Introduction: Introduction:
Rupture of the anterior cruciate ligament (ACL) stimulates angiogenesis and increased blood flow in the adjacent intact supporting structures (1). These vascular adaptations are associated with increased creep, decreased tensile strength, and decreased stress at failure in the medial collateral ligament (MCL) of ACL deficient knees (1). Vasomotor responses of the vascular bed in the ACL deficient knees are significantly attenuated or absent raising the question of whether the vasculature is adequately able to meet the metabolic demands of the injured tissue. Both a loss of contractile responses to phenylephrine and neuropeptide Y and a loss of dilatory response to acetylcholine, histamine, substance P and reactive hyperemia have been documented in the ACL deficient knee (2). However it is unknown if this microvascular pathophysiology worsens with time or if the normal vasomotor responses return to normal. We have therefore measured vascular responses to a variety of dilatory and contractile agonists in the MCL after 6 and 14 weeks of ACL deficiency. We hypothesized that the loss of vasomotor responsiveness caused by the chronic instability and progressive osteoarthritis (OA) of ACL deficiency would worsen with time.

Materials and Methods: 18 one year old female New Zealand White rabbits were used and separated into three groups: unoperated control (n=6), 6 week ACL transected (n=6), and 14 week ACL transected (n=6). Previous studies have shown no difference in blood flow between sham operated and unoperated controls at these time points and was therefore omitted(1). ACL transection was surgically induced in right knee using a lateral approach under halothane anesthesia (1L O2/min, 2-5% halothane).

At 0 weeks, 6 weeks, or 14 weeks the MCL of the right knee was surgically exposed and the overlying fascia removed with care to not disturb the underlying vasculature. Vascular responses were then measured using a Laser Speckle Perfusion Imager. Vasoactive drugs (bradykinin, substance P, and phenylephrine) were applied topically in 100μL boluses in doses ranging from 10-14 to 10-8 moles. Reactive hyperemia was induced through 4 minutes of femoral artery occlusion and release of occlusion. Blood flow images were captured every 10 seconds for 9 minutes following drug application, or femoral artery occlusion. Results were analyzed using Laser Imaging Analysis software (© Photokron Inc, Calgary, Alberta Canada). Results are expressed as mean values and were analyzed for statistical significance using students t-test.

Results: Results:
ACL deficiency induced several alterations in knee physiology. MCL thickening, synovial hyperplasia, and capsular thickening were observed 6 weeks following ACL transection, although no osteophytes or overt cartilage defects were observed. By 14 weeks following ACL transection typical osteoarthritic changes were observed, including cartilage lesions, and osteophytes, in addition to the alterations observed at 6 weeks. Knee joint diameter was found to be significantly increased in the ACL deficient knee at both 6 and 14 weeks (control: 22.1 ± 0.2 mm, 6 week: 29.4 ± 0.3 mm*, 14 week 31.2 ± 0.3 mm*, *p < 0.05 ) Bradykinin, femoral artery occlusion, and low doses of substance P increased perfusion in the MCL vasculature in control knees. Vasodilatory responses to bradykinin, substance P, and reactive hyperemia were found to be significantly decreased compared to control responses at both 6 and 14 weeks (Figure 1). Phenylephrine and higher doses of substance P decreased perfusion in control knees. Contractile responses were also diminished at 6 and 14 weeks post ACL transection (Figure 1).

Discussion: Discussion:
ACL deficiency induced diminished responses to all mediators applied in addition to reperfusion hyperaemia. Consistent with our hypothesis, these perturbed responses worsened over time, in concert with advancing OA.

Oxidative stress associated with inflammation in OA may confound normal vascular signaling mechanisms and result in the diminished vascular responses observed. The deficient vasodilatory and vasoconstrictive responses would compromise the ability of the vasculature to respond to increased metabolic demands of the tissue and hypoxia may ensue. The vasculature has become pathologically affected and may play a role in osteoarthritic pathology. Further evaluations of the mechanisms involved in these vascular aberrations are warranted.


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Figure 1. Vascular Responses of the MCL vasculature to Bradykinin, Substance P, Reactive Hyperaemia, and Phenylephrine in Control, 6 week, 14 week ACL transected knees.