Low levels of anterior tibial loading enhance knee extensor reflex response characteristics

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Abstract:
We examined whether neuromuscular reflexes were altered with anterior loads applied to the tibio-femoral joint. A ligament testing device was modified by attaching a reflex hammer to a steel mounted frame to illicit a patellar tendon tap, while anterior directed loads displaced the tibia on the femur. Five trials were acquired while anterior-directed loads (20, 50, 100 N; counterbalanced) were applied to the posterior tibia between 20 N pre ($20\,N_{Pre}$) and post ($20\,N_{Post}$ baseline conditions on two different days. Surface electromyography (sEMG) recorded mean quadriceps (Q) and hamstring (H) reflex time ($R_{Time} = ms$) and reflex amplitude ($R_{Amp} = \%MVIC$). A load cell on the anterior tibia measured the timing ($KE_{Time} = ms$) and amplitude ($KE_{Amp} = N$) of the knee extension force, and was used to calculate electromechanical delay ($EMD = ms$) and peak knee extension moment ($KEM_{om} = Nm/kg$). Data from 19 recreationally active subjects revealed good to excellent response consistency between test days and between baseline conditions for R_{Time}, R_{Amp}, KE_{Time} and KE_{Amp}. With anterior tibial loading, R_{Time} was faster at 50 N vs. $20\,N_{Post}$, and R_{Amp} was greater at $20\,N_{Pre}$ vs. $20\,N_{Post}$ (Q and H) and at 50 N vs. 100 N (Q only). KE_{Mom} was greater at $20\,N_{Pre}$ and 50 N vs. $20\,N_{Post}$, and EMD was shorter at 50 N vs. 20 N, $20\,N_{Pre}$ and $20\,N_{Post}$. These results suggest that knee extensor reflex responses are enhanced with low (50 N) but not moderate (100 N) anterior loading of the knee. Keywords: Stretch reflex; Tendon tap; Tibiofemoral displacement; Surface electromyography; Proprioception; Knee extensor moment

Article:
INTRODUCTION

The anterior cruciate ligament (ACL) provides passive restraint to anterior translation of the tibia on the femur in both weight bearing [4,14] and non-weightbearing [5], and acts as a dynamic sensory modality to regulate and maintain active muscle stiffness and neuromuscular control of joint stability [11, 15,24,25]. While a significant hamstring reflex arc [11,25] and quadriceps inhibition [25] have been demonstrated with high, dynamic levels of ACL loading, these responses are long latency in nature and have not been demonstrated at more physiological loads

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[20,25]. Hence, the ability of the ACL-hamstring reflex arc to contribute to timely stabilization of the joint and prevent ligament injury has been questioned.

In light of these findings, the ACL and surrounding articular receptors appear to play a more critical sensory role in regulating muscle stiffness and joint stability through their effects on gamma motor neurons. Johann-son and colleagues [15,24] have shown that sinusoidal loading of the cruciate ligaments results in changes in the sensitivity of primary and secondary muscle spindle afferents at relatively small tensile loads ($\leq$40 N), thereby contributing to the regulation of muscle stiffness through heightened gamma motor neuron activation. Further, while Pope et al. [19] were unable to illicit a ligament-hamstring reflex arc with an anterior drawer up to 4 mm of tibial displacement or with a direct 125 N load to the ACL via a wire loop, they found significant afferent impulses from the ACL and surrounding articular receptors with ACL loads less than 25 N. While these findings support the role of the ACL and articular receptors in providing proprioceptive feedback and regulation of muscle stiffness about the knee joint, these findings have been limited to direct mechanical tensioning of the ACL in anaesthetized cats. Although the ACL provides the majority (~85%) of passive restraint to anterior tibial translation [5], passive restraint is also provided by other capsuloligamentous and musculotendinous structures. Whether similar low to moderate loads, applied externally to the human tibiofemoral joint, are sufficient to tension articular structures and alter afferent activity and muscle activation is relatively unknown.

Exploring the modulatory effects of ligament and capsular afferents on gamma motor neuron drive and spindle sensitivity under more functional tensile loads in-vivo is relevant to our understanding of factors that can negatively or positively influence functional joint stability. Through a series of studies, Beynnon and colleagues [2,3] have demonstrated increased strain on the ACL with physiological loads including open and closed chain knee extensor activities, transitioning of the tibiofemoral joint from non-weight bearing to weight bearing [4], and when applying low to moderate anterior shear loads, external and internal torques, and varus-valgus torques in weight bearing [10]. These findings suggest that functional, weight bearing activities may pre-load the intact ACL to facilitate its sensory role in perceiving mild to moderate joint displacement and loads, potentially mediating dynamic joint stabilization strategies. This proprioceptive role of the ACL during functional weight bearing is supported by work demonstrating altered hamstring activation patterns in individuals with ACL-deficient knees [1,13,16,18] and in female athletes with increased knee joint laxity [21,23]. However, it is yet unclear from these studies whether the observed differences are the result of changes in proprioceptive input (i.e. reduced tension perceived by the ACL and capsular structures during functional loading), or due to mechanical alterations in the joint contact surfaces in weight bearing, or both.

Research examining the effect of passive capsulo-ligamentous tensioning on neuromuscular control in-vivo, using relatively low to moderate loads is lacking. Understanding the knee joint's sensitivity to a range of lower mechanical loads may help us better understand the fac tors that may modulate the contribution of the ACL and other articular structures to neuromuscular control of knee stability (e.g. ligamentous laxity, hormones, joint angle). The knee extensor reflex offers one model to examine this relationship, as the magnitude of this monosynaptic stretch reflex is primarily dependent on Group Ia afferent activity, muscle spindle sensitivity, and

motor neuron excitability [9]. To that end, our purpose was to determine whether knee extensor reflex characteristics were altered during moderate anterior loading of the tibio-femoral joint. To achieve this aim, we designed a reflex testing apparatus to illicit a knee extension perturbation (i.e. patellar tendon tap), while anterior directed loads displaced the tibia on the femur. We hypothesized that mild to moderate anterior tibial loading would alter proprioceptive sensitivity of the knee joint, as manifested by heightened hamstring and reduced quadriceps reflex activation in response to the knee extension perturbation.

METHODS

Setting and design

A mixed model, repeated measures design was used to examine myoelectric and motor knee extensor reflex responses in males and females while a series of anterior directed loads were applied to the posterior tibia. All testing was performed in the University's Applied Neuromechanics Research Laboratory. Participants consisted of 19 (10 male, nine female) healthy recreationally active college-aged students (23.8 ± 4.1 years; 168.5 ± 9.8 cm; 72.1 ± 18.7 kg) who reported no previous history of knee ligament injury or surgery, no history of connective tissue disorders or diseases, and no lower extremity injury in the past 6 months. For the purpose of examining day-to-day measurement consistency of the reflex response, subjects underwent identical testing on two separate days, spaced —24–48 h apart. Prior to participation in the study, participants signed a written informed consent form approved by the University's Institutional Review Board for the Protection of Human Subjects.

Instrumentation

Reflex testing apparatus

A custom reflex testing apparatus (RTA) was designed to initiate a patellar tendon reflex while the tibia was displaced anterior relative to the femur (patent pending) (Fig. 1(a) and (b)). The RTA was constructed using a modified Telos GA-IIE ligament testing instrument (Austin and Associates; Falston, MD), which allows the application of controlled, quantifiable loads to the posterior tibia to incrementally displace the tibia
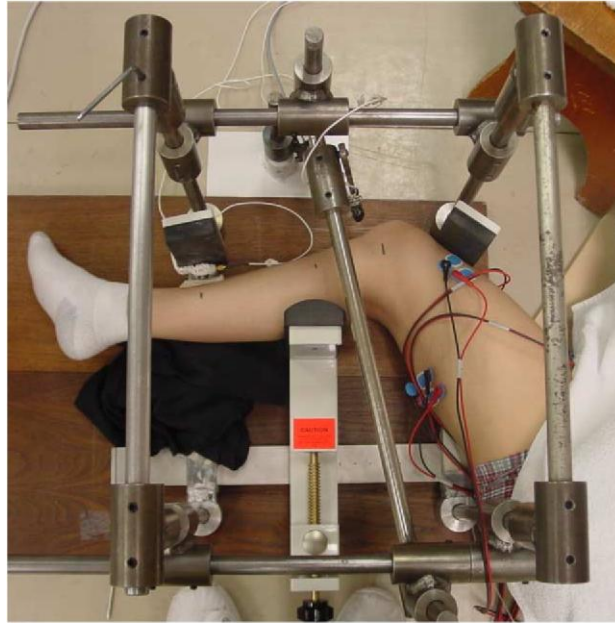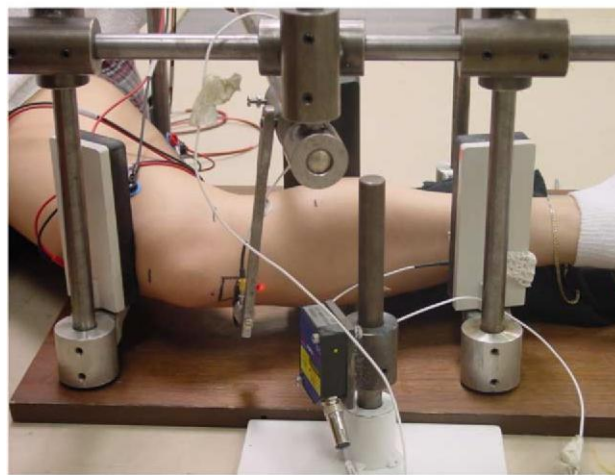
Fig. 1. (a) Superior and (b) anterior view of the reflex testing apparatus designed to elicit a patellar tendon reflex while the tibia is displaced relative to the femur.

relative to the femur. A triangulating laser (OptoNCDT ILD 1400; Micro-Optronic, Dresden, Germany; manufacturer reported accuracy ±0.01 mm) with an attached LED display (Infinity-IDP-4; Newport Electronics Inc., Santa Ana, CA) was positioned at the tibial tuberosity to quantify tibiofemoral displacements during the application of anterior directed loads.

A reflex hammer was attached to the unit via a steel mounted frame welded to each corner of the unit, which allowed the reflex hammer to be adjusted in multiple planes for proper alignment with the patellar tendon of each subject. The reflex hammer was attached to the frame using a ball bearing joint to allow unrestricted movement of the hammer, and was equipped with a pie zoelectric load cell (PCB Piezotronics, Depew, NY) to quantify the tap force, trigger data acquisition, and to ensure a consistent force application was applied to the patellar tendon across

trials and days. A second piezoelectric load cell (PCB Piezotronics, Depew, NY) was positioned on the anterior tibia 23 cm distal to the medial joint line, and interfaced with Datapac 2K2 software to measure the timing and amplitude of the knee extensor motor response (refer to Fig. 1(a)). Attached to the load cell was a 1/4" thick, small piece of rigid splint material (Orthoplast®; Johnson & Johnson Orthopaedics Inc., New Brunswick, NJ) that was heat molded to conform to the contour of the anterior tibia, to aid subject comfort and to insure consistent contact with the tibial load cell.

Surface electromyography
A 16 channel Myopac telemetric system (Run Technologies, Mission Viejo, CA) recorded surface electromyographic (sEMG) activity of the quadriceps and hamstrings in response to the reflex event. Unit specifications for this unit are as follows: amplification of 1 mV/V, frequency bandwidth of 10–1000 Hz, CMRR of 90 dB min at 60 Hz, input resistance of 1 MO, and internal sampling rate of 8 KHz. The sEMG signal for each muscle was detected with 10mm bipolar Ag–AgCl surface electrodes (Medicotest Blue Sensor Model #N- 00-S; Ambu Products, Germany), placed over the vastus medialis (VM) and vastus lateralis (VL) (midway between the motor point and distal tendon), and the rectus femoris (RF), medial hamstring (MH) and biceps femoris (BF) (mid-belly), with a center-to-center distance of 2.5 cm. The reference electrode was positioned on the contralateral anterior tibial shaft. Prior to attaching the electrodes, all skin areas were shaved and scrubbed with alcohol. All electrode placements were positioned at the horizontal mid-point of the muscle to limit cross talk from adjacent muscles, and manual muscle testing was used to confirm signal fidelity.

Prior to collection of the reflex trials, maximal EMG signals were recorded during maximal voluntary isometric contractions (MVICs) of each muscle group for later normalization of the EMG data. Participants were positioned in a Biodex System 3 isokinetic dynamometer, (Biodex Medical Systems Inc., Shirley, NY) at 60° of knee flexion (consistent with joint positioning in the reflex testing apparatus) and asked to complete three, five second maximal effort knee extension (quadriceps) and knee flexion (hamstrings) contractions with the dynamometer locked at 0°/s.

Procedures
Subjects were positioned side-lying in the RTA on the side to be tested, with the back straight (spine in neutral alignment), and the knee in 55–60° of flexion. The contralateral lower extremity was positioned so that it was resting on a cushioned stool with the hip and knee flexed to 90°. To ensure subject comfort and neutral body alignment, a standard pillow was placed under the head and a body pillow was held by the subject. Anterior stabilizing arms, comprised of metal and a dense, non-compressible rubber material, were placed on the anterior thigh 2 cm superior to the patella and on the anterior tibia (with accompanying load cell) 23 cm distal to the medial joint line. The tibial load applicator was aligned 8 cm distal to the medial joint line, and the reflex hammer was adjusted so that the striking surface was aligned perpendicular to the mid-substance of the patellar tendon. The triangulating laser was positioned with the beam directed at the tibial tuberosity (see Fig. 1(b)). Sensors for the tibial load applicator, reflex hammer and triangulating laser were zeroed before each condition.

Once properly positioned, the subjects were asked to squeeze a racquetball while tendon taps, separated by 30-s rest, were delivered by dropping the reflex hammer from a fixed parallel position. The ball squeeze was used similar to the Jendrassik's maneuver (hooking together the fingers of each hand and attempting to pull them apart) to distract the subject's attention and facilitate the elicitation of the deep tendon reflex. Subjects were instructed to squeeze the ball firmly when prompted, to focus all their attention on the ball squeeze, and to not hold their breath during the ball squeeze. Each trial consistently began with the examiner saying "squeeze (subject's name)''. Breathing and electromyographic activity were monitored by the investigator, and trials repeated if the subject was either holding their breath or tensing their leg muscles. We chose this method of distraction because the subject could not comfortably perform the standard Jendrassik's method in sidelying, and we felt it was important to insure consistency in the subject's attention across repeated trials and conditions.

Reflex trials were acquired with anterior-directed tibial loads of 20, 50, and 100 N, with their order counterbalanced between a 20 N pre-baseline condition (20 NPre) and a 20 N post-baseline (20 NPost) condition (five conditions total). Five trials were acquired for each condition on two separate days, spaced 24–48 h apart. A 20 N load for pre- and post-baseline conditions was determined during pilot testing, as this allowed the load applicator to gently snug against the posterior calf, without causing any measurable displacement of the tibia on the femur. This insured that the anterior shin remained in contact with the tibial load cell during all trials and load conditions, and that the contribution of cutaneous receptors was consistent across conditions. SEMG and force data from the tendon tap hammer and anterior tibial load cells were simultaneously acquired at a sampling rate of 1000 Hz, and stored for later analyses in Datapac 2K2 lab application software version 3.05 (Run Technologies, Mission Viejo, CA). The voltage from the load cell attached to the reflex hammer was interfaced with the computer software to trigger data acquisition and mark the time of the reflex stimulus. All data were recorded for 100 ms prior to and 500 ms following each tendon tap using a trigger sweep function. Data were coded so that the investigator processing and analyzing the data (SJS) was blinded to the load condition for each set of trials.

Data reduction of dependent variables
MVIC trials were digitally processed using a centered (symmetric) root mean square (RMS) algorithm, with a 100 ms time constant. The peak amplitude (RMS value) identified over the middle 3 s of each trial was averaged across the three trials and used to normalize sEMG reflex activation amplitudes (R_{Amp} = %MVIC) for each subject.

EMG and force signals from the reflex trials were digitally processed using full wave rectification and a high and low pass (20–500 Hz), fourth-order zero-lag Butterworth filter. The five trials for each load condition were then ensemble averaged to obtain a single representative signal from which to determine reflex characteristics. Fig. 2 shows a typical example of a representative averaged signal. As evidenced by the clear, monosynaptic (M1) wave form, we observed quite consistent reflex responses across trials. Reflex time (RTime = ms) for each muscle was defined as the time delay between the onset of the tendon tap and a five standard deviation increase in sEMG activity above baseline activity (100 ms pre- trigger) for 10 ms or longer. Reflex amplitude (RAmp = %MVIC) represented the peak sEMG monosynaptic reflex amplitude normalized to the MVIC for each muscle. For all sEMG analyses, the reflex time and

amplitude of the vastus medialis, vastus lateralis and rectus femoris were averaged to obtain a single representative quadriceps response, and the reflex time and amplitude of the medial hamstrings and biceps femoris were averaged to obtain a single representative hamstring response.

Using data from the load cell positioned on the anterior shin, knee extensor motor time (KE_{Time} = ms) was defined as the time delay from the onset of the tendon tap to a five standard deviation increase in knee extensor force above baseline activity for 25 ms or longer. Electromechanical delay (EMD = ms) was then defined as the time lag between R_{Time} of the quadriceps and the onset of the knee extension force (KE_{Time}). The amplitude of the knee extensor force (KE_{Amp} = N) was defined as the peak force recorded by the force sensor at the shin, minus any resting force recorded against the shin prior to the reflex event. Using KE_{Amp} and the known distance of the force sensor 23 cm from the medial joint line, knee extensor moment (KE_{Mom}) was calculated
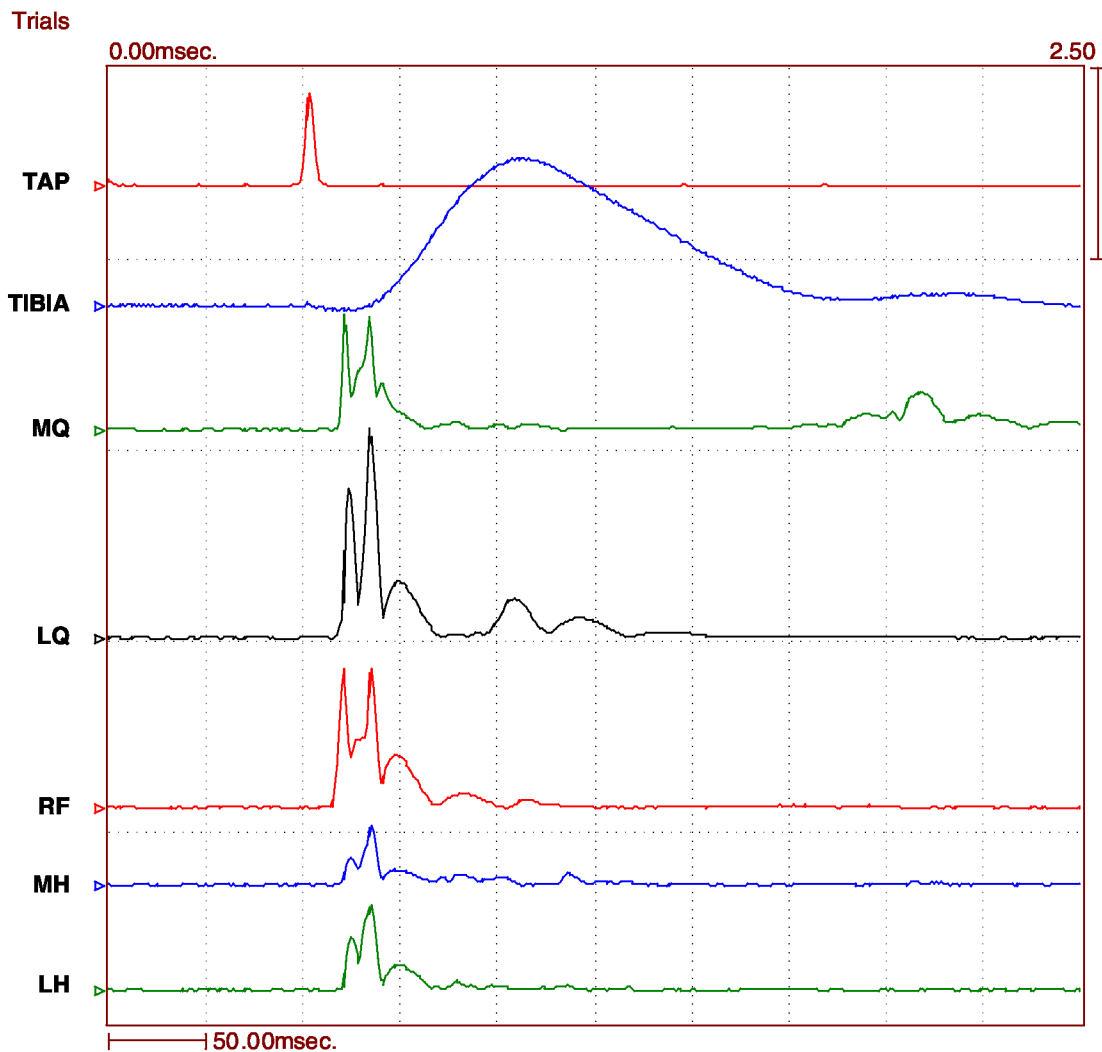

Fig. 2. Representative, average signal obtained over five trials from a single subject, illustrating trigger onset (tap), reflex activation (MQ, LQ, RF, MH, LH) and knee extensor motor response (tibia).

for each trial, and normalized to the subject's mass (N m/kg).

Statistical analyses

To insure consistency in the tendon tap force across trials and test days within each subject, a repeated measures ANOVA was used to calculate intraclass correlation coefficients (ICC formula 2, k) and the standard error of measurement (SEM). We then examined measurement consistency of reflex responses obtained from the RTA between test days (day 1 and day 2) for all load conditions, and between $20\,N_{Pre}$ and $20\,N_{Post}$ conditions within each test day. The participants' R_{Time} (ms), R_{Amp} (% MVIC), KE_{Time} (ms), and KE_{Amp} (N) measurements from the 1 st and 2nd test-sessions for each load ($20\,N_{Pre}$, 20, 50, 100 N, $20\,N_{Post)}$ and between pre- and post-baseline conditions ($20\,N_{Pre}$ vs. $20\,N_{Post)}$ within each test session, were analyzed by way of repeated measures ANOVA in order to calculate the intraclass correlation (ICC formula 2,k) and SEM. Once measurement reliability was ascertained, we examined the effect of anterior tibial loading on knee extensor reflex characteristics using separate mixed model, repeated measures ANOVAs for R_{Time} (ms), R_{Amp} (% MVIC), KE_{Mom} (N m/ kg) and EMD (ms) across the five anterior tibial load conditions ($20\,N_{Pre}$, 20, 50, 100 N, $20\,N_{Post)}$ acquired on day 1. The ANOVA model specifications for R_{Time} and R_{Amp} include the parameters to estimate the effects associated with the two muscle groups (Q and H), the five tibial loads, and the effects associated with the interaction between the muscle groups and the tibial loads. Post hoc analyses consisted of repeated contrasts for within effects, and simple main effects testing for significant interactions. Bonferroni corrections were used for multiple comparisons. Alpha was set a priori at P S 0.05.

RESULTS

Tendon tap force

Table 1 lists the mean ± standard deviations for the tendon tap force recorded for each condition and test day, as well as the intraclass correlation coefficients and standard error of measurement for consistency of tendon tap force between day 1 and day 2 for each load condition and between $20\,N_{Pre}$ and $20\,N_{Post}$ within day 1 and day 2. While the ANOVA model revealed a significant difference across load conditions for day 1 (P = 0.032) and day 2 (P = 0.014), the largest mean difference was 2.6 N between pre test baseline (20.77 N) and the 50 N load (23.3 ± 8.9 N), and post hoc analyses failed to identify this or any other pairwise comparisons as statistically different from one another.

Assessment of measurement consistency

Table 2 lists the ICC and SEM of the response consistency for timing of the myoelectric reflex response and subsequent knee extensor force production between day 1 and day 2 and between $20\,N_{Pre}$ and $20\,N_{Post.}$ Table 3 reports the ICC and SEM values of the response consistency for amplitude of the myoelectric reflex response and subsequent knee extensor force production between day 1 and day 2 and between $20\,N_{Pre}$ and $20\,N_{Post}$. All measures revealed good to excellent response consistency between test days for R_{Time} (ICC range = 0.72–0.91), R_{Amp} (ICC = 0.81–0.95), and KE_{Time} (ICC = 0.76–0.91) and KE_{Amp} (ICC = 0.76–0.90). Consistency between $20\,N_{Pre}$ and $20\,N_{Post}$ baseline conditions on day 1 and day 2, respectively, were also very consistent; R_{Time} (0.92 and 0.95), R_{Amp} (0.89 and 0.98), KE_{Time} (0.93 and 0.79), KE_{Amp} (both 0.90).

Effect of anterior tibial loading on knee extensor reflex characteristics

Table 4 lists the means and standard deviations for myoelectric reflex time and activation amplitude, and

	Tendon tap force (N)			
	Mean ± SD		ICC $(2,k)$	SEM (N)
	Day 1	Day 2		
Between day 1 and day 2				
20 N_{Pre}	20.7 ± 7.1	19.5 ± 6.6	0.88	2.5
20 N	21.8 ± 7.8	20.3 ± 6.6	0.71	4.2
50 N	23.3 ± 8.9	21.7 ± 8.1	0.86	3.0
100 N	22.6+8.7	22.0 ± 8.1	0.86	3.3
20 N_{Post}	22.1 ± 7.8	20.5 ± 6.7	0.81	3.4
	20 N_{Pre}	20 N_{Post}		
Between 20 N_{Pre} and 20 N_{Post}				
Day 1	20.7 ± 7.1	22.1 ± 7.8	0.93	2.1
Day 2	19.5 ± 6.6	20.5 ± 6.7	0.92	1.9

ICC, intraclass correlation coefficient $(2,k)$; SEM, standard error of measurement.

subsequent knee extensor moment and electromechanical delay measures for each load condition. The 50 and 100 N loads resulted in mean ±SD tibiofemoral displacements of 1.9 ± 1.0 and 4.6 ± 1.5 mm, respectively, confirming that the load stimulus was sufficient to cause joint displacement. Reflex timing (R_{Time}) differed between the quadriceps and hamstrings (P = 0.001) and between load conditions (P = 0.041), but yielded no interaction between muscle and load (P = 0.539). Post hoc analyses revealed that R_{Time} was generally faster in the quadriceps compared to the hamstrings (19.4 vs 22.2 ms), and that the pooled muscle mean (Q and H) was faster at 50 N vs. 20 N_{Post} (20.3 vs. 21.2 ms). Reflex activation amplitude (R_{Amp}) also differed by load (P = 0.001), and this difference was muscle dependent (P < 0.001). While R_{Amp} was greater in the quadriceps at 50 N vs. 100 N (211% vs. 180% MVIC) and at 20 N_{Pre} vs. 20 N_{Post} (235% vs. 174%), only 20 N_{Pre} vs. 20 N_{Post} (64% vs. 56%) was significantly different in the hamstrings. Consistent with myoelectric reflex responses, knee extensor moment (KE_{Mom}) (P = 0.004) and electromechanical delay (EMD) (P < 0.001) also differed across loads. Pairwise comparisons (Bonferroni) revealed KE_{Mom} was greater at 20 N_{Pre} (0.138 N m/kg)

	Q R_{Time}		H R_{Time}		KE_{Time}	
	ICC $(2,k)$	SEM (ms)	ICC $(2,k)$	SEM (ms)	ICC $(2,k)$	SEM (ms)
Between day 1 and day 2						
20 N_{Pre}	0.86	1.4	0.76	3.1	0.77	3.8
20 N	0.72	2.2	0.78	3.0	0.87	2.6
50 N	0.91	1.1	0.79	2.9	0.85	3.8
100 N	0.89	1.2	0.74	3.0	0.81	4.4
20 N_{Post}	0.90	1.2	0.83	2.8	0.91	1.9
Between 20 N_{Pre} and 20 N_{Post}						
Day 1	0.95	0.8	0.95	1.4	0.93	1.8
Day 2	0.92	1.0	0.95	1.5	0.79	3.6

	Q R		H R_{Amp}		KE	
	ICC (2, k)	SEM (% MVIC)	ICC (2, k)	SEM (% MVIC)	ICC $(2,k)$	SEM (N)

Between day 1 and day 2							
20 $_{NPre}$	0.94	49.3	0.86		23.4	0.85	5.9
20 N	0.94	45.8	0.86		22.5	0.85	7.2
50 N	0.92	58.4	0.89		21.7	0.81	9.0
100 N	0.91	54.4	0.89		20.2	0.76	10.4
20 $_{NPost}$	0.93	51.8	0.88		20.5	0.90	5.2
Between 20 $_{NPre}$ and 20 N$_{Post}$							
Day 1	0.89	64.4	0.98		8.7	0.90	2.4
Day 2	0.97	34.2	0.96		12.2	0.90	5.1

Table 4

Means ± SD for knee extensor reflex response characteristics for each load

	R$_{Time}$ (ms)		R$_{Amp}$ (% MVIC)			EMD (ms)
	Quad	**Ham**	**Quad**	**Ham**		
20 $_{NPre}$	19.2 ± 3.3	22.0 ± 6.3	235.8 ± 195.6[b]	66.1 ± 60.4[b]	1.35E+0.06 ± 5.99[b]	22.2 ± 5.1
20N	19.6±4.1	23.0±5.7	197.6±148.2	60.3±5	1.26E+0.06±6.00	21.3±4.0
50N	19.3 ± 3.8[a]	21.7 ± 4.3[a]	210.8 ± 175.4[c]	64.1 ± 58.3	1.38E+0.07 ± 6.95[b]	15.9 ± 6.4[d]
100N	19.2±3.5	22.7±5.3	180.4±157.5	55.3±4	1.32E+0.07±7.22	21.6±8.9
20 $_{NPost}$	20.2±3.6	22.5±5.5	173.6±134.9	55.8±5	1.16E+0.05±5.05	20.8±5.4

R$_{Time}$, myoelectric reflex time; R$_{Amp}$, myoelectric reflex amplitude; KE$_{Mom}$, knee extensor moment; EMD, electromechanical delay.

[a] Pooled muscle mean faster than 20 N$_{Post}$.
[b] Greater than 20 N$_{Post}$.
[c] Greater than 100 N.

and 50 N (0. 141 N m/kg) compared to 20 N$_{Post}$ (0.118 N m/kg), and EMD was faster at 50 N (15.8 ms) compared to 20 N$_{Pre}$ (22.1 ms), 20 N (21.3 ms) and 20 N$_{Post}$ (20.8 ms). While the difference between 50 and 100 N (21.5 ms) was of equal magnitude, the pair wise comparison (P = 0.08) was not significant secondary to the greater response variability at 100 N.

DISCUSSION
This study establishes the use of a reflex testing apparatus to provide assessment of knee extensor reflex characteristics in response to functional levels of anterior shear loading with an acceptable level of reliability and precision. Our primary findings indicate that knee extensor monosynaptic stretch reflex response characteristics are quite consistent across repeated test days as well as repeated trials within test days, and are somewhat enhanced with low (50 N) but not moderate (100 N) anterior loading of the knee.

Between day response consistency
For all load conditions, day-to-day response consistency of reflex response characteristics as measured by the RTA ranged from good to excellent, and typically exceeded an ICC value of 0.80. With the exception of quadriceps reflex timing at 20 N (0.72), reflex timing of the hamstring muscles was somewhat more variable and less precise than the quadriceps, likely due to the nature of the perturbation and its focus on the extensor mechanism. Reliability estimates for myoelectric reflex timing tended to be somewhat lower than their corresponding amplitude measures. This is due to the high degree of measurement precision and the relatively small between subject variance of the timing measures, as evidenced by the very low standard errors of measurement associated with these ICCs (all ≤3.0 ms). This level of measurement precision is also evidenced by the ability of the ANOVA model to detect significant differences across loads as small as 1.0 ms (see Table 4 and Section 3).

Conversely, measures of reflex amplitude were clearly less precise and varied considerably between subjects. While the tendon tap was performed identically across all loads and testing conditions, and showed good response consistency across conditions, baseline trials and test days (Table 1), the variations in the measured tap force between subjects was quite large (range 13.5–24.8 N). All else being equal, this would suggest substantial variations in the tendon compliance across subjects that may in turn contribute to variations in the excitability of the muscle spindle and the resultant reflex amplitude. However, when we explored this relationship in post hoc analyses, we did not find significant correlations between tendon tap force and reflex activation amplitude for any of the load conditions. Given the inherent variability in inter-subject reflex amplitude, differences of 30% MVIC or greater in the quadriceps and 8% MVIC or greater in the hamstring muscles were required to detect statistically significant differences (see Table 4 and Section 3). While these differences might appear to be excessive, these values represent approximately a 15% change in the mean activation amplitudes for each muscle, which seem reasonable in discerning clinically relevant differences.

Consistent with the myoelectric reflex responses, the subsequent motor response as measured by timing and amplitude of the knee extension force (KE_{Time}, KE_{Amp}) were also quite consistent. Collectively, these findings support our ability to obtain highly repeatable measures of reflex response characteristics between test days, allowing us to detect clinically relevant differences in knee extensor response characteristics when anterior tibial loads are applied to displace the tibia on the femur.

Comparison of 20 N_{Pre} and 20 N_{Post} baseline conditions

In this study, anterior loads of 20, 50 and 100 N were applied in a counterbalance order between pre- and post-baseline conditions of 20 N. The purpose of this design was two fold; to control for any order effect in responses by load, as well as to assess response consistency over repeated trials. Tables l–3 indicate that all variables were quite consistent between 20 N_{Pre} and 20 N_{Post}, with ICC values generally being somewhat higher (typically exceeding 0.90) and SEM values somewhat more precise than those found between test day measures. While these findings would suggest relatively little change in reflex responses over repeated trials, our ANOVA results reveal attenuation in these responses from 20 N_{Pre} (always the first set of trials for each subject) to 20 N_{Post} (always the last set of trials for each subject). Results reported in Table 4 show that 3 of the 5 variables were significantly less at 20 N_{Post} compared to 20 N_{Pre}. Further, there appeared to be substantially less between subject variability in these responses with repeated testing, as evidenced by the reduction in the standard deviations from 20 N_{Pre} to 20 N_{Post}. These findings were most apparent in R_{Amp}, suggesting a reduction in muscle spindle sensitivity and motor neuron excitability with repeated testing [9], and reinforces the need for counterbalanced designs when examining multiple conditions.

Effect of anterior loads on knee extensor reflex characteristics

Our primary findings were that a 50 N anterior directed load generally produced a heightened knee extensor reflex response. This was contrary to our hypothesis, as we expected knee extensor reflexes to be inhibited when an anterior shear load was applied to the knee. Reflex time for both quadriceps and hamstring were faster at 50 N than 20 N_{Post}, and reflex activation amplitude of the quadriceps was greater at 50 N compared to the 100 N and 20 N_{Post} loads. As would be expected, reflex amplitude was considerably higher for the quadriceps compared to the hamstring, due to

the nature of the perturbation. However, the fact that increased quadriceps reflex amplitude was not accompanied by greater activation amplitude of the hamstring muscles at 50 N suggest this heighten reflex sensitivity may be problematic, as the knee may be vulnerable to greater shear forces at this load. This contention is somewhat supported by a more rapid and greater knee extensor moment measured at the 50 N load.

The ability of the neuromuscular system to respond to joint forces in a sufficient and timely manner is dependent not only on the speed at which proprioceptive feedback is provided to the CNS to initiate an EMG response, but also the additional time required for the muscle to respond with sufficient tension to counteract the injurious load. Hence, measuring the motor components of the knee extensor reflex provides a more complete picture of functional outcomes associated with potential alterations in proprioceptive input with joint loading. The aforementioned reflex behaviors were accompanied by a greater knee extensor moment that was achieved in a shorter period of time at the 50 N anterior load. Although the greater knee extensor moment at 50 N was only found to be significantly different than the 20 N_{Post}, the speed at which this moment was generated at 50 N was faster than virtually all other conditions. Hence, while the speed of the EMG portion of the myotatic stretch reflex was not appreciably altered (i.e. ~1 ms), the mechanical force delay was reduced by as much as 25%. Collectively, this resulted in a total motor time of 36 ms at the 50 N load compared to 41 ms at all other loads. As EMD represents the portion of movement where activation of the motor units and shortening of the series elastic component is occurring [6,12,29,31], EMD is thought to be strongly dependent on the magnitude of the reflex response [7,12,26– 28,30]. Hence, the reduction in motor time at 50 N appears to be directly related to the greater reflex amplitude that was generated.

With the stimulus intensity (i.e. tendon tap) controlled in the current study, the collective findings of increased reflex sensitivity (i.e. increased R_{Amp}, decreased EMD, and increased KE_{Mom}) at the 50 N load would support modest changes in the excitability of the reflex loop via alterations in peripheral afferent sensitivity and gamma motor neuron activation [9]. While the 50 N load was most often different from the 20 N_{Post} baseline condition, suggesting these findings may in part be due to attenuation of the reflex response over time (i.e. an order effect), we do not believe this is the only explanation for these findings. It is clear from Table 4 that there is a non-linear trend in reflex responses across the counterbalanced loads (20, 50, and 100 N), with mean values for 50 N Rip and KE_{Mom} generally higher and EMD generally lower than both the 20 and 100 N loads, which were not found to be significantly different from 20 N_{Post}. These observations along with significant differences in Rip between 50 and 100 N, and in EMD between 50 and 20 N, would appear to be independent of any order effect.

A similar non-linear trend in reflex response characteristics with joint loading was found by Dhaher et al. [8] when applying incremental (5–12°) valgus positional perturbations to the extended knee. Reflex responses were of low magnitude at small deflection angles (5– 7°), increased significantly and peaked with moderate deflection angles (9–10°), then decreased significantly with greater deflection angles (11–12°). Net valgus moments corresponding to these angular valgus perturbations ranged from 18 ± 5 N (5°) to 54 ± 7 N (12°), which are well within physiological loads. Collectively, these findings would suggest that the knee is sensitive to the degree of joint loading, yielding heightened reflexes up to a certain load, then yielding diminished responses once these loads are exceeded. Hence, it is plausible that we did not

achieve the level of knee loading required to result in quadriceps inhibition and enhanced hamstring activation (our original hypothesis). While reflex activation in this study appeared to begin to decrease by 100 N, further study using a larger range of incremental loads is needed to determine if greater loads would show a clear reduction in reflex activation below baseline levels.

The non-linear trend in reflex behavior also suggests that our findings are not simply due to methodological artifacts as a result of increasing load against the tibia with increasing anterior directed loads from 20 to 50 to 100 N. One might argue that by increasing the initial forces against the restraint system, that either the damping characteristics of the device or the mechanical responses (particularly EMD) may be altered. First, we were very careful to use non-compressible materials for the thigh and leg stabilization arms, to control any effects of damping. Second, if this were simply a mechanical effect, one would expect to see a linear change in EMD with increasing loads, which was not observed. Further, because the resting force recorded against the non-compressible tibial load cell prior to the reflex event was slightly greater with increasing tibial loads, and that a 5 SD increase above baseline activity was used to determine onset, one would expect an artificial increase (rather than the observed decrease) in the length of the EMD at 50 N compared to 20 N, because a higher threshold in force would have to be exceeded. These factors, along with the heightened myoelectric reflex responses that accompanied faster and stronger mechanical responses at 50 N, further suggests are findings are due to changes in reflex excitability rather than method artifacts.

Clinical relevance and future directions
The primary limitation of this research model is that it is non-weight bearing, and therefore does not account for the proprioceptive contributions from active muscles and joint compression forces, which are also relevant to protective neuromuscular knee stabilization strategies when in weight-bearing. While Beynnon et al. [2] has shown similar strains on the ACL with both open and closed chain knee extension activities, it is clear that reflex activation characteristics are quite different in weight bearing vs. non-weightbearing [22], and when assessed under active conditions [17,22]. While we recognize that examination of this relationship using a more functional, weight bearing research design may be desired, the level of measurement precision required to test our working hypotheses were not attainable using more dynamic models. Given the variability that exists in human performance, and the many other factors that can influence neuromuscular control in weight bearing, we believe the inclusion of a controlled study such as this may further clarify the independent effect of joint loading on the sensory role of the articular structures of the knee, and their influence on neuromuscular control of knee stability. Further, this model will allow us in future studies to examine factors that may alter the proprioceptive sensitivity of the knee joint to moderate loads (e.g. absolute and sex-hormone mediated increases in knee laxity, knee joint angle, muscle pre- activation), in an effort to further clarify their contri-bution to functional knee joint stability and injury risk. Ultimately, our goal is to translate findings from this controlled experimental model to findings derived from more function models, to better understand the factors influencing neuromuscular control strategies under functional weight bearing conditions.

Acknowledgement

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