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Effect of Surgery to Implant Motion and Force Sensors on Vertical Ground Reaction Forces in the Ovine Model

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Abstract

Activities of daily living (ADLs) generate complex, multidirectional forces in the anterior cruciate ligament (ACL). While calibration problems preclude direct measurement in patients, ACL forces can conceivably be measured in animals after technical challenges are overcome. For example, motion and force sensors can be implanted in the animal but investigators must determine the extent to which these sensors and surgery affect normal gait. Our objectives in this study were to determine (1) if surgically implanting knee motion sensors and an ACL force sensor significantly alter normal ovine gait and (2) how increasing gait speed and grade on a treadmill affect ovine gait before and after surgery. Ten skeletally mature, female sheep were used to test four hypotheses: (1) surgical implantation of sensors would significantly decrease average and peak vertical ground reaction forces (VGRFs) in the operated limb, (2) surgical implantation would significantly decrease single limb stance duration for the operated limb, (3) increasing treadmill speed would increase VGRFs pre- and post operatively, and (4) increasing treadmill grade would increase the hind limb VGRFs pre- and post operatively. An instrumented treadmill with two force plates was used to record fore and hind limb VGRFs during four combinations of two speeds (1.0 m/s and 1.3 m/s) and two grades (0 deg and 6 deg). Sensor implantation decreased average and peak VGRFs less than 10% and 20%, respectively, across all combinations of speed and grade. Sensor implantation significantly decreased the single limb stance duration in the operated hind limb during inclined walking at 1.3 m/s but had no effect on single limb stance duration in the operated

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limb during other activities. Increasing treadmill speed increased hind limb peak (but not average) VGRFs before surgery and peak VGRF only in the unoperated hind limb during level walking after surgery. Increasing treadmill grade (at 1 m/s) significantly increased hind limb average and peak VGRFs before surgery but increasing treadmill grade post op did not significantly affect any response measure. Since VGRF values exceeded 80% of presurgery levels, we conclude that animal gait post op is near normal. Thus, we can assume normal gait when conducting experiments following sensor implantation. Ultimately, we seek to measure ACL forces for ADLs to provide design criteria and evaluation benchmarks for traditional and tissue engineered ACL repairs and reconstructions.

Keywords

vertical ground reaction forces; ovine; instrumented treadmill; sensor implantation; activities of daily living

1 Introduction

Treating injuries to the anterior cruciate ligament (ACL) and its graft replacements remain important clinical and basic science problems. Patients report as many as 250,000 ACL tears each year [1, 2]. Such injuries can change knee kinematics and loading patterns in the surrounding structures. Left untreated, these changes can lead to joint degeneration and osteoarthritis [2–8]. Surgeons reconstruct over half of the ruptured ligaments, often using tendon autografts [9, 10]. Early studies reported a success rate exceeding 90% after surgery [11–15]. Despite these successes, investigators have also cited post operative complications including donor site morbidity, patellar discomfort, and joint laxity [11, 12, 14, 16–20]. In the intermediate term, these ACL reconstruction techniques do not restore normal 3D knee kinematics [21, 22] and in the longer-term, patients undergoing ACL reconstruction still have a high risk of radiographic osteoarthritis within 14 yrs following their injury [23]. Given the ongoing clinical concern of osteoarthritis, current ACL reconstruction strategies do not appear to be adequately addressing the long term complications.

Several compounding factors limit our understanding of the mechanisms responsible for these in vivo complications. During activities of daily living (ADLs), the ACL experiences three-dimensional forces throughout the range of knee motion, yet researchers normally load the knee in a single direction at selected knee positions before measuring reaction forces and deformations [24–33]. Using these more simplified testing methods, investigators have determined that the ACL serves as a primary restraint to anterior tibial translation, a major secondary restraint to internal tibial rotation, and a minor secondary restraint to external tibial rotation and varus-valgus angulations [22, 34–36]. While these studies are valuable, they do not provide critical information about ACL function during normal ADLs. As a result, data are lacking regarding the complex nature of ACL forces, especially in response to real or simulated in vivo loading conditions.

To improve long term outcome following ligament injury, investigators need to understand ACL function during ADLs. Unfortunately, measuring knee motions during *any* in vivo activity has been difficult in patients and directly recording the corresponding ACL forces has been impossible. Investigators can now more accurately measure in vivo *human* knee motions during normal ADLs [37–39]. However, the inability to calibrate tissues implanted with force sensors has prevented investigators from actually measuring in vivo knee forces in human patients. Henning et al. [40] first attempted to quantify human in vivo ACL forces for different activities by implanting a strain gauge sensor. However, they could only make these recordings in patients with partially torn ACLs due to the invasiveness of sensor implantation and were unable to directly calibrate the forces. Roberts et al. [41] later attempted to measure in vivo forces in the human ACL by implanting an arthroscopically implantable force probe or AIFP (MicroStrain, Inc., Burlington, VT) in the anteriomedial bundle of the ACL of a healthy adult volunteer. Unfortunately, these measurements could only be taken during passive flexion/extension motions rather than for actual ADLs and calibration required that in vitro data be obtained from other cadaveric knees. It is this inability to functionally "isolate" the ligament and impose realistic physiologic loading patterns during calibration that has forced investigators to pursue similar measurements in animal models.

The ovine model offers an attractive alternative for relating knee and ACL structure and function. Investigators have carefully examined the anatomy of the ovine stifle joint and found it to be both a valid surgical model of the human knee and cruciate ligaments [42, 43] and an ideal experimental model for studying a range of orthopedic conditions and treatments [42]. Appleyard et al. [44, 45] used the ovine model to determine how meniscectomy alters articular cartilage biomechanics [44] and how ACL transection changes meniscal and articular cartilage function [45]. Tapper et al. [46] also characterized 3D joint motion during walking, incline walking, and trotting in the intact ovine stifle joint instrumented with a surgically implanted rigid marker system. This group also determined vertical ground reaction force (VGRF) patterns for walking prior to and 1 and 2 days after implantation. They then placed the instrumented limb in a hexapod robot to simulate normal 3D kinematics [47] that could potentially be used to determine joint or ligament forces. Although such methodologies are exciting and offer the prospects of better understanding ligament function for actual ADLs, 3D kinematics must still be verified. Our challenge remains to accurately record and replicate joint motions to measure corresponding ACL forces in order to establish effective design requirements for new and existing treatment modalities.

Our group seeks to quantify functional tissue engineering parameters (FTEPs) for ADLs to more effectively repair damaged load-bearing tissues [48]. Critical among these FTEPs for all load-bearing tissues are realistic in vivo *forces* and *displacements* for different ADLs. While our group has estimated rabbit and goat tendon forces and goat ligament forces for numerous ADLs [48–53], we could not precisely replicate in vivo joint positions during our in vitro calibration procedures. Others have even measured in vivo ligament displacements in the anterior region of the human ACL for selected activities [54–57] but could neither compute tissue strains without knowing initial transducer length at zero tissue force nor infer tissue forces without ligament material properties. Joint kinematics were also not directly measured.

Given these gaps in knowledge, we sought to directly record in vivo 3D kinematics in the ovine knee and then simulate these motions to measure ACL forces for different ADLs. Our primary objective was to determine if surgically implanting knee motion sensors and an ACL force sensor would influence gait in the adult ovine model. We also sought to determine how increasing treadmill speed and grade would alter ovine gait before and after surgery. We hypothesized that [1] surgical implantation of sensors would significantly decrease average and peak VGRFs in the operated limb, however, it should maintain a minimum of 80% of pre-op values to assume normal gait when measuring ACL force and knee kinematics using the implanted sensors. This criterion was selected based on previous experience inducing surgical treatments and implanting sensors in the stifle joint of large animals. We also hypothesized that [2] surgical implantation of sensors would significantly decrease single limb stance duration for the operated limb, [3] increasing treadmill speed would increase VGRFs pre- and post operatively in the fore and hind limbs, and [4] increasing treadmill grade would increase the hind limb VGRFs pre- and post operatively by shifting the animal's center of gravity in a posterior direction. This research study is the first step in our longer-term goal of quantifying FTEPs to establish design criteria and evaluation benchmarks for surgical therapies [58, 59].

2 Materials and Methods

2.1 Experimental Design

Ten skeletally mature, female sheep (3–4 yrs old, 50–78 kg, species: Suffolk, vendor: Purdue University, West Lafayette, IN) were used. The ovine stifle joint is morphologically and biomechanically similar to the human knee [43]. The sheep knee is large enough to (1) implant motion sensors on the medial aspect of the femur and tibia, (2) implant a force sensor in the ACL midsubstance, and (3) perform reproducible ligament reconstruction. Prior to implantation surgery, VGRFs were recorded while each sheep was subjected to daily combinations of two *speeds* (1.0 m/s and 1.3 m/s) and two *grades* (0 deg (level) and 6 deg (inclined)). We initially attempted a wider range of speeds and grades but the sheep showed abnormal gait at speeds less than 1.0 m/s and after surgery, had difficulty maintaining traction at speeds and grades greater than 1.3 m/s and 6 deg, respectively. At least 10 s of VGRF data were recorded for each treatment condition.

We determined the effects of gait surface speed and grade on three response measures. We contrasted fore and hind limb *average VGRFs, peak hind limb VGRFs, and single hind limb stance duration* during the gait cycle. These values were selected to describe various aspects of the curve that may be affected by surgery or gait surface changes. All animals were trained on an instrumented treadmill (Kistler Gaitway Instrumented Treadmill, Amherst, NY) for 10 min/day for at least 5 days before surgery. We recorded both fore limb and hind limb VGRFs, as well as the center of pressure throughout the gait cycle on the front and rear force plates. VGRFs, normalized to the animal's bodyweight, were compared across the four treatment conditions (two speeds and two grades) during the final day of each animal's presurgery training period.

Following surgery, two motion sensors and one force sensor were used to record knee kinematics and ACL kinetics, respectively. One electromagnetic motion tracker each (Polhemus Liberty, Colchester, VT) was attached to the femur and tibia near the stifle joint line, and a force sensor (AIFP, Microstrain, Burlington, VT) was implanted into the ACL. The 3D motions and ligament sensor response of each sheep were measured for all four treatment conditions at 2 days and 6–9 days after sensor implantation to determine how the animal would recover from surgery and acclimate to the transducers.

Activity order was randomized before and after surgery to avoid bias due to fatigue while monitoring VGRFs. VGRF and center of pressure data recorded after surgery were compared both to those before surgery in the same limb and to those in the contralateral limb after surgery to establish how sensor implantation affects VGRFs for each of the four treatment conditions.

This design permitted us to study two research questions. *(1) How does implanting two motion sensors and an ACL force sensor alter VGRFs and hind limb stance durations? (2) How does altering the gait surface speed and grade before and after surgery affect VGRFs?*

2.2 Detailed Methods

This study was performed in accordance with IACUC standards at the University of Cincinnati. We used an instrumented treadmill to control gait speed and grade. The treadmill includes front and rear force plates located beneath the tread to record fore and hind limb VGRFs during locomotion, respectively. The treadmill allows for VGRFs and centers of pressure to be recorded at 100 Hz for controlled speeds and grades. A handler (Bailey) walked the animal on the treadmill using a harness while a spotter (Herfat) ensured that the forelimbs contacted only the front force plate while the hind limbs contacted only the rear force plate.

2.2.1 VGRF Analysis of Locomotion—A minimum of five consecutive and “distinct” gait cycles for each treatment combination were averaged and analyzed. A distinct gait cycle was defined as a cycle with clear individual left and right hoof strikes, as indicated by the center of pressure recordings on the rear force plate. The center of pressure output from the force plate was used to determine differences in VGRFs between the left and right limbs. Center of pressure recordings also permitted us to establish single leg contact time among treatment combinations. The extracted gait cycles were analyzed using a custom MATLAB program to average the gait cycles and normalize the average gait cycle time so that 0 and 1 represented consecutive left hind limb hoof strikes. Since VGRFs can vary across animals due to different weights, VGRFs were normalized to bodyweight. Typical curves are shown in Fig. 1, relating normalized VGRFs to normalized gait cycle for walking at 1 m/s on a level surface. The peak hind limb VGRFs and the single hind limb stance durations are also indicated.

2.2.2 Surgery to Implant Electromagnetic Motion Trackers and ACL Force Sensor—The sheep was anesthetized, maintained, and surgically prepped, following all IACUC approved procedures and guidelines. A rumen tube was inserted to prevent bloating. The animal received IV Lactated Ringer’s solution, and body temperature was maintained using a heated surgical table. A skin incision was created along the medial side of the patellar tendon. Once the patellar tendon was exposed, an incision was created along the medial border of the patella tendon from origin to insertion. Care was taken to minimize the damage to the surrounding musculature and muscular attachments. The patella was then subluxed and the underlying fat pad was removed to expose the ACL. A pocket was created with a cut (<1 cm) parallel to the long axis of the ACL in the distal third of the ACL. The arthroscopically implantable force probe was inserted so that the long axis of the sensor was perpendicular to the long axis of the ACL, and the pocket opening was sutured closed to secure the arthroscopically implantable force probe (Fig. 2). Electromagnetic tracking sensors were attached just proximal to the joint line on the medial femoral condyle and just distal to the joint line on the medial aspect of the tibia (Fig. 2). Each tracker was positioned to minimize the amount of soft tissue injury induced by tracker attachment. Wires from the arthroscopically implantable force probe and the tracking sensors were passed to the gluteal muscle and then cranially to the shoulder. Post operatively, limbs were not immobilized, and animals were allowed free motion.

2.2.3 Statistical Analysis—The VGRF force data were normal and homoscedastic before and after surgery. The coefficients of variation for the pre- and post op VGRFs were calculated across the gait cycle for all four combinations of speed and grade to understand interanimal variability before and after surgery. A two-way repeated measures test was then performed on the full gait cycle to determine the effect of speed and grade, separately. Additional two-way analyses of variance (ANOVAs) were conducted for each response measure (average VGRFs, peak hind limb VGRFs, and single hind limb stance duration) to determine the effects of speed and grade on these measures. For each of the four treatment levels, gait cycles for the pre-versus post op conditions were compared using a repeated measures one-way ANOVA. The three response measures for the pre-versus post op conditions were also compared using one-way ANOVAs. Lastly, a two-way ANOVA was performed on the post surgical data to determine the effect of changes in gait surface speed and grade, separately. The p-value for all analyses was set at $p<0.05$.

3 Results

3.1 Effect of Treadmill Speed and Grade on VGRFs Before Surgery

Increasing both treadmill speed (Table 1 and Fig. 3) and grade (Table 2 and Fig. 4) affected VGRFs, particularly peak hind limb forces. Increasing treadmill speed did not significantly alter average VGRFs but significantly increased peak hind limb VGRFs. Increasing treadmill speed from 1.0 m/s to 1.3 m/s on a level surface significantly increased the sheep's *peak VGRFs* (p<0.05) in both hind limbs by more than 6% during single hind limb stance. Increasing treadmill speed to 1.3 m/s on a level surface also increased the average coefficient of variation of VGRFs in both the fore limbs (slow: 8.3 ± 0.7%, fast: 12.8 ± 0.7%, mean ± standard error of the mean) and the hind limbs (slow: 10.5 ± 0.7% and fast: 12.7 ± 1.1%). Increasing treadmill grade significantly shifted the VGRFs from the fore to hind limbs at both speeds and significantly increased hind limb peak VGRFs at the slower speed. Inclining the surface to 6 deg at 1.0 m/s decreased average VGRFs in the fore limbs by 4.4% of bodyweight while increasing average VGRFs in the hind limbs by 5.3% (p<0.05). Changes were similar at 1.3 m/s. Inclining the surface to 6 deg at 1.3 m/s produced similar decreases in fore limb average VGRFs (5.1%) and nearly identical increases in hind limb average VGRFs compared with the slower speed (5.3%). These inclination-based changes at the higher speed were also significant (p<0.05). At each speed, gait surface inclination resulted in a more even weight distribution between fore and hind limbs. Inclining the surface to 6 deg at the slower speed also significantly increased left and right hind limb peak VGRFs (p<0.05) by 4.6– 4.9%. The coefficient of variation of VGRFs across the gait cycle at 1.0 m/s on an incline averaged 8.0 ± 0.5% and 9.8 ± 0.3% for the fore and hind limbs, respectively. The coefficient of variation of VGRFs across the gait cycle at 1.3 m/s on an incline averaged 15.7 ± 1.2% and 10.9 ± 0.9% for the fore and hind limbs, respectively. Across all treatment conditions, fore and hind limb VGRFs were consistent across subjects (1.0 m/s level walking condition shown in Figs. 5) with small interanimal variability (coefficient of variation <16%).

3.2 Effect of Surgery

Implanting the motion sensors and force transducer did not significantly affect VGRF patterns among the four speed and inclination conditions (p>0.108). However, after extracting the three response measures (average VGRFs, peak hind limb VGRFs, and single hind limb stance duration), surgery did not change average VGRFs for any condition but did significantly alter peak hind limb VGRFs and/or single hind limb stance durations for each condition.

Surgery did not significantly change average VGRFs in the fore limbs (1.6 ± 2.7%) or hind limbs (−1.6 ± 3.1%) during *level* treadmill walking at 1.0 m/s (Table 3 and Fig. 6). Surgery also did not significantly change peak VGRFs in the operated hind limb at the slower speed (−3.0 ± 5.2%, p=0.457). However, surgery did significantly increase peak VGRFs in the unoperated, contralateral right limb (22.3 ± 7.0%, p=0.004). Surgery also significantly increased single limb stance duration in the unoperated right hind limb (37.6 ± 13.6%, p=0.005) but had no effect in the operated limb (−2.8 ± 3.3%, p=0.525).

Surgery did not significantly change average VGRFs in the fore limbs (3.5 ± 3.7%) or hind limbs (−3.5 ± 4.3%) during level treadmill walking at *1.3 m/s* (Table 3 and Fig. 7). Surgery significantly increased the peak VGRFs for the unoperated right hind limb (25.3 ± 10.8%, p=0.036) but had no effect in the operated hind limb (−5.7 ± 12.9%, p=0.168). Surgery did not affect the single limb stance duration in the operated (0.2 ± 11.6%) or contralateral hind limb (20.2 ± 9.2%) during level treadmill walking at the faster speed (p>0.138).

Surgery did not significantly change average VGRFs in the fore limbs (7.2 ± 2.7%) or hind limbs (−6.4 ± 2.6%) during *inclined* treadmill walking at 1.0 m/s (Table 3 and Fig. 8). Implantation surgery did not significantly affect peak VGRFs in the left (−10.0 ± 3.9%) and right hind limbs (15.3 ± 6.0%) during inclined treadmill walking at the slower speed (p>0.072). Implantation surgery significantly increased the single limb stance duration in the unoperated right hind limb (35.1 ± 15.0%, p=0.029) but had no effect in the operated limb (−7.6 ± 8.5%, p=0.525).

Surgery did not significantly change average VGRFs in the fore limbs (9.5 ± 3.7%) or hind limbs (−8.1 ± 2.5%) during inclined treadmill walking at 1.3 m/s (Table 3 and Fig. 9). Surgery significantly decreased the peak VGRFs for the operated left hind limb (−16.7 ± 5.5%, p=0.012) but had no effect in the unoperated, contralateral hind limb (16.7 ± 7.2%, p=0.198). Surgery did not affect the single limb stance duration in the operated (−7.9 ± 3.9%) or contralateral hind limb (17.8 ± 10.7%) during inclined treadmill walking at the faster speed (p>0.082).

3.3 Effect of Treadmill Speed and Inclination on VGRFs After Surgery

After surgery, increasing treadmill speed affected peak VGRFs and single limb stance duration but increasing treadmill grade had no effect. During level treadmill walking, increasing speed significantly increased both the single limb stance duration of the operated hind limb (p=0.032) and the peak VGRF for the nonoperated hind limb (p=0.01). Increasing treadmill speed to 1.3 m/s on a level surface also significantly increased the average coefficient of variation of VGRFs in both the fore limbs (slow: 12.4 ± 1.4%, fast: 17.3 ± 2.7%, mean ± standard error of the mean) and the hind limbs (slow: 12.9 ± 1.7% and fast: 16.4 ± 3.2%). During inclined treadmill walking, increasing speed significantly increased the single limb stance duration of the operated hind limb (p=0.039). Altering the treadmill grade did not significantly affect any response measure. The coefficient of variation of VGRFs across the gait cycle at 1.0 m/s on an incline averaged 13.3 ± 1.7% and 11.8 ± 2.0% for the fore and hind limbs, respectively. The coefficient of variation of VGRFs across the gait cycle at 1.3 m/s on an incline averaged 14.1 ± 2.1% and 11.4 ± 2.4% for the fore and hind limbs, respectively. Across all treatment conditions after surgery, fore and hind limb VGRFs were consistent across subjects with small interanimal variability (coefficient of variation <18%).

4 Discussion

Measuring VGRFs on an instrumented treadmill provides a noninvasive and repeatable method to establish normal gait patterns and to examine the effects of speed, grade, and surgical treatments on gait. Our VGRF measurements acquired before and after surgery are consistent among animals, with a coefficient of variation averaging no more than 18% for all activities using an instrumented treadmill. This low interanimal variability suggests that changes produced by different treatments will not be masked by differences among animals. The fact that increasing treadmill surface speed increased hind limb peak VGRFs but did not affect average VGRFs could be attributed to the small range in speeds that the sheep tolerated (1.0–1.3 m/s). Since increasing grade (at 1 m/s) significantly increased both hind limb average *and* peak VGRFs prior to surgery, it would appear that altering grade may be a more effective way to alter hind limb loading and ACL forces in future experiments.

Sensor implantation produced only modest changes in VGRFs in the hind limbs but the animals spent greater time in single limb stance in the nonoperated limb early post surgery. While we expected decreases in both average and peak VGRFs in the operated limb, we also sought to ensure that inserting these sensors would not reduce VGRF forces to less than 80% of presurgery levels. The fact that peak VGRFs in the operated limb exceeded 80% of presurgery levels and average VGRFs in both fore and hind limbs exceeded 90% across all treatment conditions indicates that we have achieved our acceptance criterion. Achieving this threshold across all conditions also means that our strategy to measure knee kinematics and kinetics in the operated animal is a suitable model for normal gait. Sensor implantation surgery also produced differing effects in the operated versus nonoperated limbs. Although the duration of single limb stance in the operated limb never decreased by more than 8% during gait, duration of single limb stance in the nonoperated limb increased by as much as 37.6 ± 13.6% (during level walking at 1.3 m/s). While we expected this initial shift from the operated to nonoperated limb early post surgery, we would have preferred to allow each animal an even longer recovery period to bring the VGRFs and durations closer to presurgery levels but the reliability of the sensor recordings decreased after the first 8 days post surgery.

Our VGRFs for an ovine walking on an instrumented treadmill are similar to previous findings in literature with even lower interanimal variability. Our hind limb *average* VGRFs on a level surface are within the range reported by Tapper et al. [46] (34.5–50.0% bodyweight) for ovine overground walking (1.0–1.6 m/s). Our *peak* hind limb VGRFs are also similar to average peak preoperative hind limb VGRFs of 48% bodyweight reported by Taylor et al. [60] for ovine overground walking (average speed of 0.97 m/s). The interanimal variability in our study is also lower than the 30% variability reported by Duda et al. [61] who recorded average hind limb VGRFs of ≈60% bodyweight when walking sheep on a walkway at a slower uncontrolled speed (≈0.7 m/s). We attribute this lower variability to the instrumented treadmill that we used to precisely control gait surface speed. Controlling such gait parameters is critical as we and others seek to determine and universally apply functional tissue engineering parameters as design criteria and evaluation benchmarks in the ovine and other model systems.

The varying effects of speed and grade on VGRFs in the ovine model have not been measured previously and will be important when designing future studies. Based on our pre-op findings that increasing speed did not have a substantial effect on gait but did significantly increase the average coefficient of variation while increasing treadmill grade did have a substantial effect on gait and did not increase the average coefficient of variation, adjusting treadmill grade at the slower speed will allow us to consistently and significantly alter the loading on the hind limbs for future experiments. Our post op findings also support this strategy as increasing treadmill speed also had minimal effects on response measures after surgery. Because increasing treadmill grade did significantly affect response measures before surgery but not after surgery, we believe that early after surgery, the sheep may tend to shift its center of gravity in an anterior direction to protect the operated hind limb, thereby lowering VGRFs in the operated limb. Since each animal's response to surgery is variable, we will test more animals to determine if there is a true effect of grade after surgery. We will also attempt to expand the range of gait surface grade for each animal to ensure significantly different activities of daily living based on VGRFs. While our finding that increasing gait surface speed increases peak VGRFs supports similar results for humans [62–66] and canines [67], the effect of increasing speed on average VGRFs has not been reported in any species. Also, the effect of increasing treadmill grade on VGRF magnitudes during walking has only been reported in the human, for which findings are few and inconsistent. For humans, peak VGRFs are conventionally reported for the early stance peak and late stance peak due to the bimodal VGRF pattern. Increasing inclination resulted in increased peak VGRFs during overground locomotion for the early stance peak [68] and the late stance peak [69]. While our findings in the sheep hind limbs are similar in that we also found that increasing treadmill grade increases peak VGRFs, caution should be exercised when comparing results for a biped and quadruped.

Our study is not without limitations. Our finding that average VGRFs were not affected by changing speed suggests that we did not impose a broad enough range of treadmill speeds. Although we attempted a wider range of speeds, the sheep did not exhibit normal gait at speeds less than 1.0 m/s and could not maintain traction on the treadmill at speeds greater than 1.3 m/s after surgery. Based on the lack of speed-related effects on VGRFs, future experiments will hold the treadmill speed at 1.0 m/s. Similarly, our results only reflect changes over a relatively small range of inclinations. Treadmill grades greater than 6 deg were also attempted but the animals were unable to maintain traction on the instrumented treadmill. To increase the range of treadmill grades and VGRFs, we are now studying the effects of downhill treadmill position. While this position decreases hind limb loading, the altered joint motions and increased knee extensor muscle forces should provide a broader range of ACL loading patterns to examine. Another limitation is that even by using individual front and rear force plates, we still cannot determine VGRF contributions of each limb throughout the entire gait cycle. For example, both hind limbs are in contact with the rear force plate for a portion of the gait cycle, preventing measurements of individual limb patterns. We could therefore analyze hind limb peak VGRFs and stance durations only when a single limb was in contact. Any minor weight redistribution made by the animal during double leg stance is thus undetectable in this system. Also, we could not rely on VGRF and center of pressure data if a hind limb contacted the front force plate or vice versa. It was important to center the animal between the two force plates throughout the gait cycle. We used a harness to keep the animal centered on the treadmill as well as a spotter who ensured proper contact of the limbs with the force plates. However, we could not be certain of the effect of the harness on our measurements.

Future experiments will examine how additional activities as well as isolated and combined knee ligament injuries affect VGRFs, knee motions, and ACL forces. An instrumented treadmill provides a repeatable setting to more efficiently measure VGRFs during normal locomotion and following surgical treatment. Using this methodology, we are now correlating VGRFs with 3D knee motions and ACL forces during realistic activities of daily living. Such measurements complement our prior study showing correlations between VGRF and in vivo ACL force in the goat model, most importantly that ACL forces increase with increasing VGRF [50]. These correlations will potentially reduce the implanted instrumentation currently needed to obtain FTEPs, as well as evaluate normal function and repair outcome in vivo. These results should permit us to establish design criteria and evaluation benchmarks for traditional ACL reconstructions as well as more novel tissue engineered treatments. Ultimately, these FTEPs will guide research by our group and others as we seek to better understand in vivo joint function during activities of daily living in large animal models and eventually in patients.

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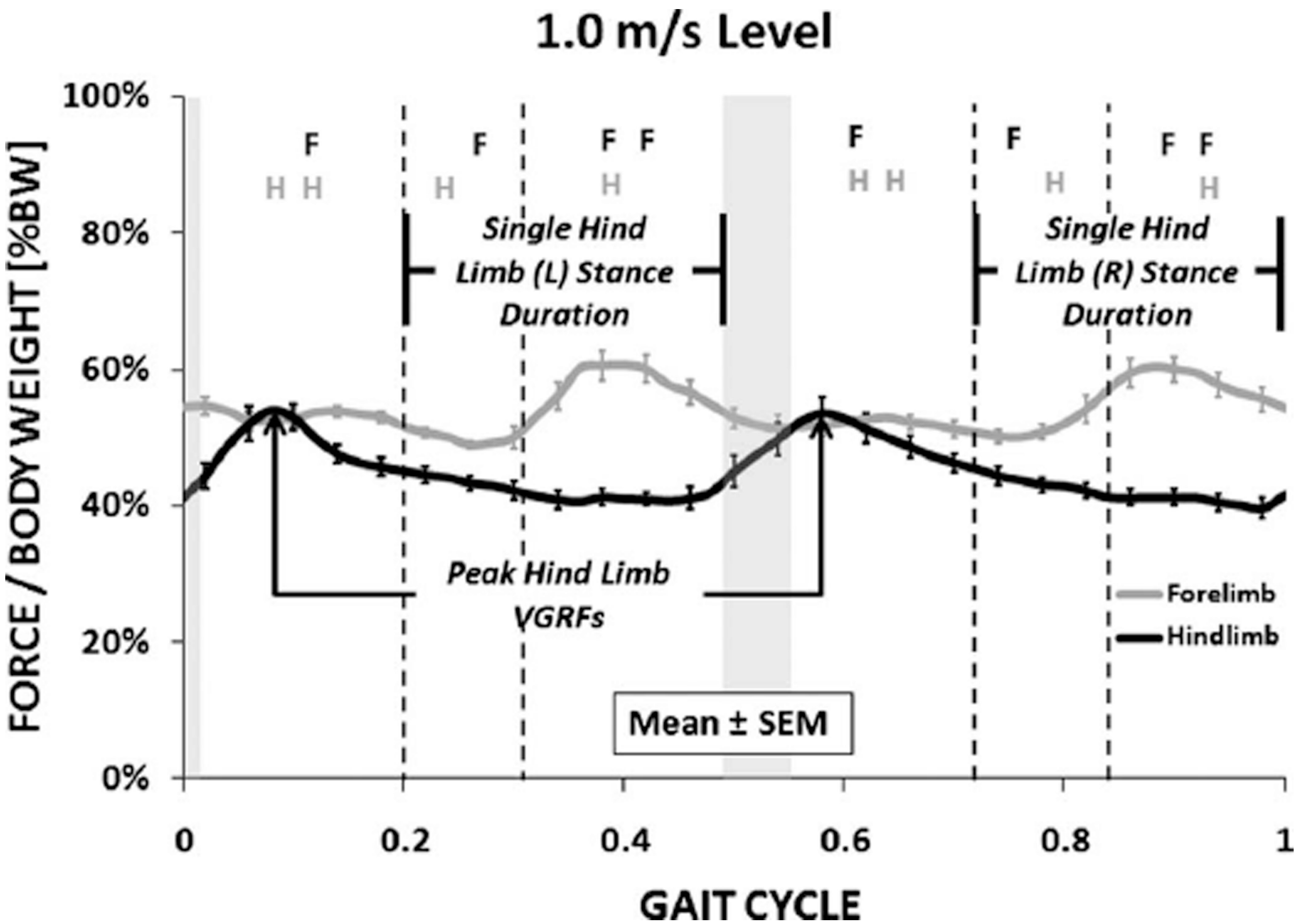

Fig. 1.

Fore and hind limb VGRFs normalized to bodyweight plotted against normalized gait cycle. 0 and 1 on the *x*-axis correspond to consecutive hoof strikes. The gray shaded regions correspond to the segment of the gait cycle when all limbs are in contact with the force plates. The letters at the top of the nonshaded regions correspond to left and right fore limb(s) (F) and hind limb(s) (H) that are in contact with the force plates.

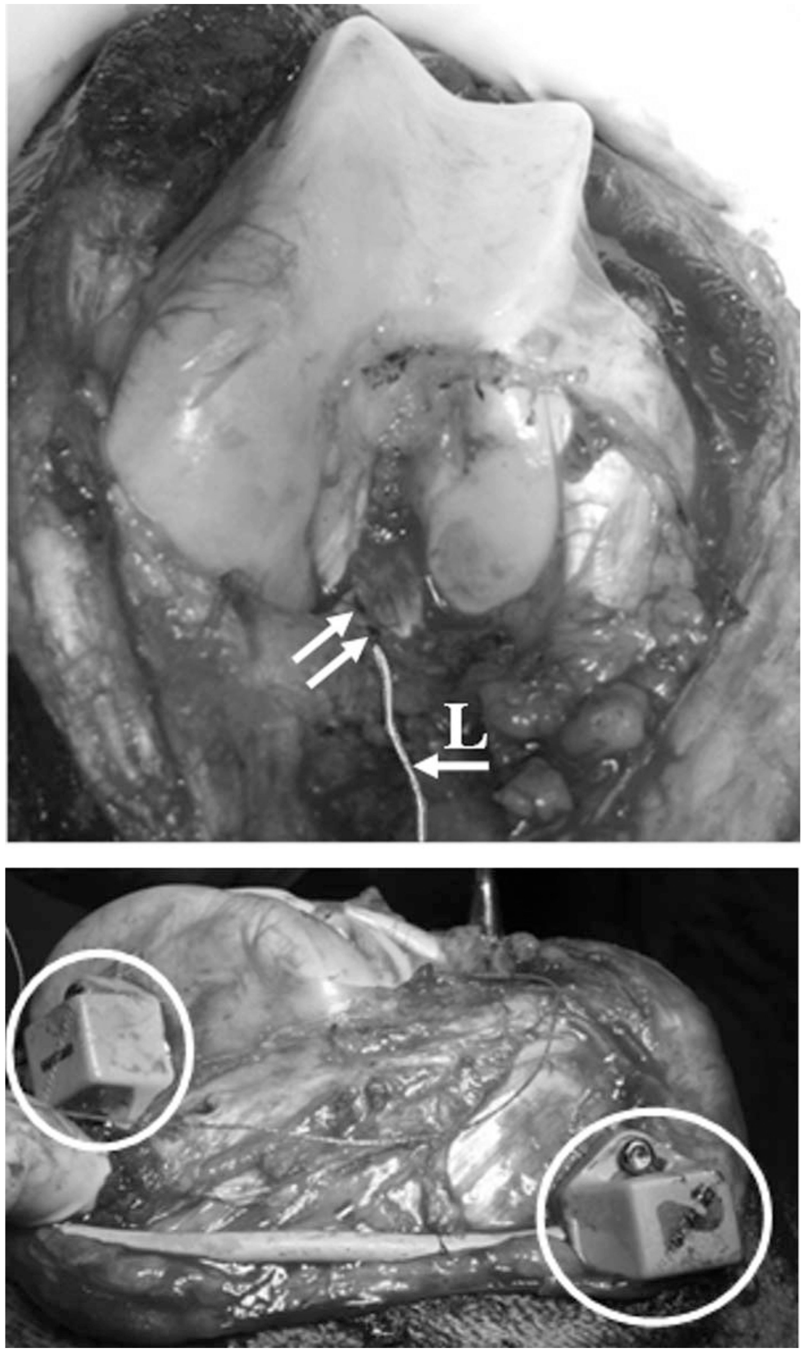

Fig. 2.

Top: AIFP implanted into a sagittal slit in the distal portion of the ACL, oriented with the open end of the sensor pointed distal, and secured into the slit by placing a suture proximal and distal (arrows) to the leadwire (L). Bottom: electromagnetic sensors (circled) implanted on the medial distal femur and medial proximal tibia.

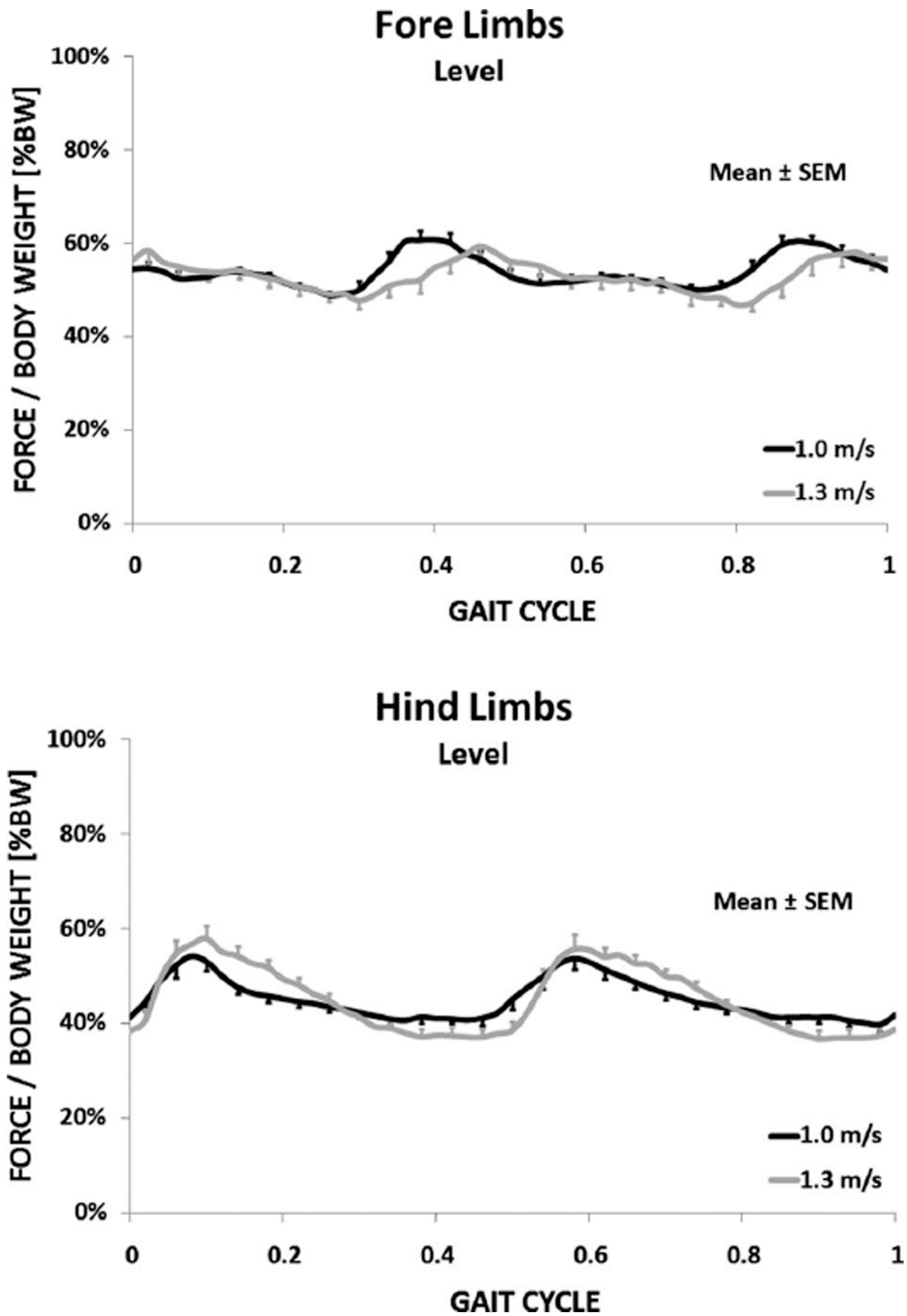

Fig. 3.

Speed did not significantly affect average VGRF (*N*=10) but did significantly alter hind limb peak VGRFs and hind limb peak timing. Average VGRF at 1.0 m/s and 1.3 m/s on a level surface. 0 and 1 on the *x*-axis correspond to consecutive hoof strikes.

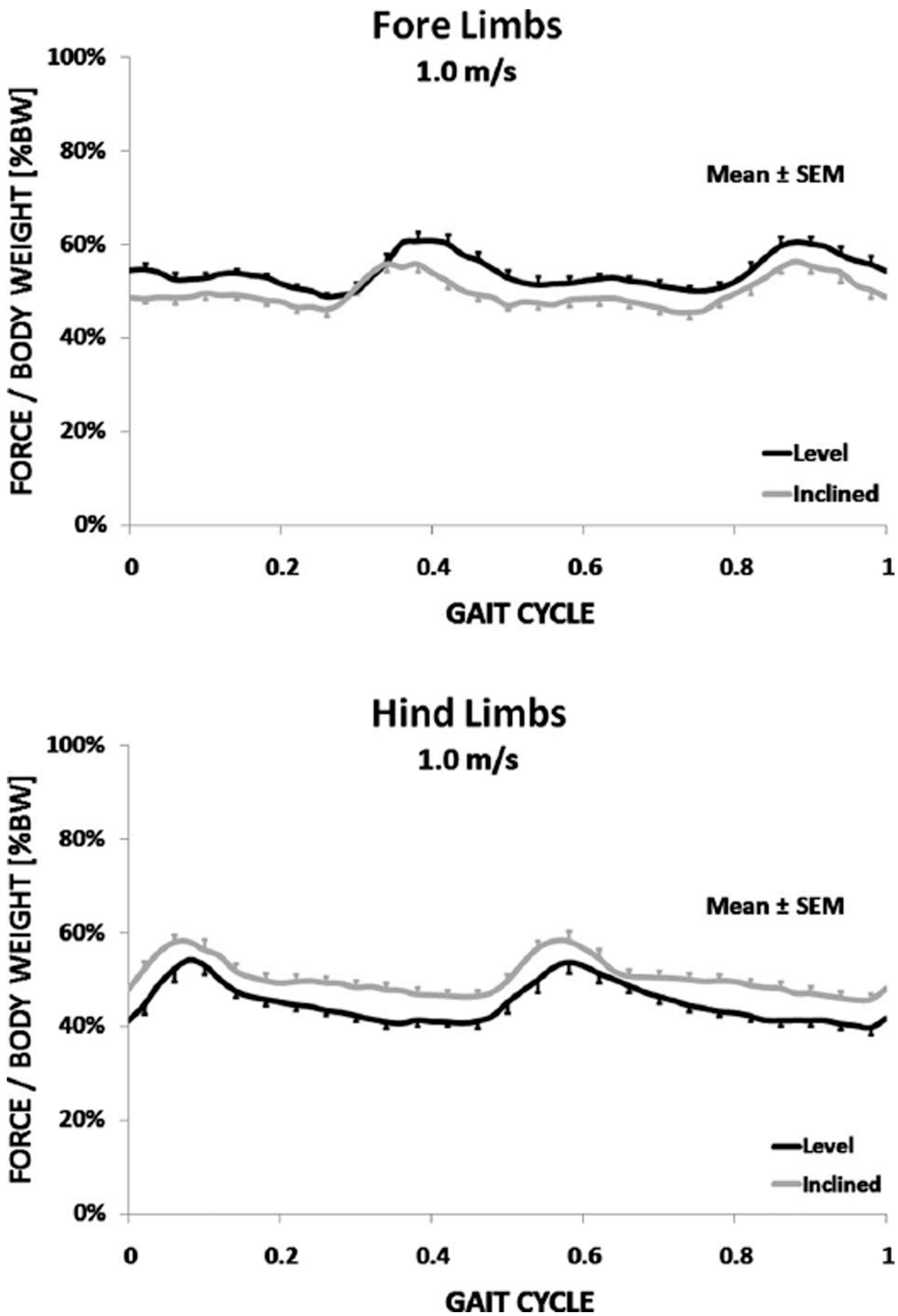

Fig. 4.

Grade did significantly affect average and hind limb peak VGRF at the slower speed (*N*=10). Average VGRF at 1.0 m/s on a level and inclined (6 deg) surface. Grade shifted VGRFs from the fore to hind limbs. 0 and 1 on the *x*-axis correspond to consecutive hoof strikes.

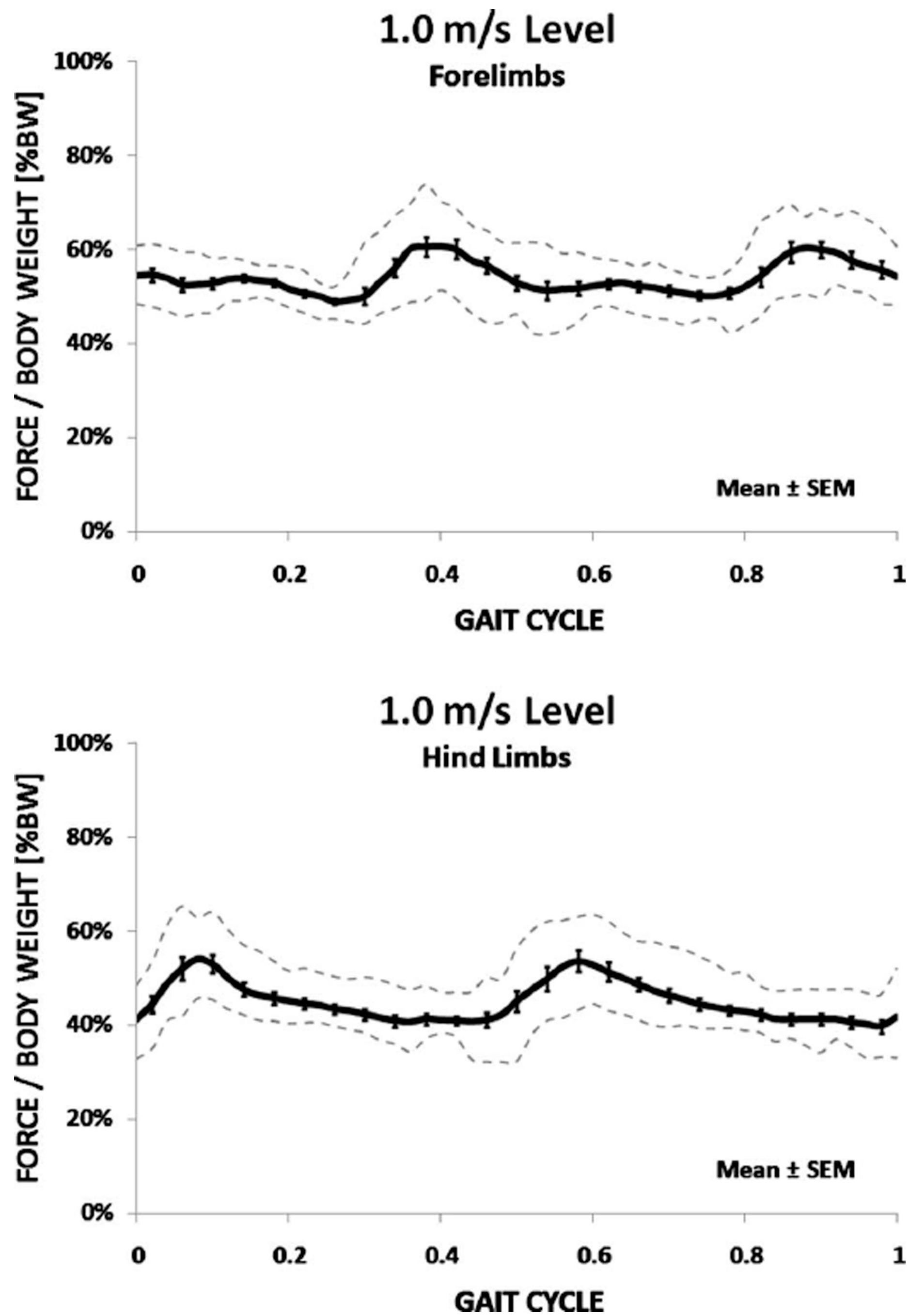

Fig. 5.

Fore and hind limb VGRFs were consistent across subjects (*N*=10) with small interanimal variability. Average VGRF at 1.0 m/s on a level surface. 0 and 1 on the *x*-axis correspond to consecutive hoof strikes. The dashed lines correspond to VGRF maximums and minimums.

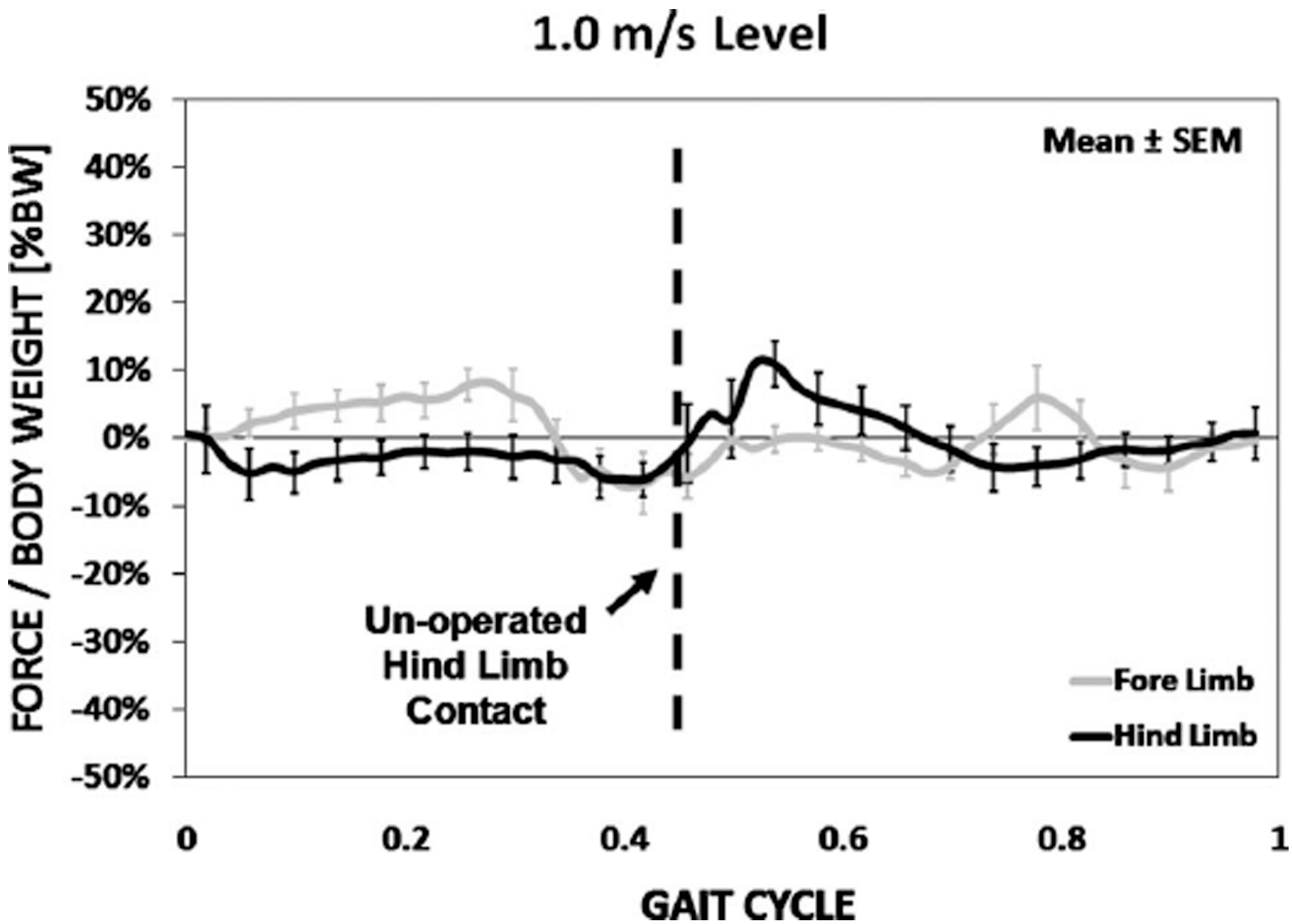

Fig. 6.
Implantation surgery did not significantly affect average VGRFs and only significantly increased the peak VGRF for the unoperated hind limb (N=6). The difference between average VGRFs before and after surgery 1.0 m/s on a level surface.

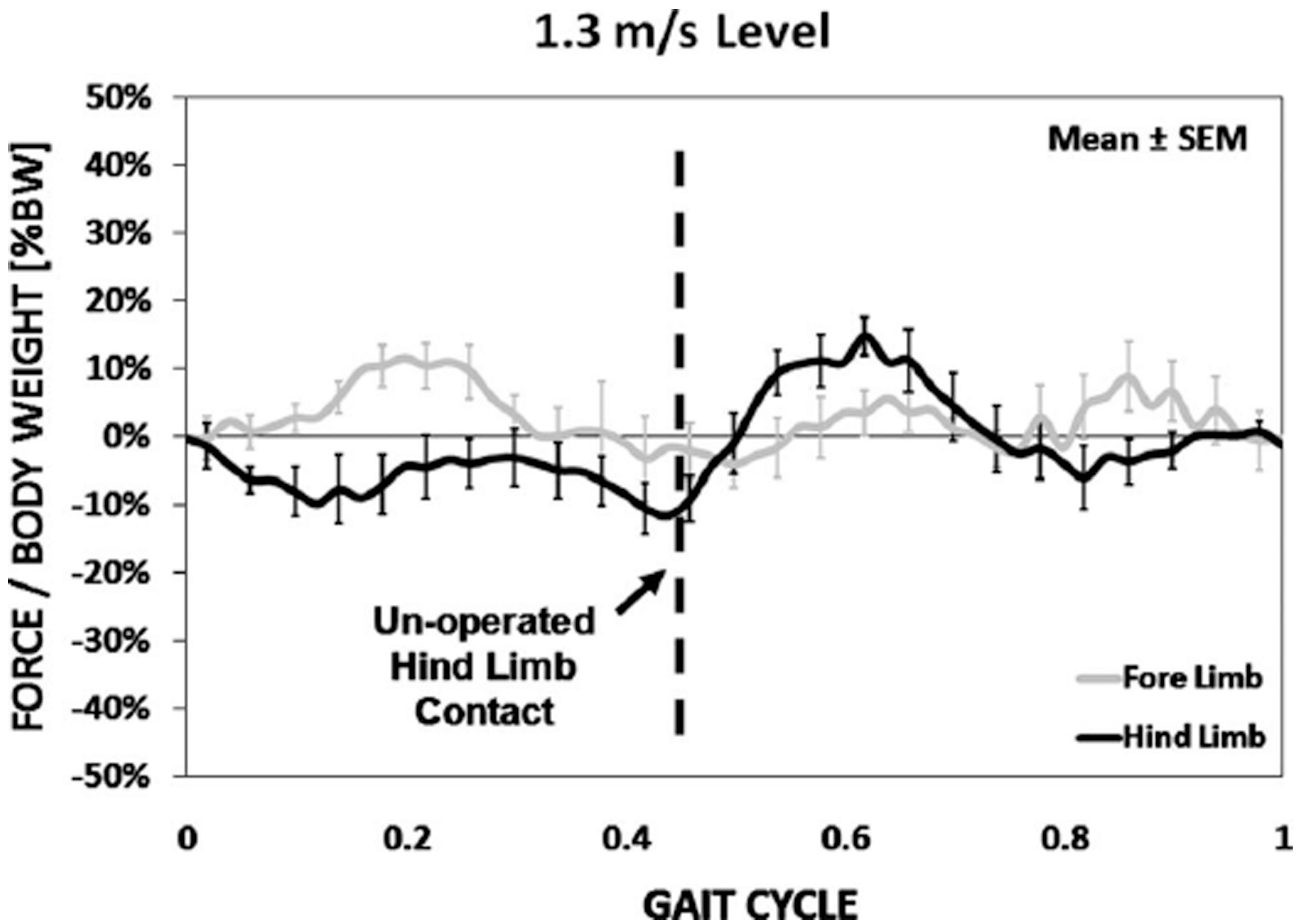

Fig. 7.
Implantation surgery did not significantly affect average VGRFs and only significantly increased the peak VGRF for the unoperated hind limb (*N*=5). The difference between average VGRFs before and after surgery at 1.3 m/s on a level surface.

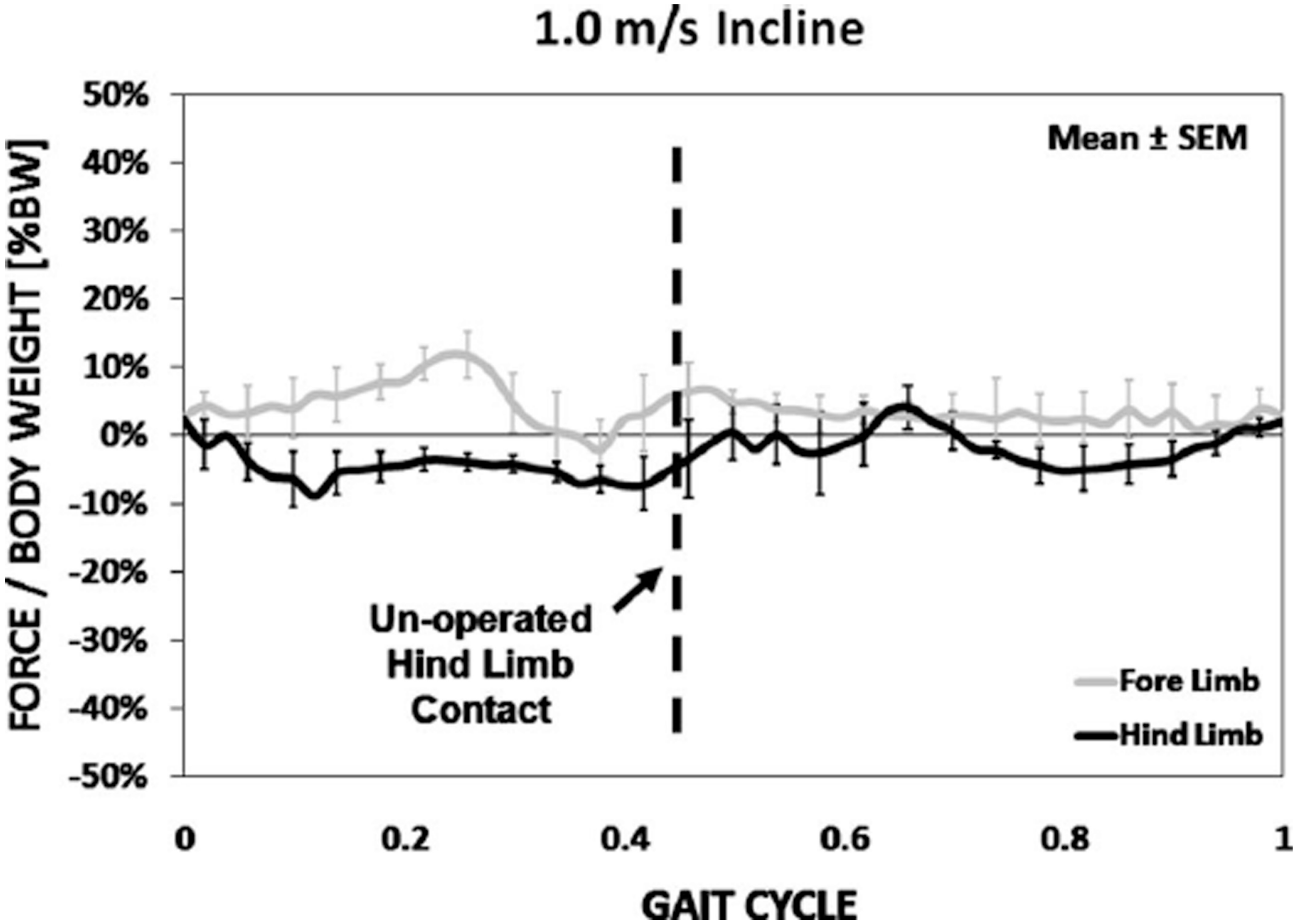

Fig. 8.

Implantation surgery did not significantly affect average VGRFs or hind limb peak VGRFs (N=5). The difference between average VGRFs before and after surgery at 1.0 m/s on an inclined (6 deg) surface.

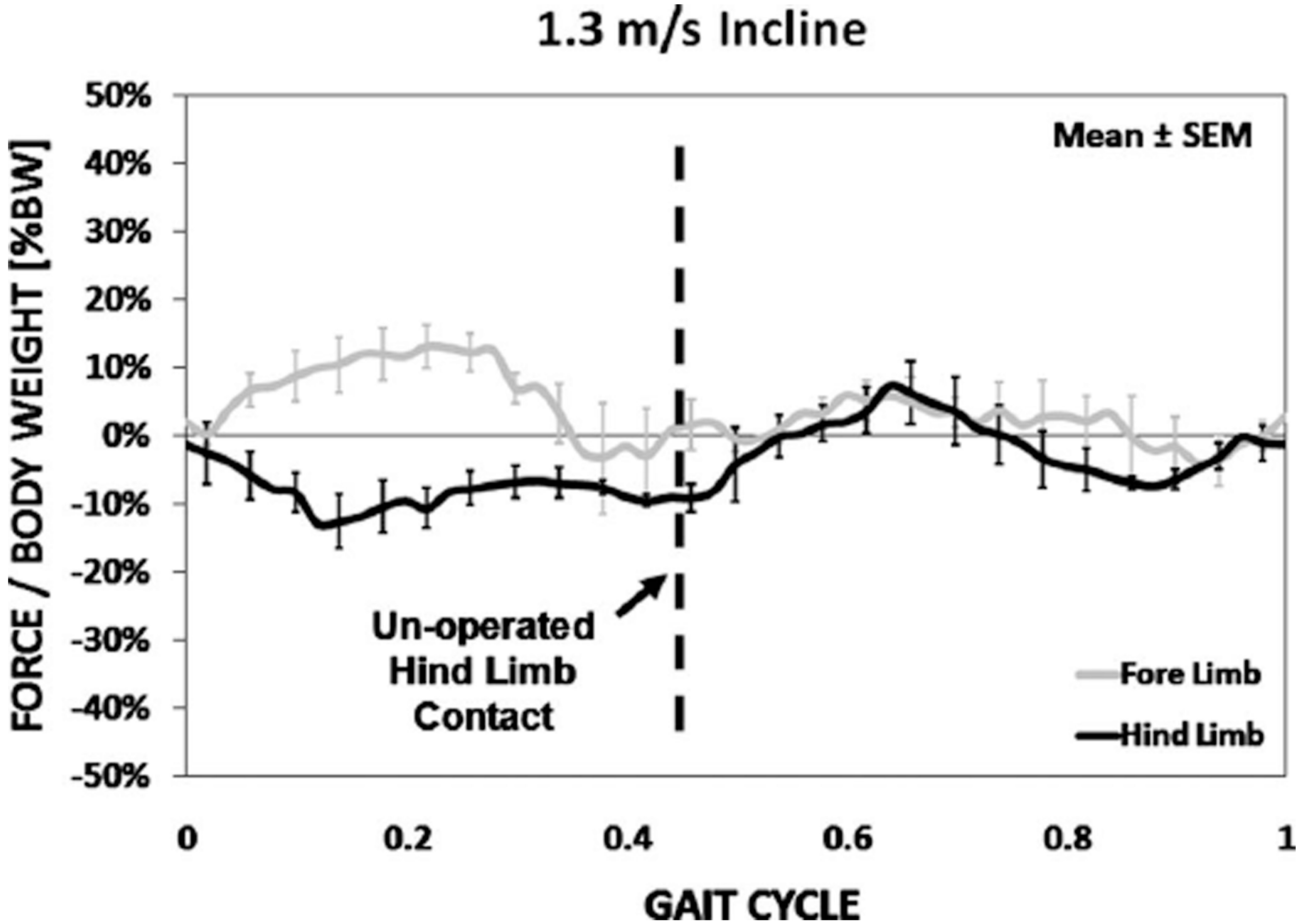

Fig. 9.

Implantation surgery did not significantly affect average VGRFs and only significantly decreased the peak VGRF for the operated hind limb (N=5). The difference between average VGRFs before and after surgery at 1.3 m/s on an inclined (6 deg) surface.

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Table 1

Effects of speed (mean±SEM) on response measures for a level surface (presurgery)

	Average VGRF (% BW)		Hind limb peak VGRF (% BW)		Hind limb contact time (% gait cycle)	
N=10	**Fore limbs**	**Hind limbs**	**Left[a]**	**Right[a]**	**Left**	**Right**
1.0 m/s	54.1 ± 0.8	45.0 ± 1.1	45.8 ± 1.4	46.0 ± 1.6	28.6 ± 1.2	29.8 ± 1.3
1.3 m/s	53.0 ± 1.0	45.0 ± 1.4	52.3 ± 2.6	52.4 ± 2.5	32.1 ± 1.2	33.2 ± 1.4

[a]Denotes a significant difference ($p<0.05$) between 1.0 m/s and 1.3 m/s.

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Table 2

Effects of inclination (mean±SEM) on response measures at 1.0 m/s (presurgery)

	Average VGRF (% BW)		Hind limb peak VGRF (% BW)		Hind limb contact time (% gait cycle)	
***N*=10**	**Fore limbs[a]**	**Hind limbs[a]**	**Left[a]**	**Right[a]**	**Left**	**Right**
Level	54.1 ± 0.8	45.0 ± 1.1	45.8 ± 1.4	46.0 ± 1.6	28.6 ± 1.2	29.8 ± 1.3
Incline	49.7 ± 0.9	50.3 ± 1.3	50.4 ± 1.6	50.9 ± 1.6	30.0 ± 1.4	29.0 ± 0.9

[a]Denotes a significant difference ($p<0.05$) between level and incline.

Table 3

Effects of surgery (mean±SEM) on response measures at 1.0 m/s (presurgery)

	Average VGRF (% BW)		Hind limb peak VGRF (% BW)		Hind limb contact time (% gait cycle)	
	Fore limbs	**Hind limbs**	**Left**	**Right**	**Left**	**Right**
1.0 m/s level (*N*=6)				*a*		*a*
Pre	53.9 ± 1.1	46.1 ± 1.1	46.3 ± 1.6	48.3 ± 2.4	27.0 ± 0.8	31.3 ± 1.8
Post	54.7 ± 1.4	45.3 ± 1.4	44.6 ± 1.5	58.3 ± 1.1	26.2 ± 0.9	42.1 ± 2.4
1.3 m/s level (*N*=5)				*a*		
Pre	53.2 ± 1.5	46.8 ± 1.5	50.1 ± 3.7	54.8 ± 3.9	30.5 ± 2.1	33.6 ± 2.1
Post	54.9 ± 1.9	45.1 ± 1.9	45.4 ± 2.1	67.1 ± 2.7	29.6 ± 1.0	40.0 ± 3.1
1.0 m/s incline (*N*=5)						*a*
Pre	48.9 ± 1.4	51.1 ± 1.4	53.4 ± 3.1	53.1 ± 2.0	30.6 ± 2.3	29.2 ± 1.4
Post	52.5 ± 1.2	47.5 ± 1.2	45.9 ± 1.7	60.1 ± 4.6	27.2 ± 0.5	37.7 ± 3.2
1.3 m/s incline (*N*=5)			*a*			
Pre	48.2 ± 2.2	51.8 ± 2.2	55.3 ± 3.2	55.8 ± 4.4	32.8 ± 1.1	32.1 ± 1.5
Post	52.5 ± 1.4	47.5 ± 1.4	45.5 ± 1.7	64.2 ± 3.7	30.1 ± 1.1	37.6 ± 3.0

[a]Denotes a significant difference (p<0.05) between pre- and post surgery.