that synovial fluid, formed from an ultrafiltrate of blood (26), may be a physiologically important nutrient delivery pathway for the anterior cruciate ligament.

Further investigations of joint structure show that the synovium, as seen by electron microscope, has no limiting basement membrane beneath the intimal cell layer (5). Intimal cells are interrupted by gaps and many of the subintimal capillaries are fenestrated to facilitate exchange of small molecules between blood and synovial fluid (5,28,30). Functionally, the synovial space has characteristics of interstitial spaces elsewhere in the body, indicating that the joint cavity is a connective tissue milieu modified for motion (7,14).

In the present study, we hypothesize that periarticular connective tissue structures of the knee...
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utilize synovial fluid as a source of nutrition. Furthermore, the use of this pathway by tissues is related to their proximity to synovial fluid, e.g., cruciates uptake, then menisci uptake, then collaterals uptake. To investigate this hypothesis, we used tritiated proline as a marker, since it is converted to tritiated hydroxyproline within the fibroblast during collagen synthesis (29). Each structure studied—the cruciate ligaments, collateral ligaments and both menisci—is 70–80% collagen in dry mass (1,6). Therefore, their labeled hydroxyproline content reflects utilization and incorporation of the synovially derived precursor, tritiated proline.

MATERIALS AND METHODS

Animal Model

Ten male New Zealand white rabbits (3.4–3.7 kg) were anesthetized using ketamine and xylazine delivered intramuscularly. Tritiated proline (specific activity 22 Ci/mmol, 1 mCi/ml), obtained from a single lot, was used for intraarticular injection at a dose of 100 μCi/kg body weight. A high-precision Hamilton syringe was used, inserting the needle into the left suprapatellar pouch of the synovial cavity, and directing the tip down the patellar groove. Injection was by manual pressure. Following the injection, the joint was moved through its full arc of motion 10 times to distribute the isotope. An average injectate volume of 0.35 ml was used. We felt that this would provide sufficient isotope to insure detection of the metabolic conversion product, tritiated hydroxyproline; and to achieve rapid distribution of tritiated proline throughout the joint.

The rabbits were placed in cages for an 8 h, postinjection period, during which time the joint tissues were metabolizing tritiated proline. After 8 h, the animals were killed and the ligaments (both cruciates and both collaterals) and menisci (medial and lateral) were carefully dissected free of all synovial covering. Only the inner halves of the menisci were utilized, so that avascular, intrasynovial structures could be compared with the ligaments.

The right knee received a sham injection of saline to serve as a control for blood–nutrient delivery. After the 8 h experimental period, ligaments and menisci were handled in the same manner as the experimental group. The control structures were analyzed in three rabbits only, since previous work with this rabbit model (25) has shown negligible tritiated proline uptake by these structures when evaluated up to 48 h following intraarticular injections of the contralateral knee.

The joint capsule anatomy and distribution of injectate was evaluated roentgenographically on five rabbits after injection of 0.35 ml radiopaque contrast (Renografin). This technique is known as arthrography. Similarly, injections were performed in five rabbits (10 knees) with simultaneous pressure recording to quantitate the joint pressures. A wick catheter (PE-20 tubing) (27) filled with saline was threaded through a 16-gauge needle, which in turn was inserted through the lateral half of the patellar tendon into the infrapatellar compartment of the synovial cavity. Once the needle was through the tendon, it was pulled back while the wick catheter was simultaneously advanced into the synovial cavity. This insertion technique minimized the amount of air admitted into the joint. Pressures were recorded following injections into the suprapatellar pouch.

Biochemistry

Within 30 min of sacrifice, the anterior cruciate, posterior cruciate, medial collateral, and lateral collateral ligaments (ACL, PCL, MCL, and LCL, respectively) and medial and lateral menisci (MM and LM, respectively) were removed, washed and placed in containers for acid hydrolysis. A 25-mg sample of tissue was hydrolyzed in 6 N HCl for 24 h at 100°C. Following the hydrolyzation, a standard aliquot (750 μl) was chromatographed on a cation-exchange column to isolate tritiated proline and tritiated hydroxyproline fractions. Counts of these fractions were obtained using a Beckman LS6800 scintillation counter. Another aliquot of hydrolysate was used to determine the total amount of hydroxyproline spectrophotometrically at 557 nm (32). Therefore, specific activity in each structure could be expressed in terms of counts per minute (cpm) of tritiated hydroxyproline per microgram of total hydroxyproline.

Autoradiography

Four rabbits had intraarticular injections of tritiated proline (100 μCi/kg) via the suprapatellar pouch as described above. At sacrifice, the ipsilateral ligaments and menisci were harvested, washed, and quick-frozen in isopentane liquid ni-
trogen for sectioning. Serial sagittal sections, 12 μm, of the structures were mounted on slides and coated with Kodak NTB-2 emulsion. Incubation in a darkroom followed for 2 weeks at 4°C, and the sections were developed in Kodak Dektol for 3 min. These sections were lightly stained with hematoxylin and eosin and viewed in the light microscope to localize the isotope.

Statistical Analysis

In order to test our hypothesis, a one-way analysis of variance (ANOVA) was performed using three grouping variables, e.g., cruciates, menisci, and collaterals. Significant differences between selective pairs were performed using a multiple t-test (BMDP program P1V) (2). Following inspection of the data, significant differences between all structures—e.g., ACL, PCL, MM, LM, LCL, MCL—were tested using paired t-test for unplanned comparison (BMDP program P1V) (2).

RESULTS

Arthrography

The injection technique was evaluated arthrographically to ascertain the distribution of the isotope within the joint. After injecting and ranging the joint 10 times, the contrast was seen at the edge of the joint, in the posterior joint cavity and in the suprapatellar pouch (Fig. 1). Therefore, the injectate volume was sufficient to fill the synovial cavity, and tritiated proline uptake could be correlated with synovial fluid exposure.

Joint Pressures

The initial joint pressures ranged from −1 to −4 cm of water. Injection of 0.35 ml of saline into the suprapatellar pouch resulted in little or no joint pressure change in the infrapatellar compartment (Fig. 2A). Infrapatellar pressures began to rise.

FIG. 1. Anteroposterior (A) and lateral renografin (B) arthrograms of a rabbit knee injected with 0.35 ml of contrast. Note that this volume outlines the entire intrasynovial cavity.
FIG. 2. Joint pressure measurements. A: Infrapatellar compartmental pressure develops a small elevation when 0.4–0.6 cc of injectate is introduced into the suprapatellar compartment. Note the initial delay and gradual pressure increase; these are properties of the viscoelastic nature of the suprapatellar pouch and the presence of synovial valves between compartments. B: Abrupt pressure elevation is evident when the infrapatellar compartment is directly injected.

when 0.4–0.6 ml was injected into the suprapatellar pouch. The magnitude of this rise varied with the rate of the injection but was generally 3–6 cm H$_2$O pressure from the baseline. The delayed pressure in the infrapatellar compartment reflected the viscoelastic nature of the suprapatellar pouch and the presence of “synovial valves” between compartments (16). Direct injection into the infrapatellar compartment caused an abrupt rise of 4–6 cm H$_2$O within that compartment (Fig. 2B). The pressures dropped slowly following the injection with the joint at rest, but as the joint was ranged several times, the pressures returned more rapidly to baseline.

### Tritiated Hydroxyproline Incorporation

Measurements of tritiated hydroxyproline incorporation showed that all six knee structures utilized synovially derived tritiated proline (Table 1). Large variability was observed in the specific activity of tritiated proline, since the amount of free, unbound tritiated proline was influenced by the washing technique. None of the comparable structures in the control knee, which received a sham injection, had measurable incorporation. Therefore, the nutrient contribution to these structures by vascular inflow was negligible.

Statistical analysis from the one-way ANOVA revealed significant differences between the three groups (e.g., cruciates, menisci, collaterals) at p < 0.0001. Subsequent planned comparison demonstrated that the cruciates have a significantly greater uptake than the menisci at p < 0.0001 and also a greater uptake than the collaterals, p < 0.0001 (see Fig. 3). However, no significant differences were seen between the menisci and collaterals, p > 0.75. Further differences were investigated using a t-test for unplanned comparison.

Uptake for the two cruciates was not significantly different (p > 0.40), and likewise the uptakes for the medial and lateral menisci were not significantly different (p > 0.75). However, statistical analysis of the isotope uptake of the LCL versus the MCL revealed a trend toward a difference between these two structures (p = 0.075). Thus, the knee ligaments studied effectively utilized synovial nutrients with the cruciate ligaments having the highest consumption.

### Autoradiography

Autoradiographs revealed that the isotope had reached the deep areas of all specimens (Fig. 4). There did appear to be more isotope at the edges of the structures than in the middle. However, concentrations of isotope matching that seen peripherally were found between individual fiber fascicles of the tissue.

### Table 1. Specific radioactivity of O$^3$H-proline in periarticular structures

<table>
<thead>
<tr>
<th>Structure</th>
<th>cpm O$^3$H-Proline</th>
<th>µg total HP</th>
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<tbody>
<tr>
<td>Anterior cruciate ligament</td>
<td>4.88 ± 0.59</td>
<td></td>
</tr>
<tr>
<td>Posterior cruciate ligament</td>
<td>5.50 ± 0.63</td>
<td></td>
</tr>
<tr>
<td>Medial meniscus</td>
<td>3.04 ± 0.44</td>
<td></td>
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<tr>
<td>Lateral meniscus</td>
<td>2.83 ± 0.39</td>
<td></td>
</tr>
<tr>
<td>Medial collateral ligament</td>
<td>2.13 ± 0.36</td>
<td></td>
</tr>
<tr>
<td>Lateral collateral ligament</td>
<td>3.42 ± 0.54</td>
<td></td>
</tr>
</tbody>
</table>

Mean counts (± SE) of labeled O$^3$H proline per microgram of total hydroxyproline are shown.

### Table 2. Blood flow

<table>
<thead>
<tr>
<th>Pig knee structures</th>
<th>Blood flow (ml/100 g tissue/min)</th>
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<tbody>
<tr>
<td>Anterior cruciate ligament</td>
<td>1.25 ± 0.20</td>
</tr>
<tr>
<td>Posterior cruciate ligament</td>
<td>1.46 ± 0.07</td>
</tr>
<tr>
<td>Medial meniscus</td>
<td>0.43 ± 0.05</td>
</tr>
<tr>
<td>Medial collateral ligament</td>
<td>0.90 ± 0.03</td>
</tr>
<tr>
<td>Lateral collateral ligament</td>
<td>1.71 ± 0.32</td>
</tr>
<tr>
<td>Resting muscle</td>
<td>3.40 ± 0.18</td>
</tr>
</tbody>
</table>

Means ± SE are given.
DISCUSSION

It has long been recognized that synovial fluid is an ultrafiltrate of blood and contains the same small nutrient molecules and electrolyte composition (26). Resting intraarticular pressures are negative (12,14) and favor a net filtration from subsynovial arterial capillaries into the joint. Absorption of joint substances then follows one of three routes: the synovial lymphatics, the synovial venous capillaries or the extrasynovial, periarticular tissues. Levick has shown in a series of experiments that the primary pathway of transsynovial flow is to the extrasynovial tissues (15,16). The effect of joint motion on fluid flow and joint pressure has been documented (12,14,15), and our measurements are in agreement with these values. The joint pressures that resulted from the intraarticular injections would not alter synovial fluid diffusion characteristics (14,15).

The ligamentous and meniscal tissue of injected joints showed incorporation of synovially derived tritiated proline, whereas control ligaments and menisci from the contralateral limb showed no detectable isotope. Serum proline concentration is reported to be 2.65 mg%, and injected proline had a concentration of 0.52 mg% (4). Thus penetration measured autoradiographically reflects concentrations within physiologic ranges. These findings indicate that the preferred flow was from the synovial cavity to the periarticular connective tissue rather than directly to subsynovial venous capillaries. The fact that synovial fluid equilibrates with periarticular interstitial fluid is reflected by the uptake and incorporation of labeled proline within the articular structures. The cruciate ligaments and menisci freely exposed to synovial fluid (9), had the greatest proline incorporation. The MCL, which showed a trend toward the lowest incorporation, is found closely applied to bone with little exposure to the joint space and the corresponding joint fluid. The LCL, although farther from the joint, lies in a low-pressure space in contact with the synovial extension surrounding the popliteus tendon (9,13). The arthrograms (roentgenograms of the joint) clearly demonstrate the distribution (Fig. 1). Contrast medium was seen in the space between the LCL and the condyles, whereas comparatively little was seen between the MCL and the joint. Thus all knee ligaments and menisci, and presumably capsular tissue, utilize synovial nutrients, with
the amount of transsynovial delivery correlating with proximity to synovial fluid.

The path of nutrient delivery is from synovial arterial capillaries to the synovial cavity and, then, by transsynovial bulk flow and diffusion to the knee ligaments and menisci. Ultimately, the effectiveness of delivery depends on tissue metabolism, thickness, and permeability, as well as synovial nutrient concentration. Although there is a paucity of data on periarticular tissue metabolism and diffusion characteristics, this in vivo model employed physiological concentrations of labeled proline and complete tissue penetration was seen. Furthermore, there was a preferential accumulation of the isotope in the intrafascicular tissue, an area where the fibroblast resides, suggesting that an efficient delivery mechanism is inherent to the ligamentous and meniscal tissues.

Our hypotheses in this study were that the periarticular connective tissue structures of the knee can derive nutrients from synovial fluid and that the consumption of these nutrients is related to the proximity of synovial fluid and level of tissue metabolism. These notions were founded on previous observations of joint anatomy and function. The results support these hypotheses using a physiologically appropriate model. We found that (a) transsynovial nutrient flow is a physiologically important nutrient pathway to ligaments and menisci of the diarthral joint; and (b) the amount of synovial nutrient delivery correlated with ligamentous and meniscal exposure to synovial fluid.

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REFERENCES