**Introduction:** Rotator cuff (RC) disease is the most common cause of shoulder pain in adults (1). The incidence of full-thickness RC tears has been reported to be as high as 40% (2). Several studies have documented the pathological changes associated with RC disease including disruption and thinning of fascicles, foci of granulation tissue, and dystrophic calcification (3). However, despite a large volume of knowledge on the incidence and management of RC disease, the etiology or pathogenesis of the disease remains controversial. This has hindered biological treatment options for RC disease.

The purpose of this study was to determine expression levels of a subset of matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMPs) in normal and torn RC tendons. We hypothesized that there would be a relative increase in MMP levels and a decrease in TIMP mRNA levels in torn rotator cuff tendons.

**Methods:** Tissue was obtained from 10 patients undergoing RC repair for full thickness tears. RC tissue was harvested from the tear site. Care was taken to ensure that there was no contamination of tissue from adjacent structures. The mean age of the patients was 59.2 +/- 4.4 years. In addition, tissue was obtained post-mortem from donors with no history of shoulder disease and no evidence of RC disease on gross examination. The mean age of the cadaveric tissue was 74 +/-.7 years. The ethics review board from our institution approved the study.

Reverse transcriptase polymerase chain reaction (RT-PCR) was performed as previously described (4) for MMP-1,-3,-8,-10,-11,-13 and TIMP-1,-2,-3,-4 and normalized to the housekeeping gene GAPDH using human-specific primer sets (5). For protein analysis, tissue was powdered using a Micro-Dismembrator S (B Braun Biotech International GmbH, Melsungen, Germany) and resuspended in protein extraction buffer. The homogenate was clarified and protein content determined using the Bradford assay. 15 ug of protein was electrophoresed through a 4-20% SDS-polyacrylamide gel under reducing conditions. Proteins were transferred to nitrocellulose, blocked, and incubated with 1:1000 dilution of anti-human MMP-13 antibody (NeoMarkers, Fremont, CA). After washing, the nitrocellulose blot was incubated in 1:1000 diluted sheep anti-mouse conjugated horse radish peroxidase antibody (Amersham-Pharmacia Biotech, UK). Membranes were developed using an ECL detection kit (Amersham-Pharmacia Biotech, UK) and exposed to X-ray film. Unpaired t-tests were used to determine differences between torn rotator cuffs and those obtained at post-mortem.

**Essential Results:** All of our no RT controls were negative. The mRNA levels for collagenases (MMP-1,-8,-13), stromelysin (MMP-3,-10,-11) and TIMP (TIMP-1,-2,-3,-4) are summarized in figs. 1, 2, and 3 respectively. MMP-13 mRNA levels were significantly increased (mean = 675% of normal values) in torn rotator cuffs. In addition, MMP-3, TIMP-2,-3, and -4 mRNA levels were significantly decreased. Western blotting results suggested an increase in the latent (pro-) form of MMP-13 but more importantly the active form of MMP-13 was present in torn rotator cuffs. MMP-3, TIMP-2, TIMP-3, and TIMP-4 mRNA levels were also decreased in torn RCs. Since TIMPs are known to bind to and inhibit MMPs in a 1:1 stoichiometric fashion, tendon degeneration may be a combination of both an increase in MMPs (MMP-13) and a decrease in TIMPs (TIMP-2 to 4) (9).

In conclusion, MMP and TIMP mRNA levels were specifically altered in torn rotator cuffs. In particular MMP-13 levels were increased both at the mRNA and protein levels (active form) in torn RC tendons. MMP-13 is known to degrade type I collagen with equal efficiency as MMP-1 or MMP-8. Since the RC is primarily type I collagen, MMP-13 therefore may be involved in the pathogenesis of rotator cuff degeneration (7,8). Interestingly, TIMP-2, TIMP-3, and TIMP-4 mRNA levels were also decreased in torn RCs. Since TIMPs are known to bind to and inhibit MMPs in a 1:1 stoichiometric fashion, tendon degeneration may be a combination of both an increase in MMPs (MMP-13) and a decrease in TIMPs (TIMP-2 to 4) (9).

**Discussion:** MMPs are a family of zinc-dependent endopeptidases which collectively degrade essentially all components of the extracellular matrix (6). MMP-13 levels were increased both at the mRNA and protein levels (active form) in torn RC tendons. MMP-13 is known to degrade type I collagen with equal efficiency as MMP-1 or MMP-8. Since the RC is primarily type I collagen, MMP-13 therefore may be involved in the pathogenesis of rotator cuff degeneration (7,8). Interestingly, TIMP-2, TIMP-3, and TIMP-4 mRNA levels were also decreased in torn RCs. Since TIMPs are known to bind to and inhibit MMPs in a 1:1 stoichiometric fashion, tendon degeneration may be a combination of both an increase in MMPs (MMP-13) and a decrease in TIMPs (TIMP-2 to 4) (9).

These findings suggest that MMPs and TIMPs may play a role in the pathogenesis of RC disease and may provide potential targets for novel therapeutic interventions.

**References:**