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The use of motion analysis to measure pain-related behaviour in a rat model of degenerative tendon injuries

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Abstract:

Chronic tendinopathy is characterized with longstanding activity-related pain with degenerative tendon injuries. An objective tool to measure painful responses in animal models is essential for the development of effective treatment for tendinopathy. Gait analysis has been developed to monitor the inflammatory pain in small animals. We reported the use of motion analysis to monitor gait changes in a rat model of degenerative tendon injury. Intratendinous injection of collagenase into the left patellar tendon of Sprague Dawley rat was used to induce degenerative tendon injury, while an equal volume of saline was injected in the control groups. Motion analyses with a high-speed video camera were performed on all rats at pre-injury, 2, 4, 8, 12 or 16 weeks post injection. In the end-point study, the rats were sacrificed to obtain tendon samples for histological examination after motion analyses. In the follow-up study, repeated motion analyses were performed on another group of collagenase-treated and saline-treated rats. The results showed that rats with injured patellar tendon exhibited altered walking gait as compared to the controls. The change in double stance duration in the collagenase-treated rats was reversible by administration of buprenorphine ($p = 0.029$), it suggested that the detected gait changes were associated pain. Comparisons of end-point and follow-up studies revealed the confounding effects of training, which led to higher gait velocities and probably a different adaptive response to tendon pain in the trained rats. The results

showed that motion analysis could be used to measure activity-related chronic tendon pain. (Word count: 247)

Keywords: motion analysis, tendon pain, patellar tendon, gait, tendinopathy

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Introduction:

Chronic tendinopathy refers to insidious onset of chronic activity-related pain that can affect virtually all tendons. The pathogenesis of chronic tendinopathy remains speculative (Riley, 2004). It is generally regarded as a result of failed healing to accumulated micro-injuries (Khan, 1999). Treatments are empirical and symptom-based, and the responses to treatment vary a lot (Alfredson, 2005). Previous experimental studies on chronic tendinopathy included analyses of clinical samples of tendinopathic tissues (Fu, 2002; Fu, 2007; Fu, 2002; Rolf 2001) Histopathological changes similar to those in clinical samples of tendinopathy were reproduced in animal models by overuse (Glazebrook, 2008), injection of collagenase (Chen, 2004) or cytokines (Stone, 1999). However, owing to a lack of measurement of pain, representative animal models for chronic tendinopathy could not be established. Breakthroughs for experimental studies for chronic tendinopathy will reside on the development of an objective measure of tendon pain in animal models.

A number of methods were developed to measure pain in small animals, including measurement of weight-bearing (Vrinten, 2003), mechanical sensitivity (Fernihough, 2004), vocalization (Han, 2005) and gait analysis such as footprint analysis (Marxen, 2004) and motion analysis [Coulthard, 2002; Coulthard, 2003; Varejao 2002]. As chronic tendinopathy is characterized with activity-related pain, it is more plausible to

detect the pain-associated changes in dynamic state (walking) instead of static state (standing). Gait analysis has been developed to monitor inflammatory pain in small animals, such as rats (Coulthard, 2003), as well as large animals such as horse (Marxen, 2004). Coulthard P (2002; 2003) demonstrated the use of motion analysis as a reproducible, objective measure for acute and chronic pain induced with intraplantar injection of irritants, characterized by significant changes in temporal gait parameters such as double stance duration. Changes in ankle angle during stance phase have been used to investigate functional recovery in rats (Varejao, 2002). Knee joint pain induced by arthritis was widely studied in animal model by motion analysis (Neugebauer, 2007), but it is still unexplored if pain associated with degenerative patellar tendon injury could be monitored by the same technique.

In the current study, we reported the use of motion analysis of the sagittal plane of walking gait to measure painful responses in a rat model of collagenase-induced degenerative tendon injury. Several unexplored areas in measurement of pain-associated gait changes were addressed. Firstly, changes in the contralateral side of the injured limb were analyzed in order to evaluate if there was compensatory change in the contralateral side. Secondly, two different double stance durations within a stride were separately measured. Double stance duration (DS) is defined the duration when both limbs touch the ground during a stride. It follows that two

separate DS are identified in a stride: one ended with the take-off of the contralateral limb, while another ended with the take-off of the observed limb. However, there was no clear distinction between the two in previous reports (Coulthard, 2002; Coulthard, 2003). Since DS is identified as a sensitive parameter to characterize pain (Simjee, 2004), it is necessary to distinguish the two for the use of DS as a valid measurement for pain. Thirdly, the influences of repeated measurements as training effect were evaluated when comparing the results of follow-up study with those of end-point measurement.

Materials and Methods:

The procedures in the following animal experiments were approved by the Animal Research Ethics Committee, the Chinese University of Hong Kong.

Experimental design

Fifty-eight male Sprague Dawley rats, at an age of eight weeks, weighting 250-300 grams, were used in this study. **In the end point study**, forty-two rats were randomly assigned into different groups. Six rats were subject to motion analysis without injection to the knee as pre-injection group. Thirty rats were assigned to receive collagenase injection and subject to motion analysis at 2, 4, 8, 12 and 16 weeks post injection (n=6). Six rats received saline injection as control and motion analysis was performed at 16 weeks post injection. These rats were sacrificed immediately after motion analysis. Patellar tendon samples were obtained for histological examination.

In the follow-up study, sixteen rats were randomly assigned into the collagenase group (n = 8) and the saline group (n=8). Motion analysis was performed before the injection, at 2, 4, 8, 12 and 16 weeks post-injection. At 18 weeks post injury, both groups of rats received one intraperitoneal injection of buprenorphine (0.25 mg/kg body weight). Motion analysis was performed at 30 minutes and 28 hours after buprenorphine administration, according to the pharmacological actions of buprenorphine as analgesics.

Collagenase-induced degenerative tendon injury

Collagenase I (bacterial collagenase type I; Sigma Chemical Inc.) was reconstituted in 0.9 % saline and filtered with a 0.22 μ m syringe filter (Millipore) for sterilization. A 20 μ l aliquot of 0.3mg collagenase I or saline was intratendinously injected (Chen, 2004) to the left patellar tendon, and the injection was monitored with high resolution ultrasound imaging (Visualsonics, Toronto, Canada). Free cage activity was allowed after injection. The body weight of the rats was measured regularly during the study.

Motion analysis

Gait recording was performed at the time points aforementioned with a similar set-up described by Messner (1999). At each time point, each rat was encouraged to walk in a 1.5-meter straight enclosed walkway with darkened ends. The walkway was made with transparent plastic to allow videotaping the rat motion from outside (Figure 1A). One day before data collection, the hair on rear body of each rat was shaved to expose the major joint positions under anaesthesia by intraperitoneal injection of 2.5% (w/v) sodium pentobarbital (45 mg/kg body weight). Hip, knee, ankle and toe were marked with black ink on both hind limbs and checked for the consistency of marks and joint movement (Figure 1B). One CCD digital video camera (JVC 9600, Japan) with a

100-Hz filming rate at a 1/250s shutter speed was used to videotape the rat motion in the sagittal plane. Video data were collected by placing each rat in one end of the walkway and allowing ambulation across the central part of the walkway (Figure 1C). A reference point was also marked on the central part of the walkway for the calculation of gait velocity (Figure 1E) and stride length with a calibration with a ruler placed on the walkway. Each rat walked in both directions along the track in order to record the gait in both limbs. When three consecutive strides with stable speed were performed, the middle stride was identified as a successful stride trial. Four successful trials were collected for each side from each rat, and the trials with median gait velocity were taken for further analysis.

The video data was processed by a motion analysis system (Ariel Performance Analysis System, USA). The video of each successful trial was trimmed from the time of foot strike to the time of the next foot strike of the observed limb, as identified visually from the video. The time of take-off after the first foot strike was also visually identified and thus the stride duration, stride length, stance duration and swing duration were measured. In addition, the time of take-off of the contralateral limb was also visually identified to determine the double stance duration (DS), and this DS was registered as a measurement for the observed limb. For example, double

stance duration ended with take-off of control side (DS-C) was measured in the video records of the injection side, while the double stance duration ended with the take-off of the injection (DS-I) was measured in the video records of the control side. For the analysis of knee motions, each frame in each trial was digitized to obtain 2-D coordinates of the markers. The knee angles were obtained and time-normalized to 0-100% stance and 0-100% swing (Figure 1D). The average knee angular velocities at mid-stance (KVMS) and mid-swing (KVMW) was calculated as the rate of change in the angular displacement of knee joint at 40-60 % stance and 40-60 % swing respectively.

Histological examination

In the end-point study, the rats were euthanized with an overdose of sodium pentobarbital (25 % w/v) immediately after motion analysis. The patellar tendons were harvested to prepared histological sections. The specimens were fixed in 10% buffered formalin overnight and paraffin-embedded for haematoxylin and eosin staining.

Statistical analysis

SPSS software version 15.0 (SPSS Inc., Chicago, IL) were used for all statistical tests.

Statistical significance was accepted at $\alpha = 0.05$. All data were checked with normal distribution by Kolmogorov-Smirnov test. For the end-point study, a multivariate analysis of variances (MANOVA) was used to detect significant differences with gait velocity, stride length, stride duration, stance duration, swing duration, double stance duration, average knee angular velocity at mid-stance and mid-swing as outcome variables. The effects of treatment (collagenase vs. saline injection) and time post injection were determined, as well as the interaction between treatment and side of injection, and the interaction between treatment and time post injection. For the follow-up study, a design of analysis of variances (ANOVA) with repeated measure was used to analyze the effects of collagenase injection (collagenase vs saline) as between-subjects factor, the effects of time after injection (6 levels: pre-injection, 2, 4, 8, 12 and 16 weeks post injection) and side of injection (2 levels: injected side and control side) as within-subject factors. The same group of gait parameters were entered into the ANOVA model as dependent variables. For the study of the effect of analgesic, ANOVA with repeated measure was used with collagenase injection (collagenase vs saline) as between-subjects factor and time after injection of buprenorphine was within-subject factor (3 levels: before injection of buprenorphine, 30 min and 28 hours after buprenorphine injection).

Results:Histology

Extensive matrix degradation and increased healing tendon cells were noticed with inflammatory response at week 2 post collagenase injection (Figure 2A). The cellularity slightly decreased in week 4 (Figure 2B) and week 8 (Figure 2C) with limited healing responses; but further matrix disturbance was developed in the absence of inflammatory cells at week 12 (Figure 2D). At 16 weeks after injection, tendon degeneration was observed in the collagenase groups with increased cellularity, increased vascularity and significant matrix disturbance with calcification (Figure 2E), while normal tendon histology was found in the group with saline injection (Figure 2F).

End-point study

The statistical results for the gait analyses of the end-point and follow-up study were presented in Table 1. Significant changes in gait velocity ($p=0.001$) with time were detected, but the difference between the collagenase-treated and the saline-treated rats was not significant ($p=0.134$) (Figure 3A). Significant decrease in stride length ($p<0.001$) and swing duration ($p<0.001$) were detected in collagenase-treated rats as compared to the saline-treated rats (Figure 3B, C). Double stance duration ended with take-off of control side (DS-C) was significantly increased in the collagenase-treated

rats ($p=0.038$) (Figure 3D). Average knee angular velocity at mid swing (KVMW) in the contralateral side of the collagenase-treated rats was higher than that in the saline-treated rats at 16 weeks post injection ($p=0.008$) (Figure 3E). Average knee angular velocity at mid stance (KVMS) did not show significant difference between collagenase and control group ($p = 0.897$) (Figure 3F), but a marginally significant interaction between treatment and side of injection was detected ($p = 0.048$). As we only collected saline-treated groups at 16 weeks post injection, the between-subject effect of Treatment*Time was not available.

Follow-up study

The body weight of the collagenase-treated rats ($545 \pm 57\text{g}$) and the saline-treated rats ($507 \pm 37\text{g}$) at 18 weeks post injury exhibited no significant difference (Student's t test, $p = 0.15$). As compared to the pre-injection levels, both the collagenase-treated and the saline-treated rats walked faster (Figure 4A) with increased stride length (Figure 4B) but the differences between the two groups were statistically insignificant. Decreased swing duration was noticed in the saline-treated rats ($p = 0.023$) but not in the collagenase-treated rats (Figure 4C). Double stance duration and KVMW exhibited no significant difference between the collagenase-treated and the saline-treated rats (Figure 4D, E). A time-dependent increase in KVMS was observed

in the contralateral side of collagenase-treated rats but not in the saline-treated rats ($p=0.003$) (Figure 4F).

Effect of buprenorphine

Buprenorphine injection induced a significant restoration in DS-C after 30 min in the collagenase-treated rats and the DS-C returned to the original level after 28 hours ($p = 0.029$) (Table 1). No significant change in gait parameters was detected in the saline-treated control after buprenorphine injection (Figure 5).

Comparisons of end point study and follow-up study

Except in the pre-injury groups, the gait velocities of the rats in follow-up study were significantly higher than those in the end-point study (Student's t test, $p<0.05$) (Figure 6). Significant time-dependent changes were detected in all gait parameters in the follow-up study, but only in some gait parameters in the end-point study (Table 1). Collagenase-induced tendon injury caused an increased DS-C (Figure 3C) in the end-point study, but a decreased DS-C (Figure 4D) was noticed in the follow-up study despite statistical significances were not detected ($p=0.068$).

Discussion:

We reported the use of motion analysis to measure chronic painful response associated with degenerative tendon injuries. It was found that altered walking gait, marked by an increase in double stance duration ended with take-off of contralateral control side (DS-C), was resulted from a non-healing degenerative tendon injuries induced by collagenase injection. The gait change was suppressible by buprenorphine and the analgesic effect could not carry over 24 hours after single injection (analgesic effect of buprenorphine lasts for 8- 10 hours), indicating that a painful response was associated with the collagenase-induced tendon injuries. These findings supported that the animal model of collagenase-induced tendon injury resembled the longstanding pain in chronic tendinopathy. Significant changes in the gait parameters of the contralateral limbs in collagenase-treated rats suggested adaptive motions to reduce mechanical allodynia in the injured limbs. Our results indicate that motion analysis was useful for the detection of knee pain associated with patellar tendon injuries at longer time points post injury as compared to previous studies which worked on relatively short-term inflammatory pain (Coulthard, 2003), Achilles tendon injuries (Messner, 1999) or arthritic knee (Simjee 2004).

Histological examination revealed that collagenase treatment induced matrix damage and healing response in early time points (2 and 4 weeks). However, the healing

responses failed to restore normal tendon histology after 8 weeks post injury, with persistent matrix disturbance including loss of collagen birefringence and appearance of tendon calcification (Lui, 2008). On the other hand, saline injection did not cause observable injury in patellar tendons up to 16 weeks post injury. The time frame of the present study encompassed the inflammatory phase of the primary injury, the healing response and the development of failed healing outcomes. As compared to previous studies of short-term follow up of gait changes which might only cover the inflammatory phase (Coulthard, 2003), the temporal gait changes within 16 weeks post injury appeared more complicated. Though it is difficult to correlate the local histopathological changes in the injured patellar tendon to the overall changes in gait parameters, the injury or healing status in relation to pain level may contribute to the variations in the walking gait of the rats.

In the end-point study, the gait change was primarily the lengthened double stance duration (DS) in the collagenase-treated rats, which might be due to a delayed take-off especially in the contralateral control limb. Delayed take-off may shorten swing duration as KVMW was also increased, and shortened swing duration would probably affect the stride length, which was also found to be shorter in the collagenase rats. These findings suggested that various gait parameters are coherent entities which

affect one another. The observed increase in DS as an indicator of pain was consistent to previous reports on inflammatory pain (Coulthard, 2002; Coulthard, 2003; Simjee 2004), which was explained as an adaptive mechanism to reduce mechanical agitation to the injured limb to avoid pain.

In the follow-up study, the differences between collagenase-treated rats and saline controls were less obvious. Relatively longer swing duration and a decrease in DS-C were observed in the collagenase-treated rats, which was just opposite to the findings in the end-point study. Comparing the results in both studies, it was found that the gait velocities of collagenase-treated rats were significantly higher in the follow-up study (Figure 6). It is probably due to the training effect on the rats to walk along the track, as all rats in the follow-up study received repeated motion analysis while rats in the end-point study only received once. Since DS exhibited an inverse relationship to gait velocity (Figure 7A), fast walking rats naturally shortened DS, which did not favor the strategy of pain avoidance by increasing the DS in high speed walking. In order to determine if gait velocity affected the pain-associated change as shown in DS, standardization of DS was performed to remove the influences of gait velocity according to van Iersel MB (2007). In brief, a linear regression was performed between DS and the inverse of gait velocity, and the regressed values of DS were

calculated from gait velocity in each trial, which represented the average DS at the specified gait velocity (Figure 7B). Residual DS was calculated by subtracting the regressed DS from the observed DS, which represented the deviations from the average DS at the specified gait velocity, and the influence of gait velocity on DS was removed (Figure 7C). It was found that the velocity-standardized DS (residual DS) in the collagenase-treated rats was still higher in the end-point study and lower in follow-up study as compared to the saline control (Figure 7D). It suggests that gait velocities did not directly affect the direction of pain-associated changes in DS. Different strategies for adaptation of degenerative tendon injuries might be employed in the rats during high speed walking and low speed walking. Since KVMS in the collagenase-treated rats was higher in the contralateral control limb in the follow-up study (Figure 4F) and lower in the injected limb in the end-point study (Figure 3F), it is speculated that during high speed walking the rats avoided mechanical agitation on the injured limb by a faster motion in the contralateral control limb; while at low speed walking the rats could simply decelerate the motion of the injured limb. It follows that gait velocity would confound the use of DS as a measurement of painful responses. As repeated motion analyses might train up the rats to complete the task faster (higher gait velocity), end-point measurement is preferable with a lower gait velocity and greater variability to detect difference. Moreover, exclusion criteria

according to gait velocity could be applied to restrict data collection of moderate voluntary walk to enable consistent measurement of pain-associated gait changes.

There are several limitations in the present study. Firstly, collagenase-induced degenerative tendon injuries may not fully represent tendinopathy; despite there is a long history of the use of collagenase model to study tendinopathy (William, 1984). Other models are established to address different aspects in the development of degenerative injuries, including the injection of cytokines (Stone, 1999), prostaglandins (Khan, 2005) and application of long-term overuse exercise (Glazebrook, 2008). Due to unknown pathogenesis, these established models for tendinopathy could only reproduce similar histopathological features of tendinopathy at advanced stage, as clinical samples of tendinopathy were obtained from patients resistant to conservative treatments. Thus the measurement of tendon pain in animals is crucial to complement the histopathological findings in these models, as chronic tendon pain is the key feature of clinical cases of tendinopathy. Further works could apply motion analysis to detect painful responses in other tendinopathy animal models in order to collect more information about histopathological changes and pain in tendinopathy.

The second limitation is the skin movement of the rats which may decrease the

accuracy in the measurement of knee angles (Filipe, 2006). Strictly speaking, it is almost impossible to determine the exact position of the knee by marking the skin over the knee. However, marking on the skin over the joints may still convey some information about joint motion, for example, we could still identify when the knee joint flex or extend, that is probably why external marker over skin is still used in many kinematic studies. In our study, the marking over the joints had been carefully checked by flexing the knee and the ankle, and these markings consistently revealed a general pattern of knee motion during walking, as demonstrated in the plot of angular displacement against time (Figure 1D). Unsatisfactory marking could be identified as significant deviations from normal knee motion in the kinematic plot.

The third limitation is about the difficulties to include kinematic data at the ankle joint for a better data interpretation. It is possible that knee pain associated with patellar tendon injury also would affect the ankle motion as compensatory motion, but it is too complicated to include ankle data in the present study. As it is a general practice to report many inter-related gait parameters in order to have a comprehensive description of the animal gait, multiple comparisons are unavoidable which may lead to increased chance of Type I error. Analysis of ankle data would further increase number of comparisons on the same set of data. Previous studies on animal gait (Coulthard, 2002; Coulthard, 2003; Simjee 2004) also faced the same problem of multiple comparisons,

but adjustments were made only to multiple 2-group comparisons. In our study, we analyzed the data with MANOVA and ANOVA with repeated measures but post hoc multiple group comparisons were not performed.

Finally, the loading force on the injured limb was not measured in this study, which may provide new information about the avoidance of mechanical loading on the injured limb.

In conclusion, motion analysis is applicable to measurement of painful responses associated with degenerative tendon injuries in rat models, but the gait velocity of the rats should be carefully controlled to optimize the measurement.

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Figure legends**Table 1****Statistical results for the motion analyses**

Multivariate analysis of variances (MANOVA) was used to analyze data in the end-point study, while analysis of variances (ANOVA) with repeated measure was used in the follow-up study and the analgesic study. * marks statistical significance at $\alpha = 0.05$. N.D. represents “Not Determined”.

Figure 1**Experimental set-up of motion analysis on the walking gait of rats**

The rats were encouraged to walk through a transparent walkway (A) during motion analysis. The video camera was positioned to capture the rat motion in the saggital plane as shown in the diagram (B). Both hind limbs of the rats were marked on the joints of hip, knee, ankle and toe and checked for consistency of marks and joint movement by moving the limbs, as shown by a consistent kinematic plot for knee angle during a stride (C). With the help of the reference point and the marks on the joints, the gait parameters and knee angles were measured by a motion analysis system (D).

Figure 2**Histology of collagenase-induced injury in patellar tendon samples**

Intratendinous injection of collagenase induced significant tissue damages and increased cellularity including inflammatory cells and healing tendon cells were observed in the injured tendons at 2 weeks post injection (A). The cellularity slightly decreased in week 4 (B) and week 8 (C) with limited healing responses. Matrix disturbance was developed in the absence of inflammatory cells at week 12 (D). At 16 weeks after injection, tendon degeneration was observed in the collagenase groups with increased cellularity, increased vascularity and significant matrix disturbance with calcification (E), while normal tendon histology was found in the group with saline injection (F). (optical magnification: 100X)

Figure 3**Results of motion analysis of the end-point study on collagenase-induced patellar tendon injury**

The significant effects of collagenase-induced patellar tendon injury were shown on various gait parameters including gait velocity (A), stride length (B), swing duration (C), double stance duration (D), and average knee angular velocity at mid-swing

(KVMW) (E) and at mid-stance (KVMS) (F). Statistical analysis was performed by multivariate analysis of variances (MANOVA) and the detailed results were shown in Table 1.

Figure 4

Results of motion analysis of the follow-up study on collagenase-induced patellar tendon injury

The results of the follow-up study were shown on the same set of gait parameters in the endpoint study. These gait parameters include gait velocity (A), stride length (B), swing duration (C), double stance duration (D), and average knee angular velocity at mid-swing (KVMW) (E) and at mid-stance (KVMS) (F). As repeated measurements for individual rats were made, these gait parameters were expressed as differences relative to pre-injection level. Statistical analysis was performed by analysis of variances (ANOVA) with repeated measure and the detailed results were shown in Table 1.

Figure 5

Effect of buprenorphine injection on double stance duration

At 30 min after injection, buprenorphine induced a significant increase in double

stance duration ended with take-off of contralateral control side (DS-C) in the collagenase-treated rats but not the saline treated rats. At 28 hours after buprenorphine injection, the DS-C returned to the original level in the collagenase-treated rats, and no significant change was detected in the saline-treated rats.

Figure 6

Comparison of gait velocities in the collagenase-treated rats in the end-point and the follow-up study

Except in the pre-injury groups, the gait velocities of the rats in follow-up study were significantly higher than those in the end points study (Student's t test, $p < 0.05$). * marks statistical significance at $\alpha = 0.05$.

Figure 7

Standardization of double stance duration with gait velocity

A scatter plot revealed an inverse relationship between double stance duration (DS) and gait velocity (A). A regression analysis between DS and gait velocity returned the regressed values of DS from observed gait velocities (B). Standardization was achieved by calculating the residual DS as the difference between regressed DS and

observed DS, which represented the deviation from averaged DS at specified gait velocity. The influence of gait velocity on DS was thus removed as shown in a plot between residual DS and gait velocity (C). A lower velocity-standardized DS (residual DS) was noticed in the follow-up study, while a higher residual DS was found in the endpoint study (D).

Table 1: Statistical results of gait analyses

	End-point study				Follow-up study				Analgesic study	
Gait parameter s	MANOVA				Repeated measure ANOVA					
	Between-subjects factor				Within-subject factor			Between-subjects factor	Within-subject factor	
	Time	Treatment	Treatment	Treatment	Time	Treatment	Treatment	Treatment	Analgesic	Analgesic
		^Time	^Side			^Time	^Side			^Treatment
Gait velocity	0.001*	N.D.	0.235	0.134	<0.001*	0.085	0.556	0.519	0.004*	0.224
Stride length	<0.001*	N.D.	0.679	<0.001*	<0.001*	0.543	0.896	0.097	0.344	0.453
Stride duration	0.672	N.D.	0.228	0.963	<0.001*	0.162	0.388	0.076	<0.001*	0.214
Stance duration	0.454	N.D.	0.348	0.274	<0.001*	0.087	0.874	0.136	<0.001*	0.210
Swing duration	<0.001*	N.D.	0.243	<0.001*	<0.001*	0.226	0.09	0.023*	0.001*	0.558
Double stance duration (DS)	0.008*	N.D.	0.614	0.047*	<0.001*	0.721	0.068	0.539	0.003*	0.029*
Knee angular velocity at midswing (KVMW)	<0.001*	N.D.	0.019*	0.008*	<0.001*	0.948	0.148	0.399	0.019*	0.858
Knee angular velocity at midstance (KVMS)	0.001*	N.D.	0.048*	0.897	0.003*	0.003*	0.568	0.850	0.001*	0.345

Figure 5

Figure 6

Figure 7