

# Simvastatin Exposure and Rotator Cuff Repair in a Rat Model

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## abstract

Simvastatin is a common medication prescribed for hypercholesterolemia that accelerates local bone formation. It is unclear whether simvastatin can accelerate healing at the tendon-bone interface after rotator cuff repair. This study was conducted to investigate whether local and systemic administration of simvastatin increased tendon-bone healing of the rotator cuff as detected by maximum load to failure in a controlled animal-based model. Supraspinatus tendon repair was performed on 120 Sprague-Dawley rats. Sixty rats had a polylactic acid membrane overlying the repair site. Of these, 30 contained simvastatin and 30 did not contain medication. Sixty rats underwent repair without a polylactic acid membrane. Of these, 30 received oral simvastatin (25 mg/kg/d) and 30 received a regular diet. At 4 weeks, 5 rats from each group were killed for histologic analysis. At 8 weeks, 5 rats from each group were killed for histologic analysis and the remaining 20 rats were killed for biomechanical analysis. One rat that received oral simvastatin died of muscle necrosis. Average maximum load to failure was  $35.2 \pm 6.2$  N for those receiving oral simvastatin,  $36.8 \pm 9.0$  N for oral control subjects,  $39.5 \pm 12.8$  N for those receiving local simvastatin, and  $39.1 \pm 9.3$  N for control subjects with a polylactic acid membrane. No statistically significant differences were found between any of the 4 groups ( $P > .05$ ). Qualitative histologic findings showed that all groups showed increased collagen formation and organization at 8 weeks compared with 4 weeks, with no differences between the 4 groups at each time point. The use of systemic and local simvastatin offered no benefit over control groups. [*Orthopedics*. 2017; 40(2):e288-e292.]

erated the formation of bone locally and triggered early expression of growth factors, including vascular endothelial growth factor, bone morphogenetic protein 2, and core-binding factor alpha 1 in rabbits.<sup>5</sup> In a mouse model, simvastatin injections stimulated murine calvarial bone growth by 53%,<sup>6</sup> and a 180% increase in bone area was noted with implantation of a polylactic acid membrane containing gel with simvastatin.

The rotator cuff tendons of the shoulder attach to the greater tuberosity via a specialized fibrocartilage zone into bone that provides a gradual transition to avoid concentration of stress.<sup>7-9</sup> After surgical repair of the rotator cuff, the tendon heals

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**S**tatins are widely prescribed for hypercholesterolemia, effectively lowering serum cholesterol levels as hydroxymethylglutaryl-coenzyme A reductase inhibitors. They also may increase

bone mineral density and reduce the risk of fracture by decreasing bone resorption<sup>1,2</sup> through an inhibitory effect on hydroxymethylglutaryl-coenzyme A reductase activity.<sup>3,4</sup> Simvastatin induced and accel-

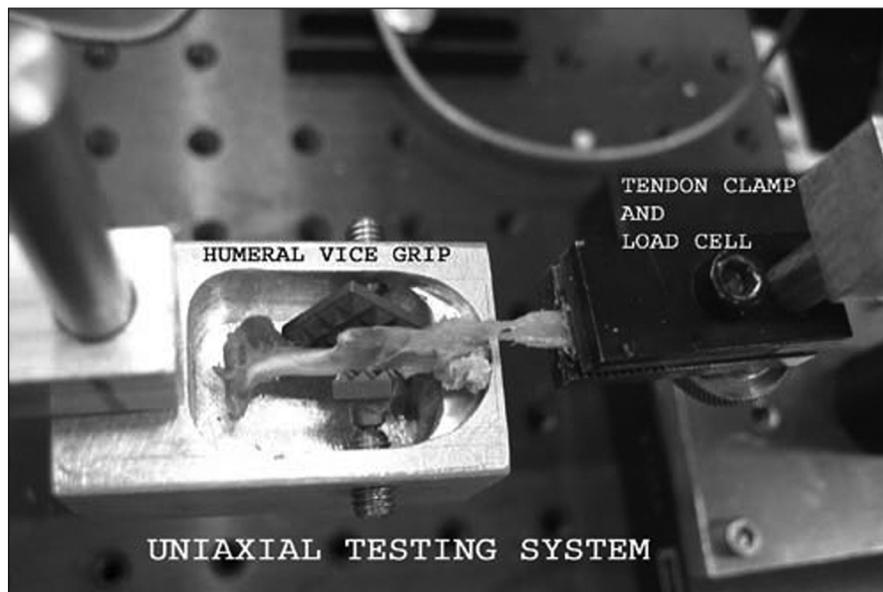
by forming a fibrous interface, followed by bony ingrowth and continuity of collagen fiber from bone to tendon.<sup>3,10</sup>

Failure rates of rotator cuff tendon-bone healing are high when evaluated objectively with ultrasound or magnetic resonance imaging.<sup>8,11-14</sup> The gradual process of repaired tendon-bone healing is most vulnerable early at the tendon-bone attachment site.<sup>3,15</sup> Factors that improve the early healing process may increase clinical success.<sup>16-18</sup> Statins, which positively affect bone turnover, may enhance tendon-bone healing.

This study was conducted to determine whether simvastatin, delivered both systemically and locally at the site of repair, improves the strength of repair healing by measuring the primary outcome of maximum load to repair failure in rat rotator cuff repairs compared with control subjects. Secondary outcomes included histologic analysis of repair sites to evaluate for increased collagen organization and fibrocartilage formation. The hypothesis was that rats treated with local and systemic simvastatin after rotator cuff repair would have higher maximum load to failure by biomechanical analysis and greater collagen organization and fibrocartilage formation by histologic analysis of the repair at both 4 and 8 weeks after surgery.

**MATERIALS AND METHODS**

After institutional animal care and use committee approval was obtained, 120 skeletally mature male Sprague-Dawley rats were obtained. The rat rotator cuff models rotator cuff healing in the human shoulder.<sup>19,20</sup> Sixty rats had a polylactic acid membrane overlying the supraspinatus repair site, held in place with the repair suture. Polylactic acid membranes were synthesized by pouring 20% weight/volume polylactide in methylene chloride (Cargill, Blair, Nebraska) into a mold.<sup>6,21</sup> Thirty rats had a polylactic acid membrane containing gel with simvastatin (2.2 mg) placed at the repair site (local statin group), and 30 had a polylactic acid mem-



**Figure 1:** Custom biomechanical testing system. [Reprinted with permission from Cohen DB, Kawamura S, Ehteshami JR, Rodeo SA. Indomethacin and celecoxib impair rotator cuff tendon-to-bone healing. *The American Journal of Sports Medicine*. 2006; 34(3):362-369.]

brane without any medication. Of the 60 rats that underwent repair without a polylactic acid membrane, 30 were given simvastatin mixed with their food pellet at a dose of 25 mg/kg/d. The rats were housed individually, and those receiving oral simvastatin were observed to fully consume the drug-containing pellet each day. The drug was started 1 week before surgery and continued for 4 weeks after surgery (oral statin group). The final 30 rats undergoing repair were fed a regular diet for the duration of the study (control group). At 4 weeks, 5 rats from each group were killed for histologic examination, and at 8 weeks, the remaining rats were killed and underwent biomechanical and histologic testing.

**Surgical Technique**

The rats underwent open transosseous rotator cuff repair with 1 modified Kessler stitch with 5-0 Prolene suture (Ethicon; Johnson & Johnson, Piscataway, New Jersey), as described previously. Postoperative weight bearing was as tolerated.

**Biomechanical Testing**

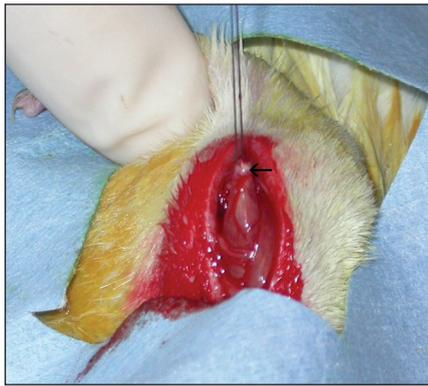
The humerus and supraspinatus were dissected from the thawed tissue on the

day of biomechanical testing. A digital micrometer was used to measure the cross-sectional area of the supraspinatus tendon at its insertion site on the greater tuberosity.

The supraspinatus tendon was then attached to a custom-designed uniaxial testing system (**Figure 1**) with ethyl cyanoacrylate (Krazy Glue; Elmer's Products Inc, Columbus, Ohio), and the humerus was connected with a custom vice.<sup>20</sup> The tendon was preloaded to 0.10 N and then loaded to failure at 14 μm/s, which corresponds to approximately 0.4% strain. The maximum load at failure and the failure site were recorded. Statistical analysis was completed with SYSTAT software, version 13 (Systat Software, San Jose, California) with comparisons between groups with 1-way analysis of variance. Statistical significance was set at *P*>.05.

**Histologic Analysis**

Histologic analysis was performed on 40 shoulders of 30 rats. Five rats from each group were killed 4 weeks and 8 weeks after surgery. The tissue specimens were fixed in 10% neutral buffered formalin for



**Figure 2:** Supraspinatus tendon detachment. The supraspinatus tendon was surgically detached from its insertion on the humerus and tagged with a suture for later repair (arrow).



**Figure 3:** Completed supraspinatus tendon repair. The supraspinatus tendon was reattached to the humerus to complete the procedure (arrow).

failure was recorded for each specimen and then averaged for the group (Table). Average maximum load to failure for rats that underwent repair without a poly-lactic acid membrane was  $35.2 \pm 6.2$  N with oral simvastatin and  $36.8 \pm 9.0$  N for the control group ( $P=.67$ ). For the 40 rats that underwent repair with a poly-lactic acid membrane, average load to failure was  $39.5 \pm 12.8$  N for rats treated with local simvastatin and  $39.1 \pm 9.3$  N for rats treated with control poly-lactic acid membrane ( $P=.95$ ). No statistically significant differences were found.

### Histologic Analysis

Histologic examination with light and polarized light microscopy showed that all 4 treatment groups had increased collagen formation and organization in the 8-week group compared with the 4-week group. Cellular infiltration decreased and fibroblast orientation increased compared with the transitional zone at 4 weeks. The fibrocartilage zone began to form at the interface of the bone-tendon junction (Figure 4A and Figure 5A). Fibrocartilage formation was seen in all treatment groups, with no appreciable difference in organization or size (Figure 4B and Figure 5B). No differences were noted between the 4 groups at each time point. Histologic analysis showed no appreciable differences between groups in collagen organization, cellularity and vascularity in the tendon-bone interface, new bony matrix deposition in the tendon-bone interface, the presence of cartilage at the tendon-bone interface, and collagen fiber continuity from the bone surface to the interface tissue.

### DISCUSSION

The findings of this study showed that simvastatin, administered locally or systemically in a rat rotator cuff repair model, made no significant difference in maximum load to failure or histologic findings. The tendon-bone junction is a specialized transition site that buffers the

and illuminated with monochromatic polarized light.<sup>22,23</sup> The greater tuberosity, repaired tendon-bone insertion site, and midsubstance of the supraspinatus tendon were examined under light and polarized light microscopy with an Olympus BH-2 light microscope (Olympus Optical, Lake Success, New York). The light microscope was interfaced with a CCD video camera (Optronics DEI-750; Optronics, Goleta, California) mounted on an eyepiece tube. Also assessed were cellularity and vascularity in the tendon-bone interface, new matrix deposition in the tendon-bone interface, the presence of cartilage at the tendon-bone interface, and collagen fiber continuity from the bone surface to the interface tissue.

### RESULTS

In this study, 80 skeletally mature rats underwent supraspinatus repair (Figures 2-3). One of the animals in the oral statin group died as a result of muscle necrosis. No other complications occurred. No statistically significant difference was noted in average animal weight between groups at the start of the experiment or at the time of death.

### Biomechanical Testing

The site of failure for all of the tested specimens was the bone-tendon interface with tendon pull-off. Maximum load to

Table	
Repair Load to Failure	
Group	Maximal Load to Failure, Mean±SD, N
Poly-lactic acid membrane alone	39.1±9.3
Poly-lactic acid membrane+local simvastatin	39.5±12.8
No membrane+oral simvastatin	35.2±6.2
No membrane alone	36.8±9.0

48 hours. After fixation, tissues were decalcified in Immunocal (Decal, Congers, New York) for 24 hours and washed in phosphate-buffered saline solution. The tissues were dehydrated and embedded in paraffin. Then 5- $\mu$ m-thick sections that included the repaired supraspinatus tendon and greater tuberosity were cut in the coronal plane and stained with hematoxylin and eosin and picrosirius red. The appearance of the repair sites was evaluated for collagen organization and fibrocartilage formation.

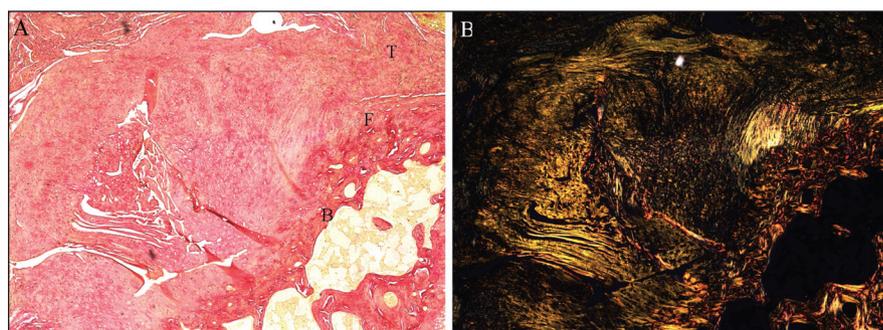
Picrosirius red staining was used for qualitative analysis of collagen content. To assess the maturity of collagenous tissue in the repaired supraspinatus tendon, sections were stained with picrosirius red

extreme biomechanical behaviors of the confluent structures.<sup>7-9,24</sup> After injury, this transitional zone is replaced with fibrovascular scar rather than repair of fibrocartilage.<sup>24-27</sup> Transforming growth factor beta-3 can increase scar tissue formation (but not organized fibrocartilage repair) and ultimate load to failure in rat rotator cuff repair.<sup>28</sup> Conversely, celecoxib and indomethacin can interfere with fibrocartilage formation in tendon-bone healing in a manner seemingly linked to inhibitory effects in the inflammatory cascade by cyclooxygenase-2.<sup>20</sup>

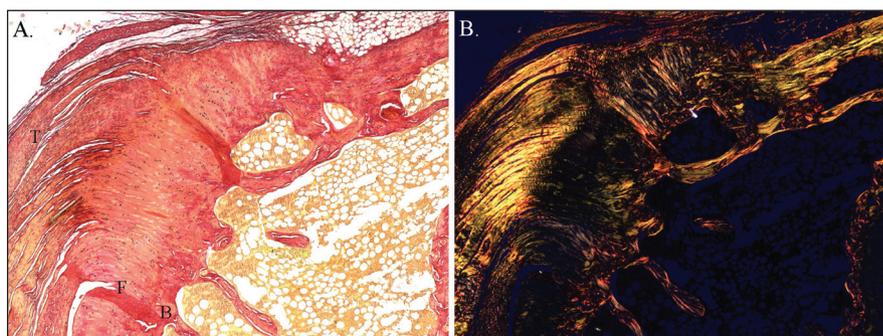
Augmentation of tendon repair with recombinant bone morphogenetic protein 2 in rabbits showed increased ultimate load to failure and bony ingrowth at the repair site compared with control subjects.<sup>29</sup> Previous studies showed increased levels of growth factors (principally bone morphogenetic protein 2) and bone formation in animal bone healing models with statin treatment.<sup>5,30</sup> The current study found equal levels of collagen formation and organization in all of the study and control groups, further supporting the biomechanical findings.

Multiple studies have reported the rare incidence of statin-associated tendinopathy, mostly through adverse drug event registries.<sup>31,32</sup> However, basic science studies in animals have not reported this effect.<sup>33</sup> In the current study, the difference in maximum load to failure of tendon repairs treated with local or systemic simvastatin compared with control subjects was not statistically significant.

The dose of simvastatin used in this study, 25 mg/kg/d, is relatively high compared with reported doses in the literature.<sup>5,34</sup> However, this level is well below the 150 to 1200 mg/kg/d dosing reported to cause skeletal muscle toxicity.<sup>35</sup> This dose was selected based on studies indicating dose-dependent effects of statins on increasing bone formation.<sup>36</sup> In this study, 1 rat died of muscle necrosis as a result of receiving simvastatin, highlighting the well-known potential risk of this commonly prescribed medication.<sup>37</sup>



**Figure 4:** Supraspinatus tendon repair with oral statin treatment. Light photomicrograph of supraspinatus repair in the oral statin group at 8 weeks (hematoxylin and eosin, original magnification  $\times 320$ ) (A). Polarized light photomicrograph to assess collagen formation and maturation at 8 weeks in a rat treated with oral statin after supraspinatus repair (picrosirius red, original magnification  $\times 320$ ) (B). Abbreviations: B, bone; F, fibrocartilage; T, tendon.



**Figure 5:** Supraspinatus repair of the control group. Light photomicrograph of supraspinatus tendon repair in a control group animal at 8 weeks showing the tendon-bone interface (hematoxylin and eosin, original magnification  $\times 320$ ) (A). Polarized light photomicrograph quantifying collagen formation and maturation at 8 weeks in a control group animal after supraspinatus repair (picrosirius red, original magnification  $\times 320$ ) (B). Abbreviations: B, bone; F, fibrocartilage; T, tendon.

Power analysis was performed before this study was conducted, based on previous work with this model,<sup>20</sup> and inclusion of 20 rats per treatment group was expected to provide sufficient power to detect a statistically significant difference in maximum load to failure between groups.

### Limitations

A potential limitation of this study was that blood serum levels of simvastatin were not monitored, and it is possible that there were differences in absorption through local and oral routes. Oral bioavailability of simvastatin in rats was well established in previous studies.<sup>38-40</sup> Multiple subcutaneous injections of simvastatin administered in the week after fracture result in increased serum levels, cholesterol-

lowering effects, and bone formation, but systemic levels and lipid-lowering effects decrease by 4 weeks.<sup>41</sup> The authors know of no study examining local simvastatin administration during tendon repair in rats, and systemic simvastatin levels are likely to be minimal at 4 and 8 weeks after repair. The clinical application of these results suggests that patients taking simvastatin at the time of rotator cuff surgery appear to have no theoretical increased or decreased risk of tendon healing. Future studies are needed to examine the effects of statins in patients who are undergoing rotator cuff surgery.

### CONCLUSION

The use of systemic and local simvastatin for tendon-bone healing had no ben-

efit over control groups in this rat rotator cuff repair study.

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