Comparison of Knee Injury Threshold During Tibial Compression Based on Limb Orientation in Mice

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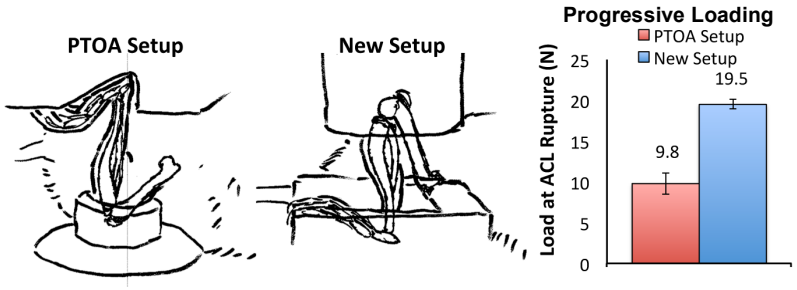
INTRODUCTION: Non-invasive tibial compression is commonly used to study bone adaptation to mechanical loading in mice. This method has also been used to study cartilage degeneration due to excessive loading of the joint. Our previous studies have used tibial compression overload to non-invasively induce anterior cruciate ligament (ACL) rupture in the knee joints of mice in order to study post-traumatic osteoarthritis (PTOA). Using our tibial compression setup, ACL injury typically occurs at 8-10 N, while other groups have reported non-injury tibial compression at loads exceeding 10 N. It is unknown which factors dictate the compressive force required for ACL rupture, but it is likely due in large part to the orientation of the mouse lower limb in the tibial compression system. In this study, we compared a newly designed tibial compression setup to our existing setup that we have used for studies of PTOA to determine if the newly designed setup will allow for tibial compression at significantly higher loads, allowing us to investigate bone adaptation or atraumatic OA without inducing ACL rupture. We hypothesized that the new tibial compression setup would allow us to apply larger loads for a greater number of cycles before rupturing the ACL compared to the existing PTOA setup.

METHODS: The PTOA tibial compression setup involves a mouse lying prone with the hip fully extended, the knee flexed near 90° and held by a shallow hemispherical fixture; the lower leg is extended upward, and the foot is held by another fixture with the ankle flexed at approximately 30°. The New tibial compression setup involves a mouse lying supine with the hip flexed, the knee fully flexed and held by a deeper fixture with a PMMA mold insert; the lower leg is extended downward, and the foot is held by a fixture with ankle flexed at approximately 10°. The study used a total of 29 mice (12 week old female C57BL/6). Mice were anesthetized using isoflourane; tibial compression using the New setup was performed on the left leg of each mouse, while tibial compression using the PTOA setup was performed on the right leg. First, a group of mice (n = 11) were subjected to progressive loading at magnitudes increasing from 2-20 N in 2 N increments until ACL rupture occurred (1 mm/s loading rate, unloaded to 1 N between loads, 1 sec. rest between each load cycle). Next, mice were subjected to cyclic loading at compressive magnitudes of 9 N (n = 9) or 14 N (n = 9). These loads were applied at 4 Hz for 1200 cycles or until ACL rupture occurred. ACL rupture was identified by an audible "pop" and an increase in baseline actuator displacement. After loading was completed, mice were euthanized and both knees were analyzed by gross dissection by an orthopedic surgeon to assess tissue damage within the joint. All procedures were approved by the UC Davis IACUC.

RESULTS: Consistent with our previous studies, ACL rupture was noted in all right knees of mice that underwent progressive loading using the PTOA loading setup at a mean compressive force of 9.8±1.3 N. Conversely, ACL rupture was noted in only 36% of left knees that underwent progressive loading using the New loading setup, at a mean compressive force of 19.5±0.6 N. With cyclic loading at 9 N, 100% of mice that were loaded with the PTOA setup experienced ACL rupture after an average of 45.4±40.7 cycles. In contrast, no mice that were cyclically loaded with the New setup at 9 N experienced ACL rupture after 1200 cycles. As expected, 100% of mice that underwent cyclic loading at 14 N using the PTOA setup experienced ACL rupture on the first cycle. However, we also observed that 89% of mice cyclically loaded at 14 N using the New setup experienced ACL rupture after an average of 15.1±8.2 cycles. Gross dissection revealed that knee injury with the PTOA setup consistently resulted in ACL rupture and joint swelling. Tibial compression with the New setup resulted in ACL rupture and joint swelling in less than half of the mice. Other occasional observations with the New tibial compression setup included tibia fracture (n = 1) and MCL rupture (n = 1); skin lacerations and hemarthrosis were also occasionally observed following 14 N cyclic compression.

DISCUSSION: Consistent with our hypothesis, we found that the New tibial compression setup was able to apply higher magnitude loads for more cycles without causing ACL rupture in mouse knees than our previously established PTOA setup. This is possibly due to the deeper, more stabilizing knee fixture, the greater flexion of the knee and hip, or the reduced flexion of the ankle. However, this New tibial compression setup was still able to cause ACL rupture and joint damage with high magnitude cyclic loading, therefore tibial compression loading protocols must take possible knee injury into account for studies of bone adaptation or atraumatic OA. High loading magnitudes also have the possibility of causing swelling, skin lacerations, and hemarthrosis in the joint, which may complicate experimental outcomes. Having the ability to perform both acute knee injury (PTOA setup) and chronic loading without ACL injury (New setup) provides us with a unique investigative tool that may be useful for comparing disease etiologies such as primary vs. secondary OA. This study also sheds light on the key differences between tibial compression setups that may dictate the threshold for ACL injury in mice.



Cyclic Loading Results				
	PTOA Setup		New Setup	
	% ACL Torn	*Avg. Cycles to Failure*	*% ACL Torn*	*Avg. Cycles to Failure*
Low Load (9N)	100%	45.4±40.7	0%	>1200
High Load (14N)	100%	1	88.9%	15.1±8.2

SIGNIFICANCE: This study compares two tibial compression configurations that can be used for studies of bone adaptation or OA. We describe the differences in limb orientation and joint fixtures for the two setups, and determine loading thresholds for knee injury in mice. The ability to customize a tibial compression system to induce or avoid knee injury is a crucial research tool that can improve reproducibility and limit confounding factors due to unwanted joint injury.

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