

# Leukocyte-Reduced Platelet-Rich Plasma Normalizes Matrix Metabolism in Torn Human Rotator Cuff Tendons

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**Background:** The optimal platelet-rich plasma (PRP) for treatment of supraspinatus tendinopathy has not been determined.

**Purpose:** To evaluate the effect of low- versus high-leukocyte concentrated PRP products on catabolic and anabolic mediators of matrix metabolism in diseased rotator cuff tendons.

**Study Design:** Controlled laboratory study.

**Methods:** Diseased supraspinatus tendons were treated with PRP made by use of 2 commercial systems: Arthrex Autologous Conditioned Plasma Double Syringe System (L<sup>lo</sup> PRP) and Biomet GPS III Mini Platelet Concentrate System (L<sup>hi</sup> PRP). Tendon explants were placed in 6-well plates and cultured in L<sup>lo</sup> PRP, L<sup>hi</sup> PRP, or control media (Dulbecco's Modified Eagle Medium + 10% fetal bovine serum) for 96 hours. Tendons were processed for hematoxylin-eosin histologic results and were scored with the modified Bonar scale. Group 1 tendons were defined as moderate tendinopathy (Bonar score <3); group 2 tendons were assessed as severely affected (Bonar score = 3). Transforming growth factor  $\beta$ -1 (TGF $\beta$ -1), interleukin-1 $\beta$  (IL-1 $\beta$ ), interleukin-1 receptor antagonist (IL-1Ra), interleukin-6 (IL-6), interleukin-8 (IL-8), and matrix metalloproteinase-9 (MMP-9) concentrations in PRP media were measured by use of enzyme-linked immunosorbent assay after 96 hours of culture with diseased tendon. Tendon messenger RNA expression of collagen type I (*COL1A1*), collagen type III (*COL3A1*), cartilage oligomeric matrix protein (*COMP*), *MMP-9*, *MMP-13*, and *IL-1 $\beta$*  was measured with real-time quantitative polymerase chain reaction.

**Results:** Leukocytes and platelets were significantly more concentrated in L<sup>hi</sup> PRP compared with L<sup>lo</sup> PRP. Increased IL-1 $\beta$  was present in L<sup>hi</sup> PRP after culture with group 1 tendons. IL-6 was increased in L<sup>hi</sup> PRP after culture with group 2 tendons. Both TGF $\beta$ -1 and MMP-9 were increased in L<sup>hi</sup> PRP after culture with either tendon group. In L<sup>lo</sup> PRP cultures, IL-1Ra:IL-1 $\beta$  in PRP used as media and *COL1A1:COL3A1* gene expression were increased for group 1 tendon cultures. Gene expression of *MMP-9* and *IL-1 $\beta$*  was increased in group 2 tendons cultured in L<sup>lo</sup> PRP. There was no significant difference in the expression of *MMP-13* or *COMP* in either group of tendons cultured in L<sup>lo</sup> PRP or L<sup>hi</sup> PRP.

**Conclusion:** L<sup>lo</sup> PRP promotes normal collagen matrix synthesis and decreases cytokines associated with matrix degradation and inflammation to a greater extent than does L<sup>hi</sup> PRP in moderately degenerative tendons. In severely degenerative tendons, neither PRP preparation enhanced matrix synthesis.

**Clinical Relevance:** L<sup>lo</sup> PRP may promote healing in moderately degenerative rotator cuff tendons.

**Keywords:** platelet-rich plasma; growth factors; biological healing enhancement; shoulder; rotator cuff

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Rotator cuff tendon abnormalities are among the most frequent causes of shoulder pain.<sup>8,36</sup> Without intervention, the prognosis for symptomatic patients with rotator cuff tears remains relatively poor.<sup>8,9</sup> Even with surgical repair, poor quality tissue regeneration is associated with anatomic and biological failure. This might be attributed to insufficient gene or protein expression or a paucity of undifferentiated cells at the healing site.<sup>6,12,14,15</sup> Relatively high failure rates after rotator cuff repair and the challenges associated with compromised tendon structure in chronic rotator cuff conditions remain important considerations for the development of techniques that enhance the biological healing environment. Current techniques include

extracellular matrices, allogenic or autogenic tendon transplants, synthetic mesh, or the addition of growth factors to the surgical site.<sup>10</sup> The use of extracellular or synthetic matrices for shoulder repair has been associated with poor results in some studies.<sup>17,32</sup> However, introduction of growth factors after a soft tissue traumatic event can stimulate enhanced repair and angiogenesis.<sup>27</sup> Platelet-rich plasma (PRP) contains growth factors of interest for tendon regeneration, making it attractive for use in rotator cuff repair augmentation.

Concurrent with the growth in PRP technologies is the increase in the diversity of preparation systems offering differing cellular concentrations in the resultant final PRP product delivered to the patient. In addition to platelet content variability, large differences in leukocyte concentration exist between PRP systems. Whether leukocytes have a positive or negative influence on healing tissues remains a controversial issue and a subject of investigation.<sup>30</sup> Multiple studies demonstrate how reduced inflammation promotes normal collagen generation and may reduce the degree of tendon damage during the healing process by limiting catabolic activity, such as extracellular matrix catabolism.<sup>24,25,35,37</sup> Additional properties of PRP that are thought to be beneficial for the treatment of tendon injuries include provision of a fibrin-matrix scaffold rich in growth factors that promotes collagen organization, fiber pattern alignment, tenocyte proliferation, and collagen type I synthesis.<sup>23</sup>

The objective of this study was to compare PRP generated from Arthrex Autologous Conditioned Plasma Double Syringe System (L<sup>lo</sup> PRP; Arthrex Inc) and Biomet GPS III Mini Platelet Concentrate Kit (L<sup>hi</sup> PRP; Biomet Inc) and their effects on diseased rotator cuff tendon metabolism. This is in contrast to a number of studies investigating the effects of PRP on healthy tendon or in animal models that do not reflect clinical reality.<sup>7,24,31</sup> With the knowledge that the L<sup>lo</sup> PRP system concentrates platelets and reduces leukocytes and that the L<sup>hi</sup> PRP system concentrates both platelets and leukocytes compared with whole blood, we hypothesized that increased leukocyte concentration would result in increased delivery of catabolic cytokines and matrix metalloproteinases (MMPs) with resultant increased tendon matrix degradation.

## METHODS

All procedures were approved by the appropriate regulatory bodies at Cornell University and Rush University Medical Center. All investigations were conducted in conformity with ethical principles of research, and informed consent for participation in the study was obtained from all participants.

### Pilot Study to Determine the Effects of Acid Citrate Dextrose in PRP on Tendon Metabolism

The use of acid citrate dextrose (ACD) anticoagulant in 1 of the 2 PRP systems (Arthrex Double Syringe ACP System; L<sup>lo</sup> PRP) is optional according to the manufacturer's directions if the PRP is used within 4 hours of initial blood

collection. The second system (Biomet GPS III Mini Platelet Concentrate Separation Kit; L<sup>hi</sup> PRP) requires 10% final volume ACD. The exclusion of ACD, which has a pH of 4.98, has the advantages of maintaining a physiologic pH, minimizing pain after injection of PRP,<sup>26</sup> and simplifying the steps and reagents needed to generate PRP. A pilot study was performed to determine the effect of inclusion or exclusion of ACD in L<sup>lo</sup> PRP on tendon metabolism.

Human biceps tendons (n = 6) were procured from cadaveric donors and dissected into 3 × 5 × 5-mm explants. Venous blood was obtained with and without ACD from healthy human volunteers to generate L<sup>lo</sup> PRP. Explants were cultured in PRP as media for 96 hours with PRP from 1 donor being used on tendon explants from 1 cadaver. Media pH was measured by use of indicator paper (Whatman) to assess exhaustion of culture media nutrients. At the time of culture termination, tendons were pulverized in a freezer-mill, and total RNA was extracted by use of the Qiagen RNeasy Kit (RNeasy Mini Handbook; Qiagen). Real-time quantitative polymerase chain reaction (RT-qPCR) was performed to measure anabolic markers collagen type I (*COL1A1*), collagen type III (*COL3A1*), cartilage oligomeric matrix protein (*COMP*), catabolic markers including *MMP-9* and *MMP-13*, tumor necrosis factor- $\alpha$  (*TNF- $\alpha$* ), and interleukin-1 $\beta$  (*IL-1 $\beta$* ) by use of ABIPRISM 7900HT Sequence-Detection with Taqman Gene Expression Assays-Inventoried (Applied Biosystems). Expression results were normalized to 18S RNA expression by use of the 2<sup>- $\Delta\Delta C_T$</sup>  method. A Shapiro-Wilks test indicated that the data were normally distributed. Gene expression and media pH were compared by use of 2-sample *t* tests.

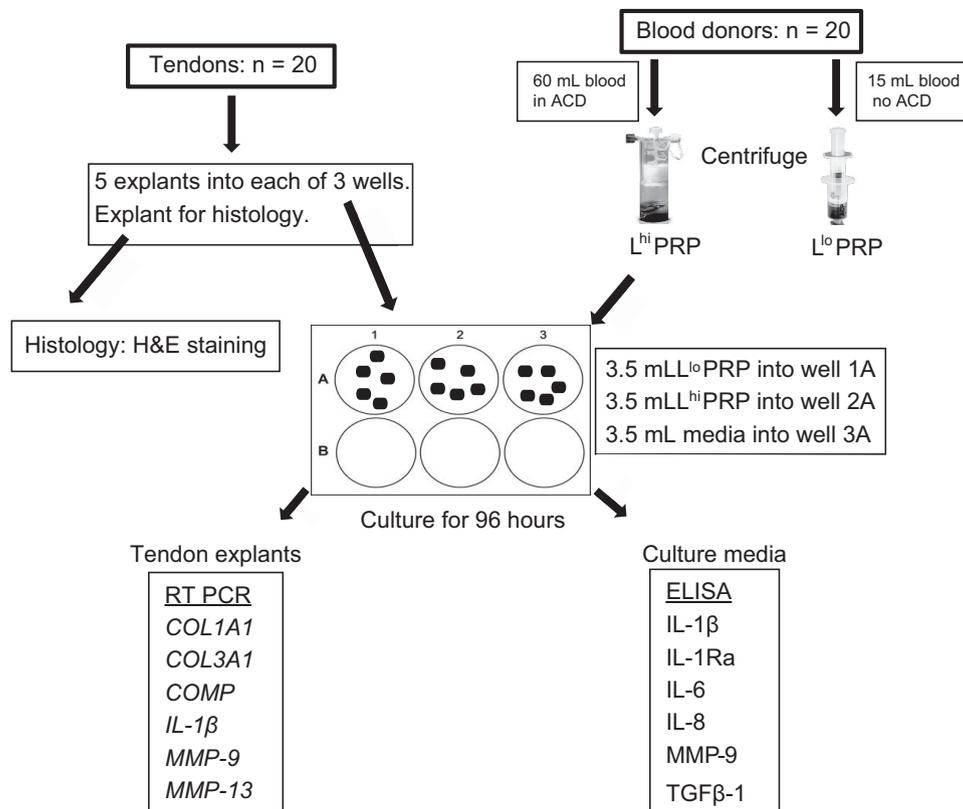
The pH of PRP without ACD (pH, 8.0) was significantly higher compared with that of PRP containing ACD (pH, 7.0; *P* < .001). There were no differences in expression genes between the PRP groups with or without ACD. Expression of *TNF- $\alpha$*  was undetectable. Results of the pilot study indicated that ACD at a final volume of 10% does not affect normal tendon metabolism and should not be a factor in outcome assessment when comparing PRPs with or without ACD. The following principal study was therefore performed using L<sup>lo</sup> PRP without ACD and L<sup>hi</sup> PRP with ACD to reflect common clinical practice.

### Rotator Cuff Tendon Acquisition and Preparation

Tendon biopsy specimens were taken from the lateral aspect of chronically torn supraspinatus tendons after routine exposure of the glenohumeral joint from 20 patients between the ages of 60 and 80 years who were undergoing reverse shoulder arthroplasty for rotator cuff arthropathy. Tendons were dissected into 3 × 3 × 5-mm explant pieces and rinsed with Hank's Balanced Salt Solution (HBSS), and 5 explants were placed per well of a 6-well plate (Figure 1).

### Platelet-Rich Plasma

Venous blood was collected from a healthy human volunteer population distinct from the rotator cuff donor patients. Blood was used to generate L<sup>lo</sup> PRP (Arthrex



**Figure 1.** Schematic of study design and method. ACD, acid citrate dextrose; *COL1A1*, collagen type I; *COL3A1*, collagen type III; *COMP*, cartilage oligomeric matrix protein; ELISA, enzyme-linked immunosorbent assay; H&E, hematoxylin and eosin; IL-1β, interleukin-1β; IL-1Ra, interleukin-1 receptor antagonist; IL-6, interleukin-6; IL-8, interleukin-8; L<sup>hi</sup> PRP, leukocyte-high platelet-rich plasma; L<sup>lo</sup> PRP, leukocyte-low platelet-rich plasma; MMP-9, matrix metalloproteinase-9; *MMP-13*, matrix metalloproteinase-13; RT-PCR, real-time polymerase chain reaction; TGFβ-1, transforming growth factor β-1.

ACP without ACD) and L<sup>hi</sup> PRP (Biomet PRP with ACD) according to manufacturer directions. One blood donor was used per tendon, and blood from that donor was used to make both L<sup>hi</sup> PRP and L<sup>lo</sup> PRP. Whole blood, platelet-poor plasma, L<sup>lo</sup> PRP, and L<sup>hi</sup> PRP samples were submitted for complete blood counts. Immediately after PRP preparation, tendon explants from a single patient were incubated in L<sup>lo</sup> PRP, L<sup>hi</sup> PRP from a single donor, or control media (Dulbecco's Modified Eagle's Medium [DMEM] + 10% fetal bovine serum [FBS]) for 96 hours. At conclusion of the incubation period, pH values for each sample were recorded to verify there were no large decreases in pH indicating exhaustion of nutrients. Tendon explants were washed with phosphate-buffered saline (PBS), snap frozen, and stored at -80°C for RNA isolation. Media were stored at -80°C in independent aliquots for each of the enzyme-linked immunosorbent assays (ELISAs) to avoid repeated freeze/thaw cycles.

#### RNA Purification and qPCR

Gene expression of *COL1A1*, *COL3A1*, *COMP*, *MMP-13*, *MMP-9*, and *IL-1β* was quantified as described for the pilot study.

#### Growth Factor and Cytokine Quantification

Concentration of transforming growth factor β-1 (TGFβ-1) was determined by use of the E<sub>max</sub> ImmunoAssay System (Promega Corp), and MMP-9 was measured with the Biotrak Activity Assay (GE Healthcare Biosciences) by use of a multiple-detection plate reader (Tecan Safire). IL-1β, IL-1 receptor antagonist protein (IL-1RA), IL-6, and IL-8 were measured with Fluorokine MAP Human Elisa Kits on a Fluorokine MAP Human Base Panel (R&D Systems).

#### Histologic Testing

Tendon samples that were not subjected to culture were histologically prepared and scored according to changes in tenocyte morphologic characteristics, collagen bundle characteristics, and variations in vascularity. Briefly, tendon explants were fixed in 4% paraformaldehyde, dehydrated in grade alcohol, cleared in xylene, and embedded in paraffin. Sequential 4-μm sections were cut, stained with hematoxylin-eosin, and then examined under light microscopy. Sections were independently scored by 2 readers (J.A.C., L.A.F.) using the modified 4-point Bonar scale (0-3, with 0 being normal).<sup>11</sup> The Bonar scale scores were

plotted, and there was a distinct separation into 2 equal groups of  $n = 7$ . For further analysis, tendons were separated into group 1 tendons, defined as those demonstrating moderate tendinopathy (Bonar score  $<3$ ), and group 2 tendons, defined as those with severe tendinopathy (Bonar score = 3). Five patients did not have enough sample for histologic examination, and 1 explant had no histologic evidence of tendon tissue. These 6 patients were removed from the study because they could not be histologically classified according to disease status.

## Statistical Analyses

A Shapiro-Wilks test indicated normal distribution of cell counts in blood and PRP (platelet, hematocrit, neutrophil, lymphocyte, and monocyte concentrations), so a 2-sample  $t$  test was used to determine differences between treatments. ELISA and gene expression data were not normally distributed (Shapiro-Wilks test). A Kruskal-Wallis 1-way analysis of variance (ANOVA) was used to determine significant differences in gene expression and ELISA data with tendons categorized as group 1 or group 2. A  $P$  value less than .05 was considered significant. Statistix 9 software (Analytical Software) was used to perform the analyses.

## RESULTS

### PRP Composition

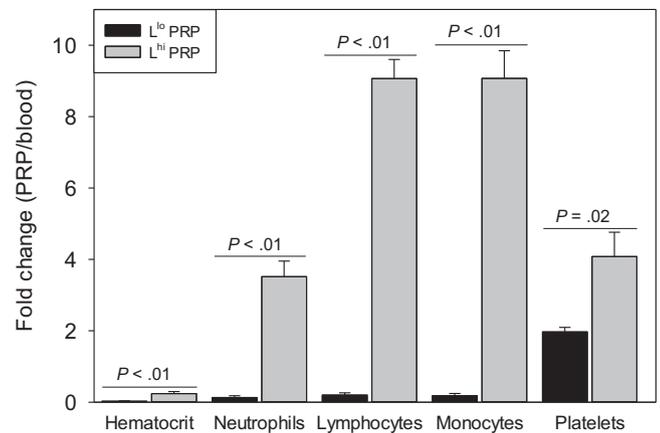
Both systems successfully generated PRP. Neutrophil, lymphocyte, and monocyte fold changes (PRP/whole blood value) were significantly greater in  $L^{hi}$  PRP compared with  $L^{lo}$  PRP (Figure 2). Platelet and RBC concentrations were significantly greater in  $L^{hi}$  PRP compared with  $L^{lo}$  PRP. Platelets were concentrated approximately  $2\times$  over venous blood in  $L^{lo}$  PRP and  $4\times$  in  $L^{hi}$  PRP.

### Histologic Results

Retrieved tendons had histologic evidence of thinning, separation, and disorganization of collagen fibers. Tendon fibroblasts were often round in shape with evidence of hyperplasia (Figure 3). There were scattered areas of mononuclear cell infiltration, vascular proliferation, fatty infiltration, and lipid and myxoid degeneration.

### Catabolic Cytokines (IL-1, IL-1RA, IL-6, IL-8, MMP-9) in PRP After Culture With Diseased Tendon

In moderately degenerative group 1 tendon cultures,  $L^{lo}$  PRP had significantly lower IL-1 $\beta$  concentration (Figure 4A), no difference in IL-1RA (Figure 4A), and increased IL-1RA:IL-1 $\beta$  ratio (Figure 4B) compared with  $L^{hi}$  PRP. In severely degenerative group 2 tendon cultures, there were no differences in IL-1 $\beta$  between  $L^{lo}$  PRP and  $L^{hi}$  PRP (Figure 4A), a significant increase in IL-1RA in  $L^{hi}$



**Figure 2.** Distribution of hematocrit, neutrophil, lymphocyte, monocyte, and platelet concentrations in leukocyte-low platelet-rich plasma ( $L^{lo}$  PRP) or leukocyte-high PRP ( $L^{hi}$  PRP) divided by corresponding venous blood values to generate fold change. Bars represent mean ( $n = 20$ )  $\pm$  standard error. A 2-sample  $t$  test was performed to compare values between  $L^{lo}$  PRP and  $L^{hi}$  PRP.

PRP (Figure 4A), but no resultant change in the IL-1RA:IL-1 $\beta$  ratio (Figure 4B).

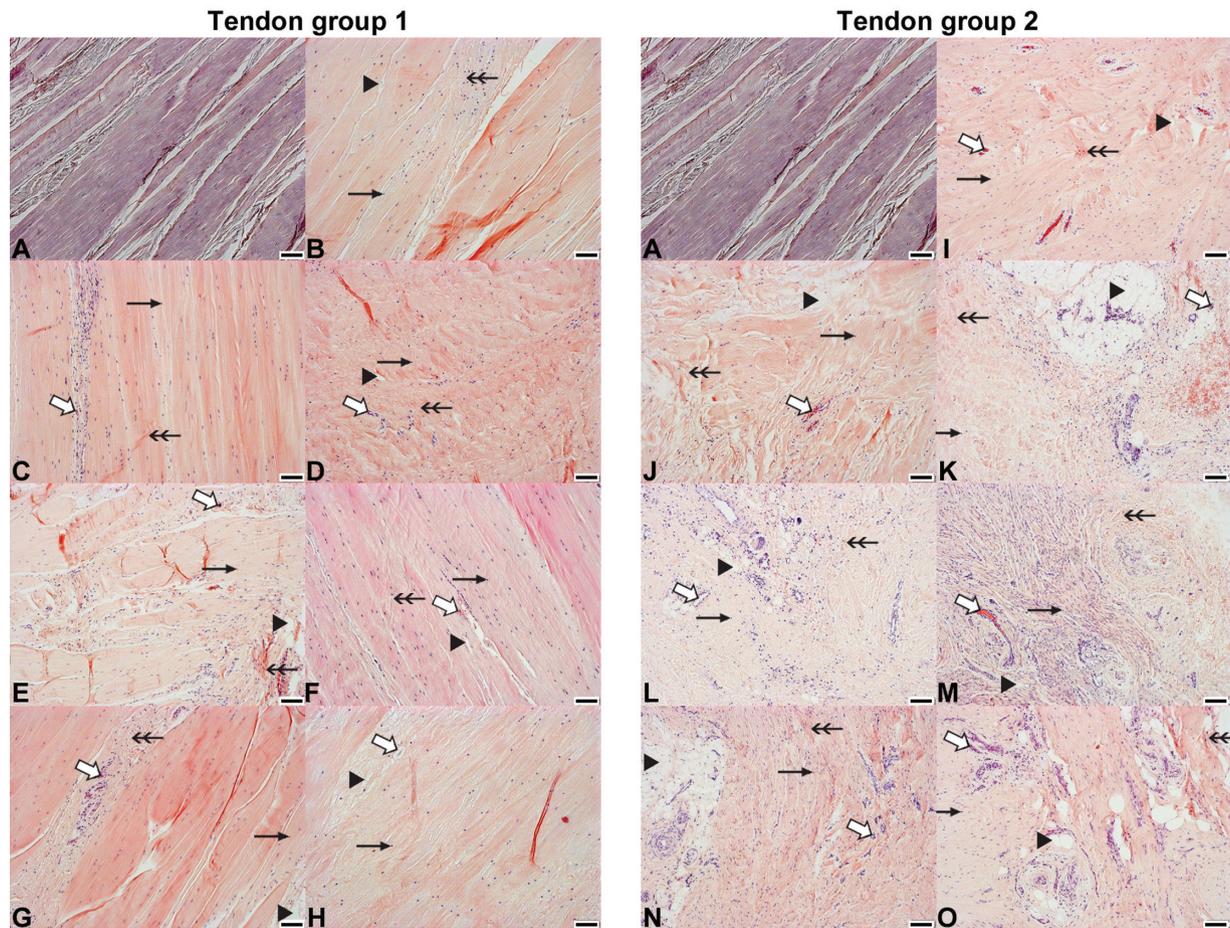
No significant difference was found in IL-6 concentration between  $L^{hi}$  PRP and  $L^{lo}$  PRP cultured with group 1 tendons (Figure 5). There was significantly greater IL-6 in group 2 tendon cultures treated with  $L^{lo}$  PRP (Figure 5). No significant difference was noted in IL-8 concentration after PRP treatment in either group 1 or group 2 cultures (Figure 5). MMP-9 was significantly increased in  $L^{hi}$  PRP when cultured with either group 1 or 2 tendons compared with  $L^{lo}$  PRP treated tendons (Figure 6).

### Growth Factor (TGF $\beta$ -1) in PRP After Culture With Diseased Tendon

The concentration of TGF $\beta$ -1 was significantly greater in  $L^{hi}$  PRP used to treat both group 1 and group 2 tendons compared with  $L^{lo}$  PRP treated groups (Figure 7).

### Matrix Gene Expression (COL1A1:COL3A1, COMP) in Tendons Cultured in PRP

In moderately degenerative group 1 tendons, COL1A1:COL3A1 gene expression was significantly increased in tendons cultured in either  $L^{lo}$  PRP or  $L^{hi}$  PRP, with expression greatest in tendons cultured in  $L^{lo}$  PRP ( $P = .04$ ) (Figure 8A). In severely degenerative group 2 tendons, there was no change in expression of COL1A1:COL3A1 between tendons cultured in  $L^{lo}$  PRP,  $L^{hi}$  PRP, or control ( $P = .29$ ). There was no significant difference in the expression of COMP after PRP treatment for either group 1 ( $P = .73$ ) or group 2 ( $P = .36$ ) tendons.



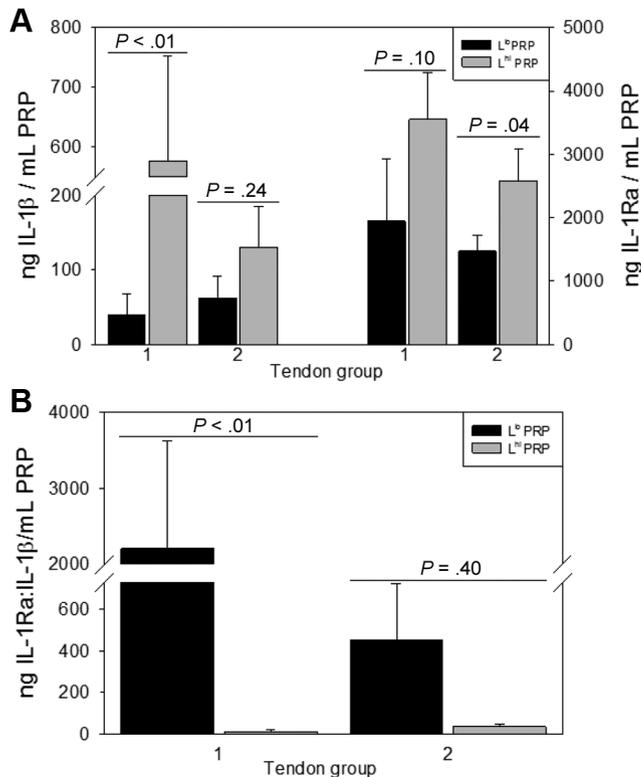
**Figure 3.** (A) Normal supraspinatus tendon demonstrating closely arranged, parallel fibers; sparse distribution of elongated fibroblasts; and vascular, lymph, and nerve bundles contained within the epitenon and running parallel with collagen fibers. (B-O) diseased supraspinatus tendon from patients who underwent reverse shoulder arthroplasty demonstrating thinning, separated, and disorientated collagen fibers (double arrows); rounded fibroblasts (arrows); fibroblastic hyperplasia; infiltration of mononuclear cells; random vascular proliferation (white arrows); fatty infiltration or lipoid degeneration (arrowheads); and myxoid degeneration. (B-H) Group 1 tendons classified as mild tendinopathy. (I-O) Group 2 tendons classified as severe tendinopathy. Hematoxylin-eosin stain, scale bar = 100  $\mu$ m.

#### Catabolic Gene Expression (*MMP-9*, *MMP-13*, *IL-1 $\beta$* ) in Tendons Cultured in PRP

Expression of *MMP-9* was similar in control tendons but was stimulated to a greater extent in group 2 tendons cultured in either  $L^{lo}$  PRP or  $L^{hi}$  PRP compared with group 1 tendons, with  $L^{lo}$  PRP being greater than  $L^{hi}$  PRP ( $P < .001$ ) (Figure 8B). No significant change in *MMP-9* expression was noted after PRP treatment in group 1 tendons ( $P = .09$ ). There was no significant difference in the expression of *MMP-13* after  $L^{lo}$  PRP or  $L^{hi}$  PRP treatment for either group 1 ( $P = .99$ ) or group 2 ( $P = .12$ ) tendons. Expression of *IL-1 $\beta$*  in group 2 tendons had a significant response to PRP ( $P = .03$ ), but group 1 tendons did not ( $P = .17$ ) (Figure 8C). In group 2 tendons, both  $L^{lo}$  PRP and  $L^{hi}$  PRP treatments resulted in significantly increased *IL1- $\beta$*  expression, with  $L^{lo}$  PRP being greater than  $L^{hi}$  PRP.

#### DISCUSSION

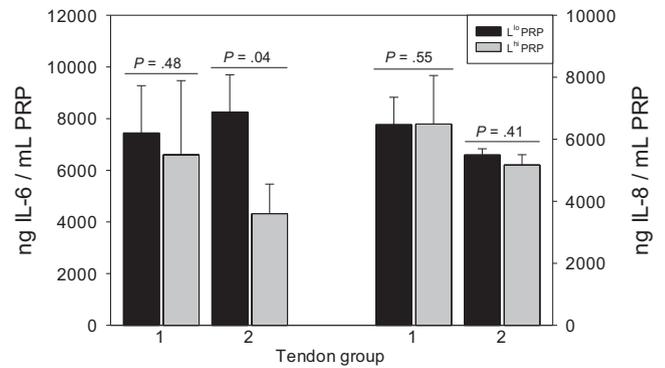
The primary objective of this study was to evaluate the effect of low versus high leukocyte and platelet concentration PRP products on catabolic and anabolic mediators of diseased tendon matrix metabolism. The findings of this study support our hypothesis that  $L^{lo}$  PRP contains less catabolic cytokines than  $L^{hi}$  PRP, resulting in a balance toward tendon matrix regeneration in mild to moderately affected tendons. However, the results observed in severely degenerative tendons were dissimilar, with neither PRP capable of enhancing matrix synthesis. This could be due to the infiltration of mononuclear cells and neovascularization in severely affected tendons, which was visible on the histologic images. This assumption is supported by the observation that even though the same  $L^{lo}$  PRP and  $L^{hi}$  PRP were used as culture media for group 1 and group 2 tendons, group 2 severely degenerative tendons responded



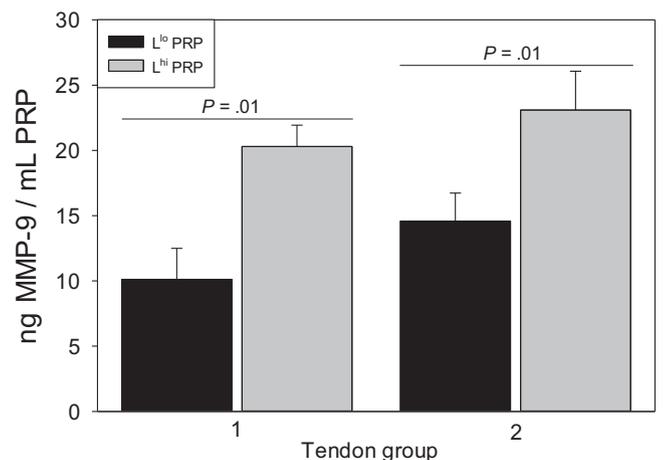
**Figure 4.** (A) Concentration of interleukin-1β (IL-1β), interleukin-1 receptor antagonist protein (IL-1Ra), and (B) the resultant IL-Ra:IL-1β ratio in leukocyte-low platelet-rich plasma (L<sup>lo</sup> PRP) or leukocyte-high PRP (L<sup>hi</sup> PRP) after 96 hours of incubation with moderately degenerative (group 1) or severely degenerative (group 2) supraspinatus tendons. Bars represent mean (n = 7) ± standard error. Significance was determined by use of Kruskal-Wallis 1-way analysis of variance.

to a greater extent—by nearly an order of magnitude—in matrix gene expression of *IL-1β* and *MMP-9*. This would suggest that there is not just a single PRP preparation for all situations, and determining the optimal preparation for each tissue should be pursued in clinical studies. The results of this study can be further extrapolated to patients with rotator cuff disease. Improved methods to classify tendon disease such that moderate versus severely degenerative rotator cuff tendinopathy can be identified preoperatively would provide information to design a treatment approach tailored to the extent of the disease process.

This ex vivo study has some inherent limitations. First, in naturally occurring tendinopathy, numerous anabolic and catabolic mediators are already present that could alter the effect of PRP on tissues, and those confounding factors would be excluded in an ex vivo study. Second, since this was a retrieval study, we did not know how long patients were affected by rotator cuff tears. Histologic assessment and scoring of the samples allowed for grouping the tendons based on severity of degeneration to accommodate for the varying levels of degeneration of tendon



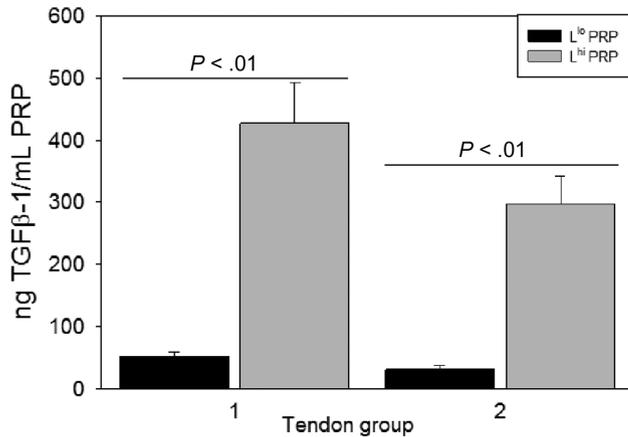
**Figure 5.** Interleukin-6 (IL-6) and IL-8 concentrations in leukocyte-low platelet-rich plasma (L<sup>lo</sup> PRP) or leukocyte-high PRP (L<sup>hi</sup> PRP) used as culture media for 96 hours for moderately degenerative (group 1) or severely degenerative (group 2) supraspinatus tendons. Bars represent mean (n = 7) ± standard error. Significance was determined by use of Kruskal-Wallis 1-way analysis of variance.



**Figure 6.** Matrix metalloproteinase-9 (MMP-9) concentration in leukocyte-low platelet-rich plasma (L<sup>lo</sup> PRP) or leukocyte-high PRP (L<sup>hi</sup> PRP) used as culture media for 96 hours for moderately degenerative (group 1) or severely degenerative (group 2) supraspinatus tendons. Bars represent mean (n = 7) ± standard error. Significance was determined by use of Kruskal-Wallis 1-way analysis of variance.

between patients. In addition, a dose-response study could not be performed given the small quantity of tissue retrieved from these patients. Finally, the pilot study investigating the effects of ACD was completed on normal tendon, and thus it is possible that the conclusions from this portion of the study may have differed if the presence or absence of ACD had been investigated on diseased tendon.

Leukocytes and the proinflammatory cytokine IL-1β were significantly increased in L<sup>hi</sup> PRP. IL-1β is produced by activated macrophages, blood neutrophils, B-lymphocytes, and endothelial cells. Increased expression of IL-1β in the

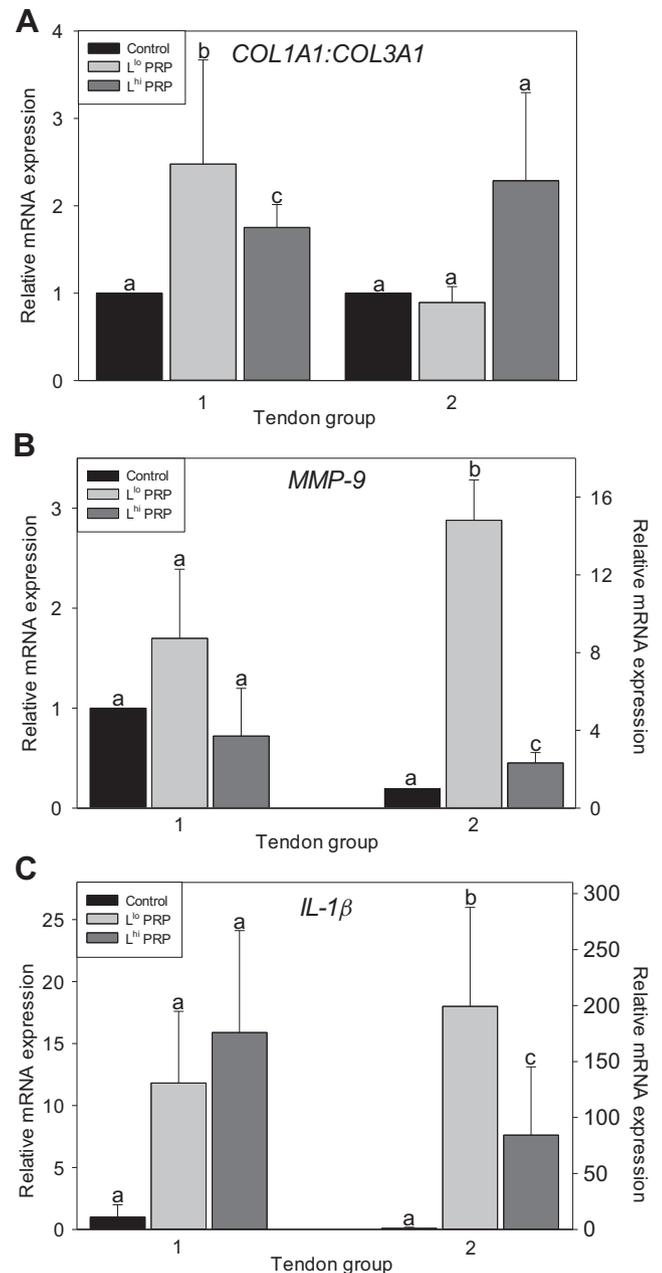


**Figure 7.** Concentration of transforming growth factor  $\beta$ -1 (TGF $\beta$ -1) in leukocyte-low platelet-rich plasma (L<sup>lo</sup> PRP) or leukocyte-high PRP (L<sup>hi</sup> PRP) after 96 hours of culture with moderately degenerative (group 1) or severely degenerative (group 2) supraspinatus tendons. Bars represent mean (n = 7)  $\pm$  standard error. Significance was determined by use of Kruskal-Wallis 1-way analysis of variance.

subacromial bursa of diseased rotator cuffs has been correlated with increased pain in patients due to its capacity to incite inflammation and hyperesthesia.<sup>37</sup> An increased ratio of IL-1Ra:IL-1 $\beta$  suggests a greater capacity to competitively inhibit binding of IL-1 $\beta$  to type I and II cell surface receptors, thereby decreasing the catabolic effects of IL-1 $\beta$ .<sup>33</sup> In group 1 tendons, but not in group 2 tendons, the ratio of IL-1Ra:IL-1 $\beta$  was increased in L<sup>lo</sup> PRP, but gene expression of IL-1 $\beta$  was not significantly affected in these tendons by either PRP. The results in group 2 tendons were dissimilar, with no change in the ratio of IL-1Ra:IL-1 $\beta$  but significant increases in IL-1 $\beta$  gene expression in tendons cultured in either PRP. Regulation of the IL-1 pathway in tendinopathies is not well understood, but these data suggest that the entire pathway should be considered, particularly in cases of severe tendinopathy where gene expression levels in the tendon tissue suggest the presence of a potentially proinflammatory environment.

In this study, L<sup>lo</sup> PRP had a significantly greater concentration of IL-6 compared with L<sup>hi</sup> PRP for group 2 tendons, and there was no significant difference between the biologics for either group for IL-8 concentrations. Limited data are available detailing the interaction between IL-6 and IL-8 in rotator cuff tendon tissue, but recent studies involving patients with acute Achilles tendon rupture showed that IL-6 and IL-8 concentrations were significantly increased during the healing phase, while proinflammatory cytokines TNF- $\alpha$  and IL-1 $\beta$  were below detectable levels. It was also observed that infusion of IL-6 into peritendinous tissue around the human Achilles tendon increased the synthesis of collagen type I.<sup>1,2</sup> These studies suggest that IL-6 and IL-8 may be associated with anti-inflammatory and regenerative effects in healing tendon.

Increased expression of MMPs has been shown to be associated with complete rotator cuff tears and tendinopathy.<sup>22</sup>



**Figure 8.** Gene expression in moderately degenerative (group 1) or severely degenerative (group 2) tendons after culture for 96 hours in leukocyte-low platelet-rich plasma (L<sup>lo</sup> PRP) or leukocyte-high PRP (L<sup>hi</sup> PRP). (A) Ratio of collagen type I (COL1A1) to collagen type 3 (COL3A1) genes associated with tendon matrix synthesis. (B) Matrix metalloproteinase-9 (MMP-9) and (C) interleukin-1 $\beta$  (IL-1 $\beta$ ) gene expression associated with tendon catabolism. Bars represent mean (n = 7)  $\pm$  standard error. Superscript letters indicate significance difference between groups by use of Kruskal-Wallis 1-way analysis of variance.

These zinc-dependent MMPs are capable of degrading intact and degenerative tendon extracellular matrix. More

specifically, MMP-13 can degrade all subtypes of collagen, including those that provide mechanical strength to tendons such as collagen type I.<sup>13</sup> Smaller collagen fragments are primarily the target of the gelatinase MMP-9. In this study, L<sup>hi</sup> PRP had a significantly larger concentration of MMP-9 than did L<sup>lo</sup> PRP in both groups. This is expected because L<sup>hi</sup> PRP had more neutrophils and platelets than did L<sup>lo</sup> PRP. MMP-9 is stored in circulating neutrophils and platelets and is released upon their activation.<sup>16</sup> However, severely degenerative group 2 tendons cultured in L<sup>lo</sup> PRP had significantly larger MMP-9 gene expression compared with L<sup>hi</sup> PRP, and both were increased compared with control tendon cultures. Much of the information regarding regulation of MMP-9 in tendinopathy is unknown, but it is thought that the action of MMP-9 to cleave denatured collagen and collagen type III in degenerative tissue allows for formation of structural collagens, such as collagen type I, in the remodeling process.<sup>28</sup>

Platelets are a natural reservoir of TGF $\beta$ -1, which is a key growth factor for stimulation of collagen synthesis, cell proliferation, recruitment, and migration and is associated with reduced scar formation in healing tendons.<sup>3,5,18</sup> In rotator cuff repair procedures, TGF $\beta$ -1 improves healing and mechanical strength at the tendon-bone interface.<sup>4,19,20,29,34</sup> The increased concentration of TGF $\beta$ -1 in L<sup>hi</sup> PRP compared with L<sup>lo</sup> PRP in the present study is not surprising since platelet concentration was also greater in L<sup>hi</sup> PRP. Although the quantity of TGF $\beta$ -1 was 3 to 4 times greater in L<sup>hi</sup> PRP than in L<sup>lo</sup> PRP, normal matrix synthesis, as measured by the ratio of *COL1A1:COL3A1* gene expression in tendons, was increased only in group 1 moderately degenerative tendons, and within that group, L<sup>lo</sup> PRP stimulated greater synthesis than did L<sup>hi</sup> PRP. Normal rotator cuff tendon is predominantly composed of type I collagen (>95%) and small amounts of type III, IV, and V collagen (<5%).<sup>38</sup> Type III collagen is normally restricted to the endotenon surrounding fiber bundles. It is increased after injury and is abundant in wound bed granulation tissue. In the natural healing of torn rotator cuff tendons, successful remodeling is typified by replacement of type III collagen with type I collagen.<sup>21</sup> The data in this study suggest that in moderately diseased tendon, L<sup>lo</sup> PRP influences normal tendon matrix metabolism but is unable to elicit a specific metabolic response in more degenerative cases of rotator cuff tendinopathy.

Combined, the results of this study suggest that in moderately degenerative rotator cuff tendons, L<sup>lo</sup> PRP promotes normal collagen matrix generation and decreases cytokines associated with matrix degradation and inflammation to a greater extent than does L<sup>hi</sup> PRP. In severely degenerative tendons, no specific recommendation can be made, as neither PRP preparation enhanced collagen synthesis and both were associated with increased inflammation as indicated by IL-1 $\beta$  synthesis in tendons. The findings of this study emphasize the concept that the effects of PRP in tendon regeneration are contextual depending on the local environment. Further methods to classify tendinopathies before injecting PRP should help customize and define the specific type of PRP to optimize tissue regeneration.

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