Journal of Visualized Experiments
Comparative analysis of lower limb kinematics between the initial and terminal phase of 5km treadmill running
--Manuscript Draft--

Article Type:	Methods Article - JoVE Produced Video
Manuscript Number:	JoVE61192R3
Full Title:	Comparative analysis of lower limb kinematics between the initial and terminal phase of 5km treadmill running
Section/Category:	JoVE Behavior
Keywords:	Long distance running, treadmill running, lower-limb kinematics, injuries
Corresponding Author:	F Dr. Ren Ningbo University Ningbo, Zhejiang CHINA
Corresponding Author's Institution:	Ningbo University
Corresponding Author E-Mail:	renfengnb@yeah.net
Order of Authors:	Wenjing Quan
	Meizi Wang
	Gongju liu
	Gusztáv Fekete
	Julien S Baker
	F Dr. Ren
	Yaodong Gu
Additional Information:	
Question	**Response**
Please indicate whether this article will be Standard Access or Open Access.	Standard Access (US$2,400)
Please indicate the **city, state/province, and country** where this article will be **filmed**. Please do not use abbreviations.	Ningbo, Zhejiang, China

TITLE:

Comparative Analysis of Lower Limb Kinematics between the Initial and Terminal Phase of 5 km Treadmill Running

AUTHORS AND AFFILIATIONS:

Wenjing Quan[1], Meizi Wang[2], Gongju Liu[2], Gusztáv Fekete[3], Julien S Baker[4], Feng Ren[1], Yaodong Gu[1]

1. Faculty of Sports Science, Ningbo University
2. Faculty of Engineering, University of Pannonia Veszeprem, Hungary
3. Savaria Institute of Technology, Eötvös Loránd University, Hungary
4. Department of Sport, and Physical Education,Hong Kong Baptist University, Hong Kong

Wenjing Quan: 516042610@qq.com
Meizi Wang: shensiqinnbu@sohu.com
Gongju Liu: andywutong@foxmail.com
Gusztáv Fekete: zhouhuiyunb@qq.com
Julien S Baker: jsbaker@hkbu.edu.hk
Feng Ren: renfengnb@yeah.net
Yaodong Gu: guyaodong@hotmail.com

Corresponding author:
Feng Ren
renfengngnb@yeah.net

KEYWORDS:

Long distance running, treadmill running, lower-limb kinematics, injuries

SUMMARY:

This study investigated the biomechanical characteristics of the lower extremity kinematic variables between the initial and terminal phase of 5 km treadmill running. The lower-limb kinematic data of 10 runners were collected using a three-dimensional motion capture system on a treadmill at the initial phase (0.5 km) and the terminal phase (5 km), respectively.

ABSTRACT:

Running is beneficial for physical health, but it is also accompanied by many injuries. However, the main factors leading to running injury remain unexplained. This study investigated the effects of long running distance on lower-limb kinematic variables and the lower limb kinematic difference of between the initial (IR) and terminal phase (TR) of 5 km running was compared. Ten amateur runners ran on a treadmill at the speed of 10 km/h. Dynamic kinematic data was collected at the phase of IR (0.5 km)and TR (5 km), respectively. The peak angle, peak angular velocities, and range of motion were recorded in this experiment. The main results demonstrated the following: ankle eversion and knee abduction were increased at TR; ROMs of ankle and knee were increased in the frontal plane at TR than IR; a larger peak angular velocity of ankle

dorsiflexion and hip interrotation were found in TR compared to IR. These changes during the long distance running may provide some specific details for exploring potential reasons of running injuries.

INTRODUCTION:

Running is the most popular sport around the world. There are a large number of individuals that run and this number increases substantially every year[1]. It has been suggested that participation in regular exercise including running can promote health, reduce the risk of cardiovascular diseases and thus improve life expectancy[2-4]. Despite the significant health benefits of running, the incidence of running injuries has increased from 25% to 83% over the years[5,6]. There are some risks associated with running, especially to the lower extremities, which are mainly focused on musculoskeletal injuries[7]. The majority of common running-related injuries are related to patellofemoral pain, ankle sprain, tibial stress fractures, and plantar fasciitis[8]. Running injuries can be induced by many factors, such as incorrect foot striking patterns, incorrect shoe selection, and other individual biomechanical factors[9]. For instance, running with a heel-strike pattern can lead to greater pronation, and is accompanied by larger plantar pressure on the medial side of foot, which may lead a higher risk for Achilles tendinopathy and patellofemoral pain[10]. In addition, running with a greater knee internal rotation has been previously reported to be associated with the iliotibial band syndrome for female runners[11], especially when running long distances.

Parameters of kinetics, kinematics, and time-space components can provide a precise analysis of gait biomechanics, and is currently considered to be an important parameter for clinical gait analysis[12]. Lower vertical ground reaction forces and larger impact accelerations are recoded after long-distance running[13,14]. Higher hip excursion and smaller knee flexions have also been found along with fatigued muscles[15], and the increased stride frequency can result in reduced stride lengths[13,16].

However, changes in biomechanical features of lower limbs at the phase of initial and terminal running have not been fully analyzed, since most studies measured biomechanical variation after running. Additionally, only a few studies use standard laboratory techniques to assess the effects of long-distance running on gait biomechanical changes in amateur runners. The main factors leading to running injuries are still unclear. Therefore, in order to reveal the underlying reasons for lower extremity injuries caused by long distance running, this study aims to compare the biomechanical changes of the lower extremity between the IR and TR phases in treadmill 5 km running in amateur runners.

PROTOCOL:

Written informed consent was obtained from subjects and the testing procedures were approved by the university ethics committee. All participants were informed of the requirements and process of the trial.

1. Laboratory preparation

1.1. During calibration, switch off the lights and remove other possibly reflective objects. Ensure that eight cameras are appropriately placed and have a clear view without reflection.

1.2. Open the Vicon Nexus 1.8.5 program, and then initialize the cameras. Select **System | Local System | MX Cameras** in the **Resources** pane and the cameras will engage.

NOTE: In the **Properties** pane, the parameters need to be adjusted. The range of values of the strobe intensity are adjusted to 0.95-1, and the value range of the threshold is set to 0.2-0.4. Set the grayscale mode to Auto. The Minimum circularity ratio is set to 0.5, and the Gain to times 1 (x1), the Max blob height to 50, and select **Enable LEDs**.

1.3. Place the T-frame in the center of the capture area, select all the cameras in the system, and use 2D mode. Confirm that the T-frame is in the camera view without any interference points. Select the first item **System Preparation** in the toolbar. In the T-Frame drop-down list, select the **5 Marker Wand & T-Frame** calibration object.

1.4. In the **System Preparation Tools** pane, click the **Start** button under the **Mask Cameras** section. Then click the **Start** button under **Calibrate MX** camera section.

NOTE: When the calibration process is completed, the progress bar is restored to **0%**.

1.5. Place the T-frame in the center of the camera to establish the origin of the coordinates.

1.6. In the **Tool** pane, click the **Start** button under the **Set Volume Origin** section.

1.7. Put the treadmill in the center of the test zone. The eight cameras are displayed around the treadmill (**Figure 1**).

1.8. Attach a total of 22 reflective markers (diameter: 14 mm) with double-sided tape on the subjects in advance.

[Figure 1 insert here]

2. Subject preparation

2.1. Before the test, interview subjects in the laboratory and give a simple explanation of the experimental procedures. Then, have the participants complete a questionnaire. Summarize the results of these questionnaires.

2.1.1. Use the following questions:
(i) How often do you run in a week?
(ii) How many years have you been running for?
(iii) Have you suffered any lower extremity injuries or received lower extremity surgeries in the last six months?

(iv) How many kilometers do you run per week?

2.2. Use the following inclusion criteria: all participants were right leg dominant and without any lower extremity injuries in the previous six months before the study. All participants ran at least 15 km per week.

NOTE: Ten healthy recreational female runners (ages: 23.4±1.3 years; height: 160.7±3.8 cm; mass: 50.3±2.3 kg; running years: 3.2±1.2 years) were selected.

2.2.1. Obtain written informed consent from participants who meet the inclusion criteria.

2.3. Require that participants wear uniform tights and pants.

2.4. Record the subjects' height (mm), weight (kg), lower limb length (mm), knee width (mm) and ankle width (mm) for the statistics model.

2.5. Place 16 reflective markers on subjects at the following locations: anterior-superior iliac spine, posterior-superior iliac spine, lateral mid-thigh, lateral knee, lateral mid-shank, lateral malleolus, second metatarsal head, and calcaneus. Place the markers on the second metatarsal head and calcaneus on the corresponding anatomical points of the socks and shoes.

2.6. Instruct the participants to wear uniform sport running shoes. Have the participants warm up with light running and stretching for 5 min.

3. Static calibration

3.1. Click the **Data Management** button on the toolbar, select **Data Management**. Click the **New Database tab** on the toolbar, select the **location**, describe the trial name and the **Clinical Template**, and click the **create** button.

3.2. In the **Open Database** window, select the name of the database that was created. In the open interface, click the green **New Patient Classification** button, the yellow **New Patient** button, and the grey **New session** button to create the experimental information including subject type, subject name, and different action status.

3.2.1. Go back to the **Nexus** pane, in the left toolbar, click **Subjects** to create a **New Subject** data set, and choose the trial model. In the **Properties pane**, fill in all the anthropometric measurements: height (mm), weight (kg), lower limb length (mm), knee width (mm) and ankle width (mm).

3.3. Click the **Go live** button, select **Spilt horizontally** and choose the **graph** to check the **Trajectory** count.

NOTE: Ensure that all the markers are visible in the 3D Perspective view. This indicates that all markers can be captured for analysis.

3.4. Prepare to capture the static model. On the **Capture Tools** pane, click the **Start** button in the **Subject Capture** section.

NOTE: During the whole data collection process, the subjects should remain stationary in the capture area to collect 140-200 frames of images. Then click the **Stop** button.

3.5. In the perspective pane, view the capture marks. Click the **Pipeline** button in the **Tools** pane, select **Running The Reconstruct Pipeline** to create a 3D image of the captured markers. Then, manually label the static model. When the identification is completed, save and press **ESC** to exit.

3.6. In the toolbar, choose the subject preparation and subject calibration. Select the **Static plug-in gait** option from the drop-down list. In the **Static Settings** pane, choose the **left foot** and **right foot**, click the **start** button and save the static model.

4. Dynamic trials

4.1. When finished collecting the static data, select **Capture** in the right toolbar. Choose **Trial Type** and **Session** from top to bottom, and fill in the trial description.

4.2. Ask participants to run on the treadmill in the following manner.

4.2.1. Warm up by walking at 8 km/h for 1 min.

4.2.2. Ask the participant to run on the treadmill at speed of 10 km/h. After an adaption period of 4 min at this velocity, record the running data for 40 s. Collect the kinematic data at a distance of 0.5 km and 5 km, respectively.

4.2.3. Ask the subjects to wear a heart rate monitor to record the heart rate and monitor the subjects' fatigue status while running.

4.3. In the **Tool Capture** pane, click the **Start** button. After collecting the dynamic trials, click **Stop** to end the collection.

5. **Post-processing**

5.1. Open the **Data Management** window, double-click the trial name. Click the run **Reconstruct Pipeline** and **Labels** button in the toolbar to reconstruct the mark point position.

5.2. In the **Perspective** window, move the blue triangles on the time bar to set the required range of time.

5.3. Shift the view of the timeline so that it shows only the selected range, click on the time bar, and click **Zoom to Region-of-Interest**.

5.4. At this point, select the **Label** button to identify and check the label points, with the same steps as the static identification process. If necessary, supplement some incomplete identification points. Delete the unlabeled marks.

5.5. In the **Subject Calibration** pane, select the **Dynamic Plug-in Gait**. Click the **Start** button to run the data. **Export** motorial trials in c3d format for post-processing.

6. Data analysis

6.1. Process the kinematics data. Apply a fourth-order low pass Butterworth filter with a cut off frequency of 10 Hz (kinematic) before exporting the joint angle data. Export the data of the joint angle.

6.2. Calculate the range of motion (ROM), peak angle and peak angular velocity of the lower limb joints (hip, knee, and ankle) in three planes (sagittal, frontal, and transverse) during one stance phase.

7. Statistical analysis

7.1. Use paired-sample T-test to compare lower limb kinematics (peak angles, ROM, peak angular velocity) between the initial (IR) and terminal phase (TR) of 5 km running.

7.2. Calculate mean values and standard deviations of the five valid trials from each subject for different running distances. Set the significance level at $p < 0.05$.

REPRESENTATIVE RESULTS:

The results showed that no differences in the peak angle of the ankle and hip were observed in the sagittal plane. Compared with IR, the peak angles of the ankle and the knee in the frontal plane were significantly increased at TR. A larger internal hip angle was found in TR as contrasted to IR. However, TR presented a smaller peak angle in hip abduction, ankle interrotation, and knee interrotation than IR (**Figure 2**).

In the sagittal plane, the ROMs of the ankle and the knee were significantly increased in IR when compared to TR. In the frontal plane, hip ROM was significantly decreased in TR compared to IR, whereas the ROMs of the ankle and the knee was increased in TR than IR. In the transverse plane, knee ROM was found to be significantly lower in the TR compared to the IR running, but no differences were found in the ROMs of the ankle and the hip (**Figure 3**).

Changes in peak angular velocity between IR and TR were also assessed. In the sagittal plane, there was no significant difference in the peak angular velocity of the hip and knee joints throughout the experiment. A larger peak angular velocity of ankle dorsiflexion was noted in TR.

In the stance phase, the smaller peak angular velocity of hip abduction and knee abduction velocity were revealed at TR. The peak angular velocity of hip interrotation increased at TR. There was no significant difference in ankle eversion, knee and ankle interrotation velocity throughout the running.

FIGURE LEGENDS:

Figure 1: Test site layout. Cameras capture lower-limb motion while the subjects run on the treadmill.

Figure 2. Peak angle for ankle, knee, and hip in sagittal (A), frontal(B), and transverse planes(C) during one gait cycle (IR N=10; TR: N=10). Significant differences between the IR and TR are denoted with an asterisk (*).

Figure 3. **Changes in Joint ROM during the gait cycle IR- vs.TR (mean values).** * Statistical significance.

Table 1. Comparisons of knee, hip and ankle peak angular velocity before and after running. Significant differences between the IR and TR are denoted with an asterisk (*).

DISCUSSION:

This study compared the effect of long distance running on the biomechanical characteristics of the lower extremity in amateur runners. It was found that the peak angle of ankle eversion and knee abduction increased after 5 km running, which is consistent with a previous study[17]. Studies have shown that excessive ankle eversion and eversion velocity are important factors that increase the risk of ankle injuries[18,19]. It is not surprising that the knee ROM increased at TR of 5 km running because studies have shown that knee kinematics are affected by long-distance running[15,17].

Similarly, the knee rotation angle range is reduced in the transverse plane. One of the reasons can be explained because the runner did not experience fatigue at TR[20]. Compared with IR, the hip interrotation peak angle was larger in TR. Previous studies indicated that an increased angle of hip interrotation can lead to stress fractures of the tibia[21]. It was also reported that hip interrotation angular velocity was associated with muscle injury[22,23]. In this study, the angular velocity of the hip interrotation was greater at TR. Hip instability is considered as an important mechanism for lower limb injury[24].

The results presented here are dependent on many procedures during the experiment. Firstly, lights must be switched off and other possible reflective objects must be removed. It is important to ensure that capture volume is entirely free from objects that may cause unwanted reflections. Secondly, it is vital to select the desired parameters in the **Tools Capture** pane for capturing a trial. Thirdly, before starting the test, the treadmill must be placed in the center of the test zone. Also, there are other potential limitations in this study. Only 10 amateur runners were recruited for this experiment. A further limitation of this study could relate to the running distance. Future studies should focus on the effect of different distances with different running shoes on muscle activities and joint moments.

The results of this study indicate that different levels of injury risk may exist for IR and TR of 5 km running. Runners should arrange running training plans scientifically, strengthen balance abilities prior to and during training, and choose running shoes with cushioning functions to reduce the injury risks of ankle and knee joint.

ACKNOWLEDGMENTS:
This study sponsored by the National Natural Science Foundation of China (81772423), K. C. Wong Magna Fund in Ningbo University, and the National Key R&D Program of China (2018YFF0300903).

DISCLOSURES:
No potential conflict of interest was reported by the authors.

REFERENCES:
1 Lee, D. C. et al. Running as a Key Lifestyle Medicine for Longevity. *Progress in Cardiovascular Diseases.* **60** (1), 45-55 (2017).
2 Dugan, S. A., Bhat, K. P. Biomechanics and analysis of running gait. *Physical Medicine & Rehabilitation Clinics of North America.* **16** (3), 603-621 (2005).
3 Hart, L. Disability and mortality among aging runners. *Clinical Journal of Sport Medicine Official Journal of the Canadian Academy of Sport Medicine.* **19** (4), 338 (2009).
4 Schnohr, P., Marott, J. L., Lange, P. & Jensen, G. B. Longevity in male and female joggers: the Copenhagen City Heart Study. *American Journal of Epidemiology.* **177** (7), 683-689 (2013).
5 Bovens, A. M. et al. Occurrence of running injuries in adults following a supervised training program. *International Journal of Sports Medicine.* **10** (S 3), S186-S190 (1989).
6 Blair, S. N., Kohl, H. W., Goodyear, N. N. Rates and Risks for Running and Exercise Injuries: Studies in Three Populations. *Research Quarterly for Exercise & Sport.* **58** (3), 221-228 (2016).
7 Lun, V., ., Meeuwisse, W. H., Stergiou, P., Stefanyshyn, D. Relation between running injury and static lower limb alignment in recreational runners. *British Journal of Sports Medicine.* **38** (5), 576-580 (2004).
8 Fukuchi, R. K., Fukuchi, C. A., Duarte, M. A public dataset of running biomechanics and the effects of running speed on lower extremity kinematics and kinetics. *PeerJ.* **5** (5), e3298- (2017).
9 Iii, E. B. L., Sackiriyas, K. S. B., Swen, R. W. A comparison of the spatiotemporal parameters, kinematics, and biomechanics between shod, unshod, and minimally supported running as compared to walking. *Physical Therapy in Sport Official Journal of the Association of Chartered Physiotherapists in Sports Medicine.* **12** (4), 151-163 (2011).
10 Dowling, G. J. et al. Dynamic foot function as a risk factor for lower limb overuse injury: a systematic review. *Journal of Foot & Ankle Research.* **7** (1), 53.
11 Aderem, J., Louw, Q. A. Biomechanical risk factors associated with iliotibial band syndrome in runners: a systematic review. *BMC Musculoskeletal Disorders.* **16** (1), 356 (2015).
12 Anderson, T. Biomechanics and running economy. *Sports Medicine.* **22** (2), 76-89 (1996).
13 Degache, F. et al. Changes in running mechanics and spring-mass behaviour induced by a 5-hour hilly running bout. *Journal of Sports Sciences.* **31** (3), 299-304.

14 Millet, G. Y. et al. Running from Paris to Beijing: biomechanical and physiological consequences. **107** (6), 731-738.

15 Mizrahi, J., Verbitsky, O., Isakov, E., Daily, D. Effect of fatigue on leg kinematics and impact acceleration in long distance running. *Human Movement Science.* **19** (2), 139-151.

16 Bisiaux, M., Moretto, P. The effects of fatigue on plantar pressure distribution in walking. *Gait & Posture.* **28** (4), 0-698.

17 Dierks, T. A., Davis, I. S., Hamill, J. The effects of running in an exerted state on lower extremity kinematics and joint timing. **43** (15), 2993-2998.

18 Rolf, C. Overuse injuries of the lower extremity in runners. *Scandinavian Journal of Medicine & Science in Sports.* **5** (4), 181-190 (1995).

19 Marti, B., Vader, J. P., Minder, C. E., Abelin, T. On the epidemiology of running injuries: the 1984 Bern Grand-Prix study. *The American Journal of Sports Medicine.* **16** (3), 285-294 (1988).

20 Dierks, T. A., Davis, I. S., Hamill, J. The effects of running in an exerted state on lower extremity kinematics and joint timing. *Journal of Biomechanics.* **43** (15), 2993-2998 (2010).

21 Noehren, B., Davis, I., Hamill, J. ASB Clinical Biomechanics Award Winner 2006: Prospective study of the biomechanical factors associated with iliotibial band syndrome. *Clinical Biomechanics.* **22** (9), 951-956 (2007).

22 Noehren, B., Pohl, M. B., Sanchez, Z., Cunningham, T., Lattermann, C. Proximal and distal kinematics in female runners with patellofemoral pain. *Clinical Biomechanics.* **27** (4), 366-371 (2012).

23 Souza, R. B., Powers, C. M. Differences in hip kinematics, muscle strength, and muscle activation between subjects with and without patellofemoral pain. *Journal of Orthopaedic & Sports Physical Therapy.* **39** (1), 12-19 (2009).

24 Ferber, R., Hreljac, A., Kendall, K. D. Suspected mechanisms in the cause of overuse running injuries: a clinical review. *Sports Health.* **1** (3), 242-246 (2009).

Figure

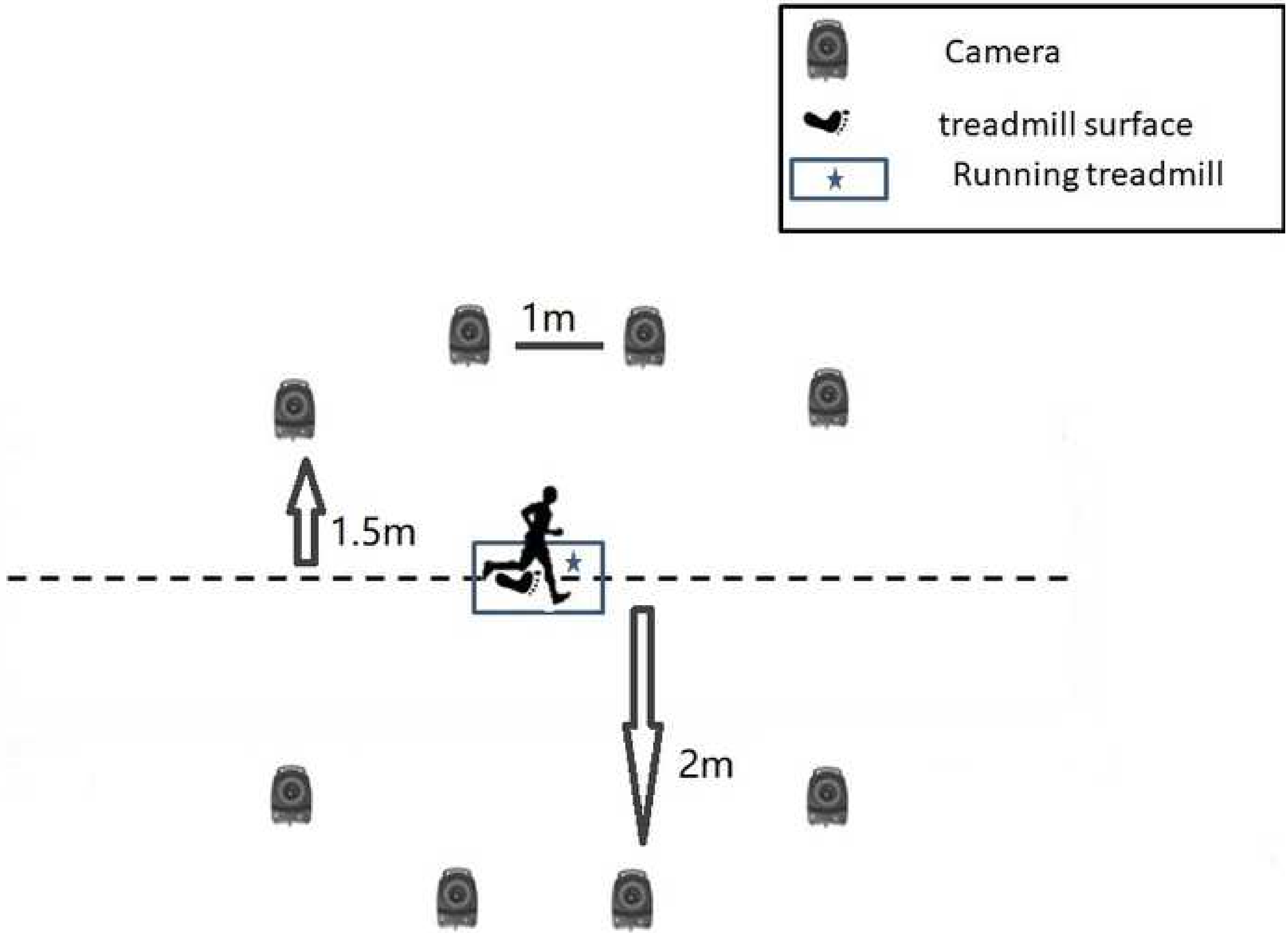

Figure 2

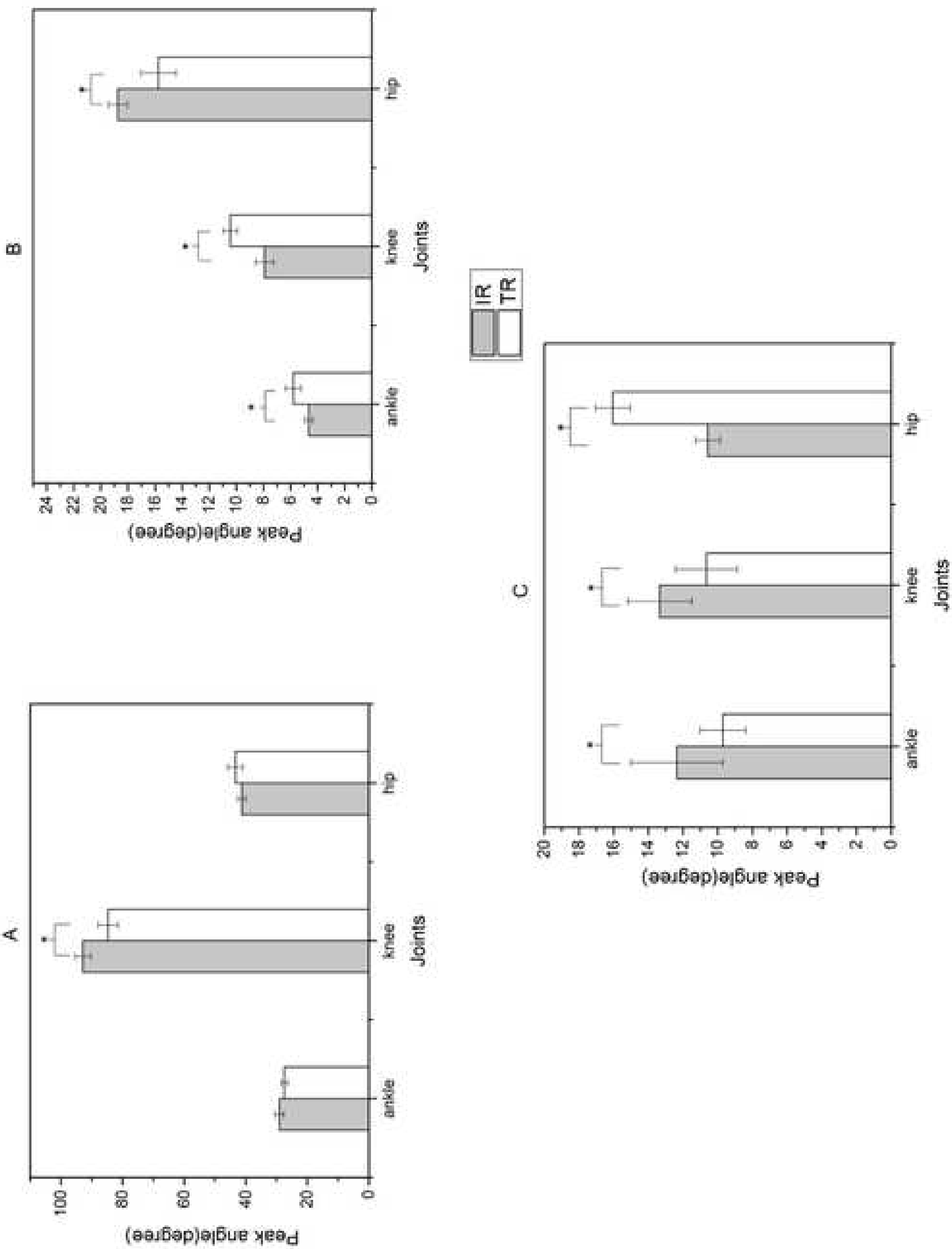

Figure 3 Click here to access/download;Figure;Figure 3.jpg

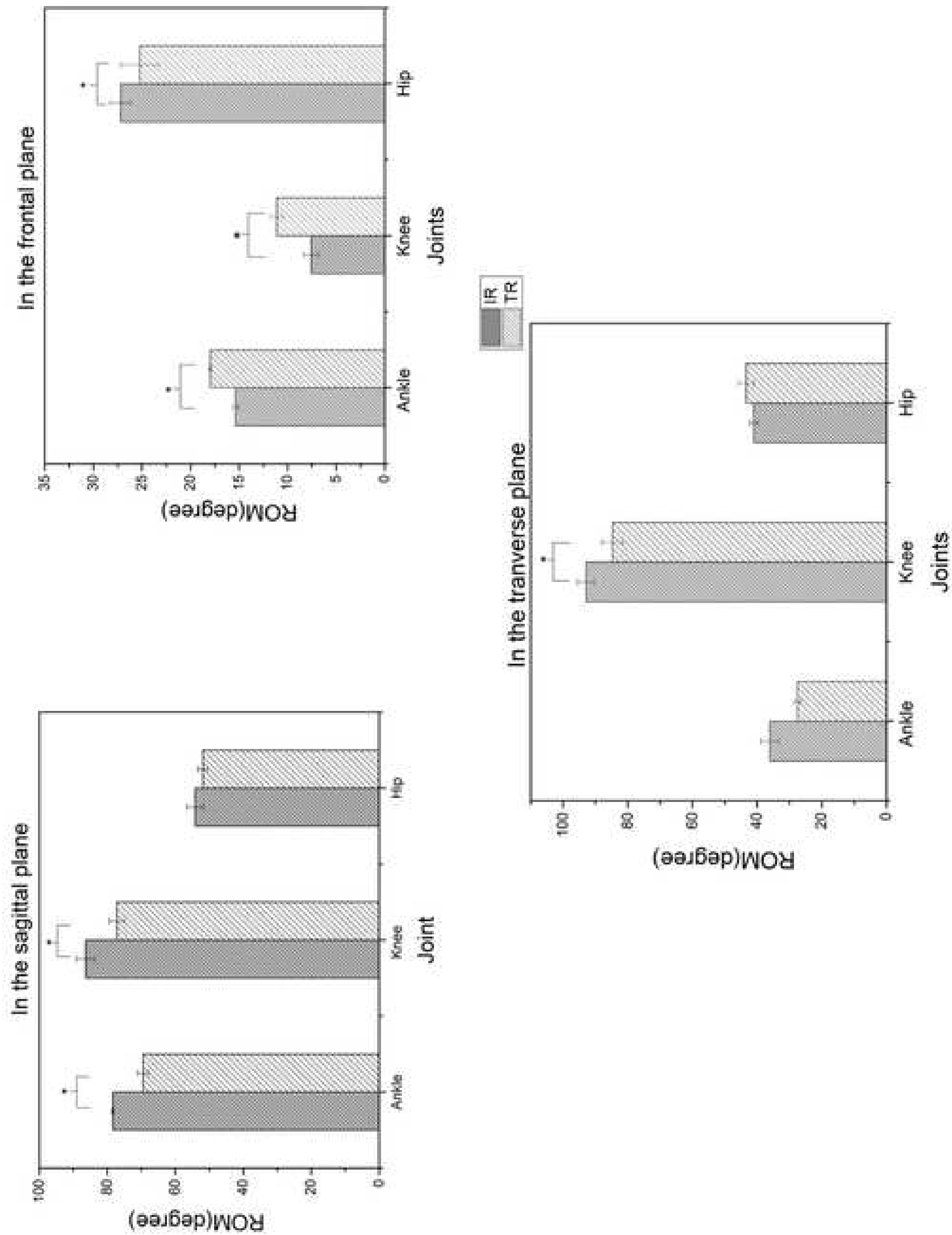

Table 1

Peak angular velocity (deg/s)	IR	TR	p-value
	Mean±SD	Mean±SD	
Hip flexion	182.58±38.38	130.00±47.80	0.075
Knee flexion	221.88±22.90	266.00±26.36	0.07
Ankle dorsiflexion	326.11±20.49	344.85±43.76	**0.046***
Hip abduction	256.06±47.31	245.54±38.17	**0.000***
Knee abduction	128.65±17.04	96.14±15.50	**0.041***
Ankle Eversion	235.43±41.68	232.95±11.60	0.915
Hip int. rotation	195.92±7.85	302.32±29.14	**0.012***
Knee int. rotation	353.83±66.05	355.26±39.74	0.912
Ankle int. rotation	135.01±42.77	146.85±23.60	0.664

Table of Materials

Click here to access/download;Table of Materials;JoVE_Materials.xlsx

Name of Material/ Equipment	Company	Comments/Description
14 mm Diameter Passive Retro-reflective Marker	Oxford Metrics Ltd., Oxford, UK	n=22
Double Adhesive Tape	Oxford Metrics	For fixing markers to skin
Heart Rate	Garmin, HRM3-SS, China	Detection of fatigue state
Motion Tracking Cameras	Ltd., Oxford, UK	n= 8
T-Frame	Ltd., Oxford, UK	-
Treadmill	Smart Run,China	the process.
Valid Dongle	Ltd., Oxford, UK	Vicon Nexus 1.4.116
Vicon Datastation ADC	Ltd., Oxford, UK	-

Dear Editor,

We would like to sincerely thank the reviewers for their helpful recommendations. We have seriously considered all the comments and carefully revised the manuscript accordingly. Revisions are highlighted in the manuscript using a red font to indicate where changes have taken place. We feel that the quality of the manuscript has been significantly improved with these modifications and improvements based on the reviewers' suggestions and comments. We hope our revision will lead to an acceptance of our manuscript for publication in the Journal of Visualized Experiments.

Several Editorial concerns have not been adequately addressed. In addition to revising the manuscript, please attach a rebuttal document responding these concerns:

We have a strict limit of 2.75 pages of protocol to be filmed, due to filming and video length concerns. Please highlight (e.g., using the 'text highlight' tool in Word) 2.75 pages or less of the protocol that will be filmed.

Response: Thank you for your suggestion. We have revised the manuscript, especially the protocol issues that you were concerned about. We have rewritten the protocol for 2.75 pages.

1. 'Pre'-5k is still ambiguous-is this the recording at 0.5 km in step 4.2?

Response: Thank you for your comments. The distance of 0.5km and 5km we chose as the initial and terminal phase of 5K running. When the subjects ran to 0.5 kilometers at the initial phase of running, they have adapted to the experimental environment, which is relatively stable in collecting data. We also collected the data at the point of when the subjects ran to 5 kilometers, as the end phase of long-running distance compares with the initial phase (0.5k) that conforms with our experimental purpose.

Reviewer: 1

Comments to the Author

General comments:

P3, L62: Avoid abbreviations. Write Hasn't in full - Has not been....

Response: Thank you for your suggestion. We have rewritten the text. The words are now revised as Has not been.

P4, L107: Delete 'Detailing position was shown on Fig.1.' Replace with 'See Fig.1.'

Response: Thank you for your comments. We have deleted "Detailing position was shown on Fig.1". The corresponding words are now revised as See Fig.1.

P4, L118. ADD 'for' ...you been running for?

Response: Thank you for raising the issue. The corresponding statement are now revised "How many years have you been running for?".

P4, L131: Spelling error - Replace 'makers' with 'markers'

Response: Thank you for your suggestion. We have rewritten the words. The corresponding word are now markers.

P5, L133: 22 markers seem a lot to be placed on one limb. Do you mean left and right limbs.

Response: Thank you for your suggestion. We mean right and left limb. We have checked the number in the manuscript. Then revised statement as “a total of 16 marks on right and left limb”.

P5: Check lines 134 - 139 to make sure that all markers add up to 22. Preferably you may be best producing a figure of one of your subjects showing all the markers labeled.

Response: Thank you for your comments. We have shown the figure that show all the marks labelled. The marker locations included: anterior-superior iliac spine, posterior-superior iliac spine, lateral mid-thigh, lateral knee, lateral mid-shank, lateral malleolus, second metatarsal head and calcaneus. The markers on the second metatarsal head and calcaneus were placed on the corresponding anatomical points of the socks and shoes.

P6, L208: Be careful with your tenses. Add 'before the joint angle data was exported' to the end of the sentence. i.e. Processing the kinematics data. A fourth-order low pass Butterworth filter with cut off frequency of 10 Hz (kinematic) was applied before the joint angle data was exported.

Response: Thank you for your suggestion. We have rewritten the text. The corresponding statement are now revised as you suggested. Now the sentence reads “A fourth-order low pass Butterworth filter with cut off frequency of 10 Hz (kinematic) was applied before the joint angle data was exported” .

P7, L227: Do you mean before and after the 5km running distance? If so then update.

Response: Thanks for your suggestion. This study investigated the effects of long running distance on lower-limb kinematic variables, the difference of lower limb kinematics between the initial (IR) and terminal phase (TR) of 5K running was compared. According to your suggestion we have update all the words in the manuscript.

Authors seem to completely neglect my criticisms, be they regarding study's aim, protocol's efficacy, English language quality, useless information, etc. Again, MS does not deserve publication at all.

Response: We have made major changes to the article as suggested and endeavored to revise the whole paper, including highlight the study purpose, improve protocol efficacy, delete repeat and useless information, and enhance the language quality of the article. All changes have been marked with red in the manuscript.

Major Concerns:

(lines 23-24 and 188) "When ran at 0.5km and 5km repeated collection of subjects' kinematics parameters… This procedure was repeated at the distance of 0.5km and 5km" Why were those distances chosen? Please, explain it clearly.

Response: Thanks for this concern, it is an important question in our article. The distance of 0.5km and 5km we chose as the initial and terminal phase of 5K running.

When the subjects ran to 0.5 kilometers at the initial phase of running, they have adapted to the experimental environment, which is relatively stable in collecting data. We also collected the data at the point of when the subjects ran to 5 kilometers, as the end phase of long-running distance compares with the initial phase (0.5k) that conforms with our experimental purpose.