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Short communication

Comparison of knee injury threshold during tibial compression based on limb orientation in mice

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ABSTRACT

Our previous studies used tibial compression overload to induce anterior cruciate ligament (ACL) rupture in mice, while others have applied similar or greater compressive magnitudes without injury. The causes of these differences in injury threshold are not known. In this study, we compared knee injury thresholds using a "prone configuration" and a "supine configuration" that differed with respect to hip, knee, and ankle flexion, and utilized different fixtures to stabilize the knee. Right limbs of female and male C57BL/6 mice were loaded using the prone configuration, while left limbs were loaded using the supine configuration. Mice underwent progressive loading from 2 to 20 N, or cyclic loading at 9 N or 14 N (n = 9–11/sex/loading method). Progressive loading with the prone configuration resulted in ACL rupture at an average of 10.2 ± 0.9 N for females and 11.4 ± 0.7 N for males. In contrast, progressive loading with the supine configuration resulted in ACL rupture in only 36% of female mice and 50% of male mice. Cyclic loading with the prone configuration resulted in ACL rupture after 15 ± 8 cycles for females and 24 ± 27 cycles for males at 9 N, and always during the first cycle for both sexes at 14 N. In contrast, cyclic loading with the supine configuration was able to complete 1,200 cycles at 9 N without injury for both sexes, and an average of 45 ± 41 cycles for females and 49 ± 25 cycles for males at 14 N before ACL rupture. These results show that tibial compression configurations can strongly affect knee injury thresholds during loading.

1. Introduction

Tibial compression loading of mice is widely used in musculoskeletal research to investigate bone adaptation to increased mechanical loading (Brodt and Silva, 2010; De Souza et al., 2005a, 2005b; Lynch et al., 2010; Zaman et al., 2006). Mouse tibial compression has also been used to investigate osteoarthritis (OA) development after mechanical loading (Christiansen et al., 2012, 2015; Ko et al., 2013, 2016; Onur et al., 2014; Poulet et al., 2015, 2011; Rai et al., 2017; Wu et al., 2014). Some of these studies use multiple cycles of tibial compression without acute injury, while other studies use "tibial compression overload" to acutely injure soft tissue structures in the joint, often in a single compressive load.

We previously described using tibial compression overload in mice to examine development of PTOA after joint injury (Anderson et al., 2016; Christiansen et al., 2012; Hsia et al., 2016; Khorasani et al., 2015; Lockwood et al., 2014; Satkunananthan et al., 2014). This method consistently injures the anterior cruciate ligament (ACL) of a mouse knee using a single compressive load at magnitudes of approximately 8–10 N (Christiansen et al., 2012; Lockwood et al., 2014). Other groups have applied similar or even greater tibial compression loading magnitudes (12 N or more) for multiple cycles without acute injury (Berman et al., 2015; Govey et al., 2016; Kelly et al., 2016; Shirazi-Fard et al., 2015). The factors contributing to variable knee injury thresholds during tibial compression are currently unclear, but likely include the position of the mouse limb within the loading system and the fixtures (or "cups") used to contact the knee and ankle joints. Identifying factors that affect knee injury threshold would inform studies of both bone adaptation and OA development, allowing researchers to design tibial compression systems to either induce or avoid soft tissue injuries during loading.

In this study, we compared two tibial compression configurations: our previously described "prone configuration" that

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reproducibly induces ACL rupture, and a "supine configuration" that is more representative of those used by other groups for non-injury tibial compression loading. We sought to identify factors contributing to the disparate knee injury thresholds reported during tibial compression in mice. We hypothesized that the supine configuration would be able to apply greater compressive loads for a greater number of cycles before inducing ACL rupture.

2. Methods

2.1. Animals

A total of 29 female and 29 male 10 week-old C57Bl/6 mice were obtained from Envigo (Indianapolis, IN). Mice were randomly assigned to experimental groups, and each mouse was subjected to tibial compression using both the prone and supine configurations (described below). Mice were anesthetized via isoflurane inhalation, and tibial compression loading was performed using an electromagnetic materials testing machine (ElectroForce 3200, TA Instruments, New Castle, DE). All mice were euthanized via carbon dioxide inhalation immediately following tibial compression. Mice were maintained and used in accordance with National Institutes of Health guidelines on the care and use of laboratory animals. All procedures were approved by the UC Davis Institutional Animal Care and Use Committee.

2.2. Prone loading configuration

The right hindlimb of each mouse was loaded using the prone configuration (Fig. 1). The hip joint was fully extended so that the femur was approximately parallel to the plane of the body, and the knee joint was held at nearly 90°. The ankle joint was constrained by the top loading platen in 30° of flexion, and the knee was supported by a shallow aluminum cup on the bottom platen.

2.3. Supine loading configurations

The left limb of each mouse was loaded using the supine configuration (Fig. 1). The hip joint was flexed so that the femur is approximately perpendicular to the plane of the body, and the knee was constrained in full flexion with the tibia held vertical. The knee joint was supported by a deeper aluminum cup that included a molded polymethylmethacrylate insert. The ankle joint was supported by the bottom platen in 10° of flexion.

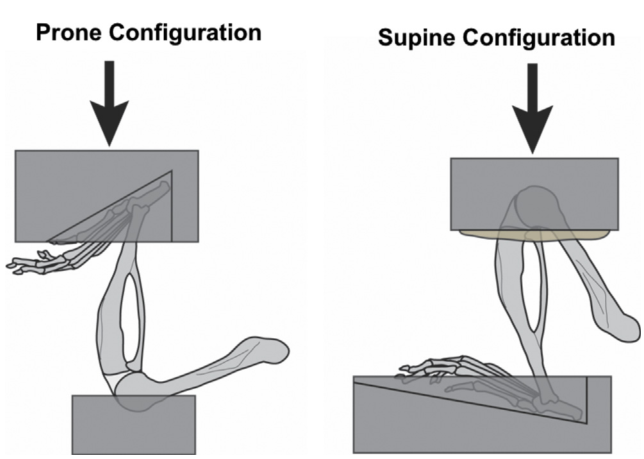

Fig. 1. In the prone configuration (left), the hip is fully extended, the knee joint is held at nearly 90° of flexion, and the ankle joint is held at 30° of flexion. In the supine configuration (right), the hip is flexed, the knee joint is fully flexed, and the ankle is held at 10° of flexion. The prone configuration uses a shallow metal cup to hold the knee, while the supine configuration uses a deeper metal cup with a molded PMMA insert. Arrows indicate the direction of loading.

2.4. Progressive magnitude loading

Mice were subjected to progressive loading (n = 11 females, n = 10 males) to identify the injury load. Tibial compression was applied at 1 mm/s, with magnitudes increasing from 2 to 20 N in increments of 2 N (Fig. 2A). Force-displacement curves were monitored for ACL rupture, identified as a characteristic sharp drop in recorded force with a subsequent translation in resting (zero-force) displacement (Fig. 2A). Compressive loads greater than 20 N were not investigated because this approaches the compressive force needed to induce tibial fracture.

2.5. Cyclic loading

Mice were subjected to cyclic tibial compression loading at 4 Hz, previously determined as the average mouse stride frequency (Lee et al., 2002), for 1,200 cycles or until ACL rupture. A saw-tooth loading protocol was used, with a 0.1 s dwell between each load cycle at the pre-load level of 0.5 N, similar to commonly used tibial compression loading protocols (Berman et al., 2015; Govey et al., 2016; Kelly et al., 2016; Shirazi-Fard et al., 2015) (Fig. 3A). Peak compressive loads were 9 N in the low magnitude group (n = 9 females, n = 10 males), and 14 N in the high magnitude group (n = 9 females, n = 9 males).

2.6. Qualitative analysis of joint injury

Following tibial compression, joints were qualitatively assessed to characterize typical damage to joint structures created by each of the loading configurations. The knee joints of a total of 30 randomly selected mice (n = 5/sex/loading method) were examined by an orthopaedic surgeon (TJS) immediately following euthanasia. Knees were dissected and evaluated under microscope for gross tissue damage, swelling, and hemarthrosis. Cruciate and collateral ligaments were specifically inspected for injury. Ligamentous injury was assessed by both physical exam to assess ligamentous laxity and by gross dissection to assess ligamentous rupture or tear. All observable trauma and the number of mice displaying each instance of damage were recorded.

2.7. Statistics

3-way ANOVA stratified by loading configuration, sex, and loading magnitude was used to compare cyclic loading results (JMP 11, SAS Institute, Inc., Cary, NC). 2-way ANOVA stratified by loading configuration and sex was used to compare progressive loading results. Paired t-tests were used to determine differences between loading configurations for each sex. The primary outcome of the progressive loading protocol was failure load; the primary outcome of the cyclic loading protocol was number of cycles to ACL rupture. Mean ± standard deviation is presented for all data. Significance was defined as $p < 0.05$.

3. Results

3.1. Progressive magnitude loading

As expected, tibial compression using the prone configuration induced ACL injury in all mice at an average magnitude of 10.2 ± 0.9 N for females and 11.4 ± 0.7 N for males (Fig. 2B). In contrast, tibial compression with the supine configuration induced ACL injury in only 36% of female mice (4/11) at an average force of