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Lower limb biomechanical analysis of healthy participants
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Dr Elizabeth Heppenheimer
Science Editor
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Dear Dr. Heppenheimer,

We would like to thank the editorial board and also the reviewers for their constructive feedback which we believe will strengthen our paper. In line with our responses to the reviewers, we agree with all of the comments made and I can confirm that we have addressed all points.

We hope that you find our revised article more suitable for publication.

I can be contacted at any time and look forward to hearing from you soon.

Yours sincerely,

S. Bahadori

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TITLE:
Lower Limb Biomechanical Analysis of Healthy Participants

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KEYWORDS:
Biomechanics, Gait, Kinematic, Kinetic, Spatial-temporal, Isometric, Muscle Strength

SUMMARY:
This article introduces a comprehensive experimental methodology on two of the latest technologies available to measure the lower limb biomechanics of individuals.

ABSTRACT:
Biomechanical analysis techniques are useful in the study of human movement. The aim of this study was to introduce a technique for the lower limb biomechanical assessment in healthy participants using commercially available systems. Separate protocols were introduced for the gait analysis and muscle strength testing systems. To ensure maximum accuracy for gait assessment, attention should be given to the marker placements and self-paced treadmill acclimatization time. Similarly, participant positioning, a practice trial, and verbal encouragement are three critical stages in muscle strength testing. The current evidence suggests that the methodology outlined in this article may be effective for the assessment of lower limb biomechanics.

INTRODUCTION:
The discipline of biomechanics primarily involves the study of stress, strain, loads and motion of biological systems - solid and fluid alike. It also involves the modelling of mechanical effects on the structure, size, shape and movement of the body[1]. For many years, developments in this field have improved our understanding of normal and pathologic gait, mechanics of neuromuscular control, and mechanics of growth and form[2].

The main objective of this article is to present a comprehensive methodology on two of the latest technologies available to measure lower limb biomechanics of individuals. The gait analysis system measures and quantifies gait biomechanics by using a self-paced (SP) treadmill in combination with an augmented reality environment, which integrates a SP algorithm to regulate the treadmill's speed, as described by Sloot et al[3]. The muscle strength testing equipment is used as an assessment and a treatment tool for upper extremity rehabilitation[4]. This device can objectively assess a variety of physiological patterns of movement or job simulation tasks in isometric and isotonic modes. It is currently recognized as the gold standard for upper limb strength measurement[5] but the evidence related specifically to the lower limb remains unclear. This paper explains the detailed protocol for completing an assessment of gait and isometric strength for the lower extremity.

Within biomechanical analysis, it is useful to combine assessments of functional performance (such as gait analysis) with specific tests of muscular performance. This is because whilst it may be assumed that increased muscle strength improves functional performance, this may not always be apparent[6]. This understanding is required for the improved future design of rehabilitation protocols and research strategies to assess these approaches.

PROTOCOL:

The method reported was followed in a study that received ethical approval from the Bournemouth University Research Ethics Committee (Reference 15005).

1. Participants

1.1. Recruit healthy adults (aged from 23 to 63 years, mean ± S.D.; 42.0 ± 13.4, body mass 70.4 ± 15.3 kg, height 175.5 ± 9.8 cm; 15 males, 15 females) to participate in the study. Thirty participants were recruited for this study.

1.2. Ensure that there is no self-reported history of dizziness, balance problems or walking difficulties in the participants.

1.3. Ensure that participants did not suffer from any known neuromuscular injury or condition affecting balance or walking.

2. Setup and procedures for gait analysis

2.1. Use a gait analysis system (**Figure 1**) comprised of a dual-belt force plate-instrumented treadmill, a 10-camera motion capture system and a virtual environment that provides optic flow.

2.2. Ensure that the participant is wearing very tight non-reflective clothing such as cycling shorts or leggings.

2.3. Using double sided adhesive tapes attach 25 passive reflective markers and place according to the lower body configuration of the Human Body Model (HBM)[7] as detailed in **Table 1** and **Figure 2**. The information in this document is taken from the HBM Reference Manual[8].

2.4. Use a joint ruler to take measurements of the required knee and ankle widths for the HBM[6].

2.5. Secure participant to a safety harness that is fastened to an overhead frame.

2.6. Start a new session in the database and make sure it is active (highlighted).

2.7. Using the subject tab, create a new participant from the **Labeling Skeleton** button.

2.8. Browse to the 'LowerLimb HBM_N2.vst' file and then enter the name of the participant. The new participant appears in the Subjects pane.

2.9. Go to the Tools pane and open the **Subject Preparation** tab.

2.10. **Zero level** the forceplates via the **Hardware** tab. Make sure that no weight is exerted on the force plates.

2.11. Prepare the participant for the ROM trial by having them ready in the middle of the treadmill.

2.12. To ensure the participant can accustom themselves to the self-paced treadmill, ask them to walk at a comfortable speed for 5 min at the beginning of the session[9,10].

2.13. Following the acclimatization and without any delay time, ask the participant to walk for a minimum of 5 min[10,11].

2.14. Ensure participants are blinded to the timing of the recordings.

2.15. Ensure to start the treadmill and start data recordings by clicking the **Start recording** button[12]. This can be done with integrated software (**Table of Materials**).

2.16. Stop the recording after acquiring the desired amount of data. It is recommended to collect three sets of 25 cycles.

2.17. Open the processing software (**Table of Materials**) and remove the high-frequency noise on data, by selecting a low-pass filter to the marker data such as a second order Butterworth filter with a cut-off frequency of 6 Hz.

2.18. Go to **File**, and then select **Export** to save as a .csv.

2.19. Determine individual strides from vertical force data and use the foot markers to ascertain gait events[13].

2.20. Analyze the gait parameters such as kinematic, kinetic and spatial-temporal data in Matlab R2017a (**Supplementary File**).

3. Setup and procedures for muscle strength test

3.1. Use the muscle strength testing equipment (multimodal dynamometer) (**Figure 3**), to measure participants' muscle strength based on Maximum Voluntary Isometric Contraction (MVIC)[14].

3.2. Attach the tool/pad number 701 to the dynamometer exercise head.

3.3. Test participant's right and left knee isometric muscle strength.

3.4. Test participants in a seated position on a chair with a backrest.

3.5. Using the up/down switch, align the dynamometer axis with the knee joint's anatomical axis of rotation. Place the pad of the tool centrally at the lower part of the shin of the tibia.

3.6. Keep the knee at 90° flexion, the hip in neutral rotation and abduction, and the foot in plantar flexion.

3.7. Place the participant's hands on their abdomen and stabilize the trunk, hips, and mid-thigh on the chair with Velcro straps.

3.8. Run a practice trial for participants to get accustomed to the testing maneuver.

3.9. Instruct the participant to extend their knee (exert pressure upwards on the pad) followed by flex (exert pressure downwards on the pad) to exert a maximum contraction on the command **Go** for 3 s.

3.10. Provide verbal prompts and encouragement ("Push" for upwards and "Pull" for downwards) during the strength testing.

3.11. Ensure that participants are aware they can stop the test immediately if they experience any unusual pain or discomfort.

3.12. Allow participants to rest for 2 min.

3.13. Repeat steps 3.1 – 3.12, three times for the left leg and right leg and record the data in newtons (N).

3.14. Save all the data and export as a report for the analysis.

REPRESENTATIVE RESULTS:

The mean and standard deviation of the spatial-temporal, kinematics, and kinetic gait parameters are given in **Table 2**. MVIC data for all 30 participants are summarized in **Table 3**. A typical set of data for the left and right side of one participant showing graphical representation of gait parameters is provided in **Figure 4** and **Figure 5**, respectively.

The data presented are representative of the results obtained across all participants, and are consistent with textbook reference results obtained for gait and isometric strength testing[15].

FIGURE AND TABLE LEGENDS:

Figure 1: Gait analysis system. The GRAIL system is used to measure gait parameters. This system consists of a split-belt instrumented treadmill, 160° semi-cylindrical projection screen, force sensors, video cameras and optical infrared system.

Figure 2: Diagram of markers used in Human Body Model (HBM). This figure shows the exact placements of all markers in the HBM lower body model. Special attention should be paid to the placement of the markers printed in green (bold in **Table 1**); these are used during initialization to define the biomechanical skeleton. This figure is adapted from the HBM Reference Manual[8].

Figure 3: The muscle strength testing equipment (multimodal dynamometer) used to measure participants lower limb muscle strength. This system is used to measure the participants' muscle strength based on Maximum Voluntary Isometric Contraction (MVIC).

Figure 4: A sample report produced from offline analysis of the gait assessment using the proposed technique. Spatial temporal data and kinematic and kinetic gait cycle for the left side of one participant. Each line represents one gait cycle. The Y-axis represents the joint angles in degrees for the kinematic plots and joint moment in newton meter per kilogram for the kinetic plots. Red lines represent left side gait parameters.

Figure 5: A sample report produced from offline analysis of the gait assessment using the proposed technique. Spatial temporal data and kinematic and kinetic gait cycle for the right side of one participant. Each line represents one gait cycle. The Y-axis represents the joint angles in degrees for the kinematic plots, and joint moment in newton meter per kilogram for the kinetic plots. The Green lines represent right side gait parameters.

Table 1: Markers used in the Human Body Model (HBM). This table shows the exact placements of all markers in the HBM lower body model. Special attention should be paid to the placement of the markers written in bold; these are used during initialization to define the biomechanical skeleton. This table is adapted from the HBM Reference Manual[8].

Table 2: The mean and standard deviation of the spatial-temporal, kinematics, kinetic gait parameters for the 30 participants. Gait parameters are reported for the left and the right side separately.

Table 3: The mean and standard deviation of the Maximum Voluntary Isometric Contraction (MVIC) for knee joint using the muscle strength testing equipment for the 30 participants.

DISCUSSION:

The contribution of this study is to accurately and comprehensively describe within one protocol the techniques for combined gait analysis and muscle strength testing that have not previously been described together.

In order to achieve accurate results for gait analysis, there are two areas that require maximum attention: 1) marker placements and 2) acclimatization time. The accuracy of the measured data is heavily dependent on the accuracy of the model used. The other key factors that affect accuracy include erroneous marker movement due to superficial skin deformation relative to the underlying skeletal structure, and the resolution of the tracking system[16]. **Figure 2** shows the exact placements of all markers in the HBM lower body model. Special attention should be given to the placement of the markers printed in green; these are used during initialization to define the biomechanical skeleton. Participants were asked to walk for at least 5 min to adapt to SP treadmill walking[17,18]. The SP mode was chosen in order to allow participants a more natural stride variability[3]. However, studies have shown that walking speed varies more during SP walking and gait disturbance could occur through acceleration or deceleration of the belt[3]. In line with other studies[13,19], to minimize this effect, we recommend at least five minutes[19] should be allowed for acclimatization.

To measure participants' muscle strength using the muscle test equipment, there are three critical stages: 1) alignment of knee joint with the dynamometer axis, 2) practice trial, and 3) verbal encouragement. Inappropriate alignment between the dynamometer and knee joint axis of rotation can introduce a factor confounding accurate isometric assessment[20]. Throughout the study, all participants were given precise instruction about the system prior to taking part. However, a practice trial and verbal encouragement are two factors that can greatly affect the MVIC[14]. Many of the individuals who underwent the strength test have very limited or no experience in performing strength testing maneuvers. Strength testing has generally been shown to be reliable[21], but it has been shown that strength scores of novice participants are likely to improve on subsequent testing as they become more comfortable and familiar with the test and the system[22]. Verbal encouragement during exercise testing has been shown to enhance maximal force[23], rate of force development[23], muscle activation[24], muscular endurance[25], power[26], maximal oxygen consumption[27], and time to exhaustion[27,28]. Therefore, we highly recommend adopting this step.

Overall, the data presented here are representative of textbook reference results for gait and isometric strength testing obtained on other equipment. Therefore, it is proposed that the methodology outlined in this article may be considered effective in the assessment of gait and muscular strength in healthy individuals. Further studies should evaluate the reliability of these systems before they are used in clinical applications.

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DISCLOSURES:
The authors have nothing to disclose.

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Figure 1

Figure 2

Click here to access/download;Figure;Figure 2.psd

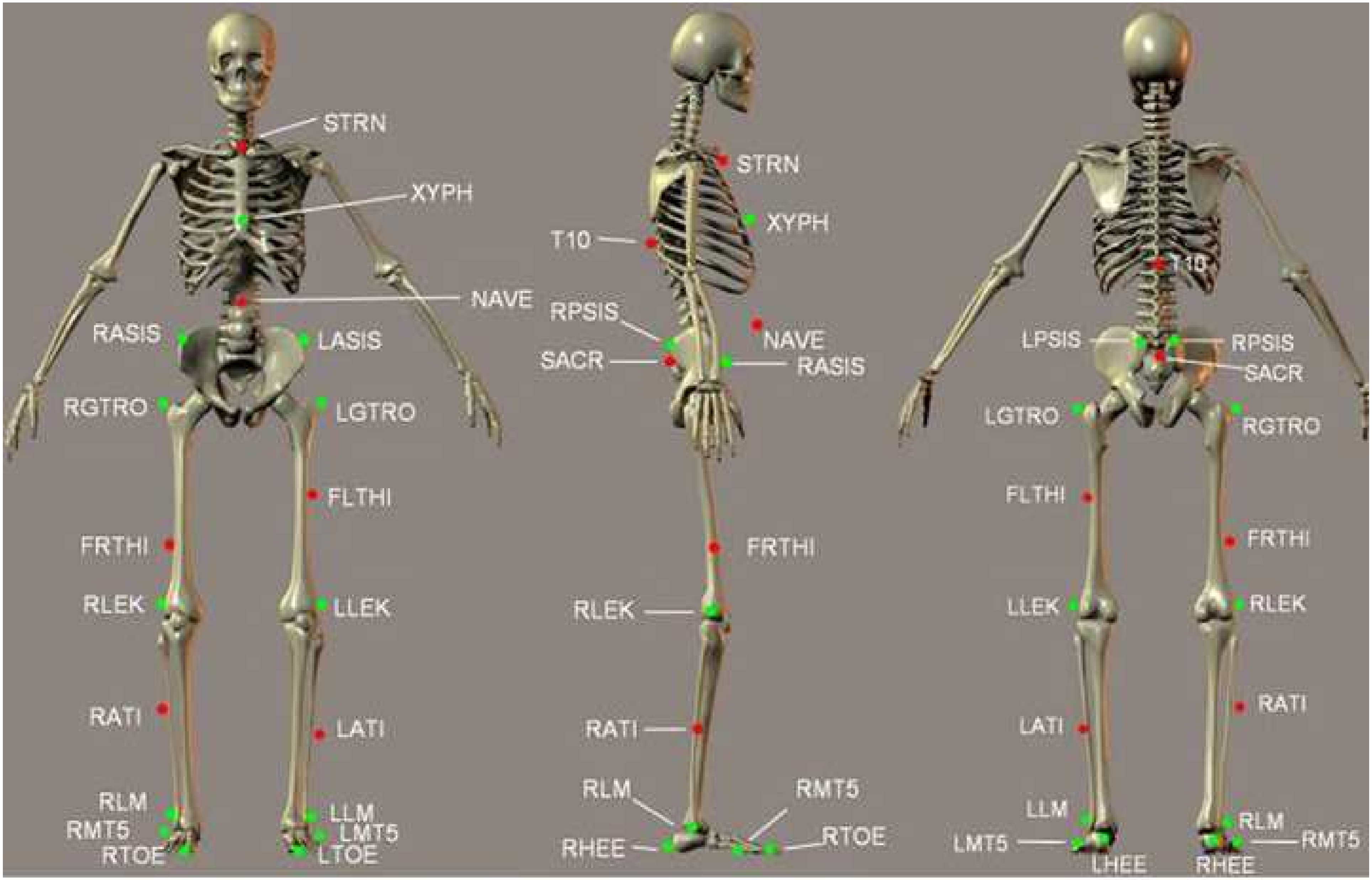


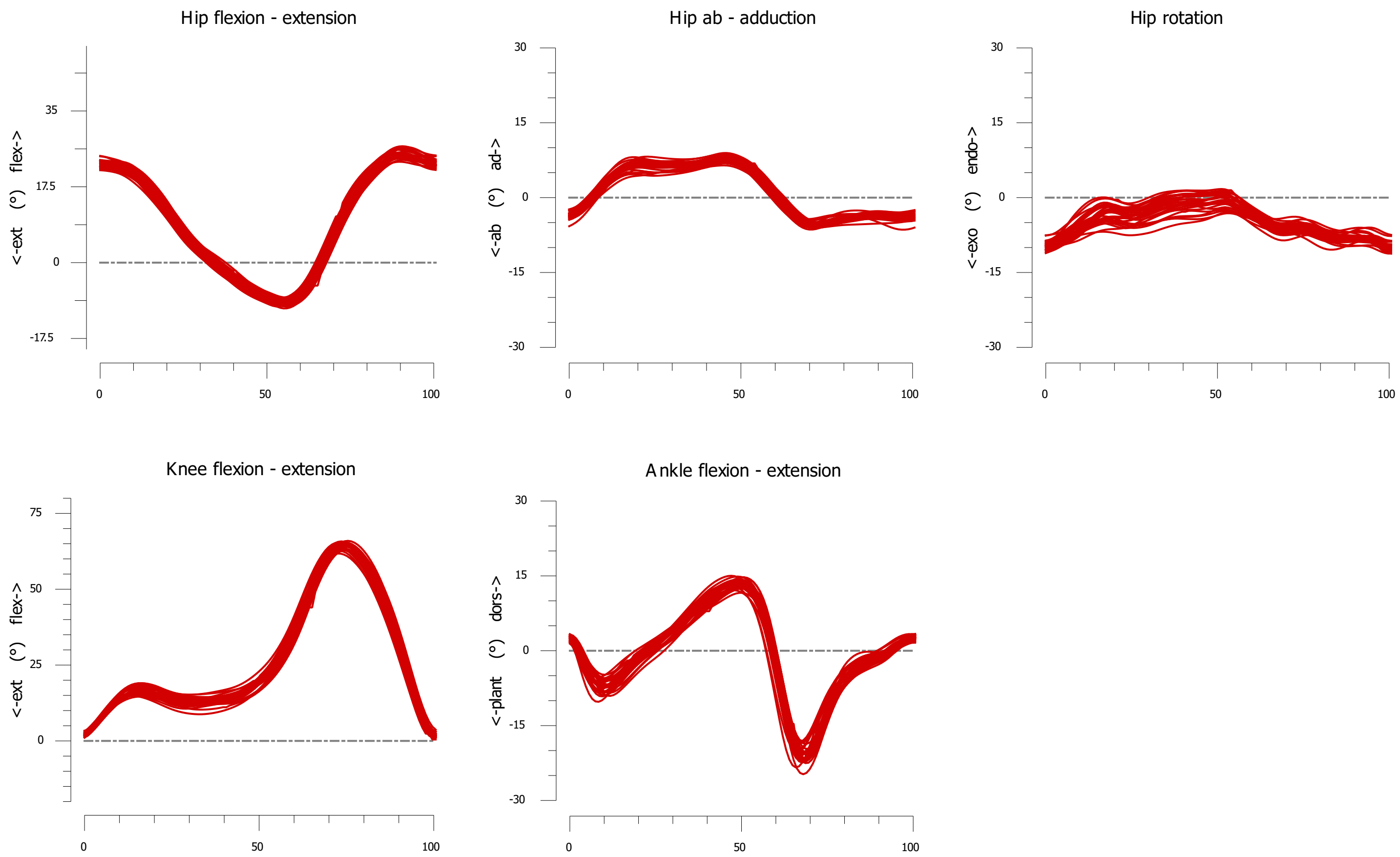
Figure 3

Left leg:

Spatial-temporal parameters

Mean stance time: 0.70 s **Mean swing time:** 0.38 s

Kinematics - Joint angles



Kinetics - Joint moments

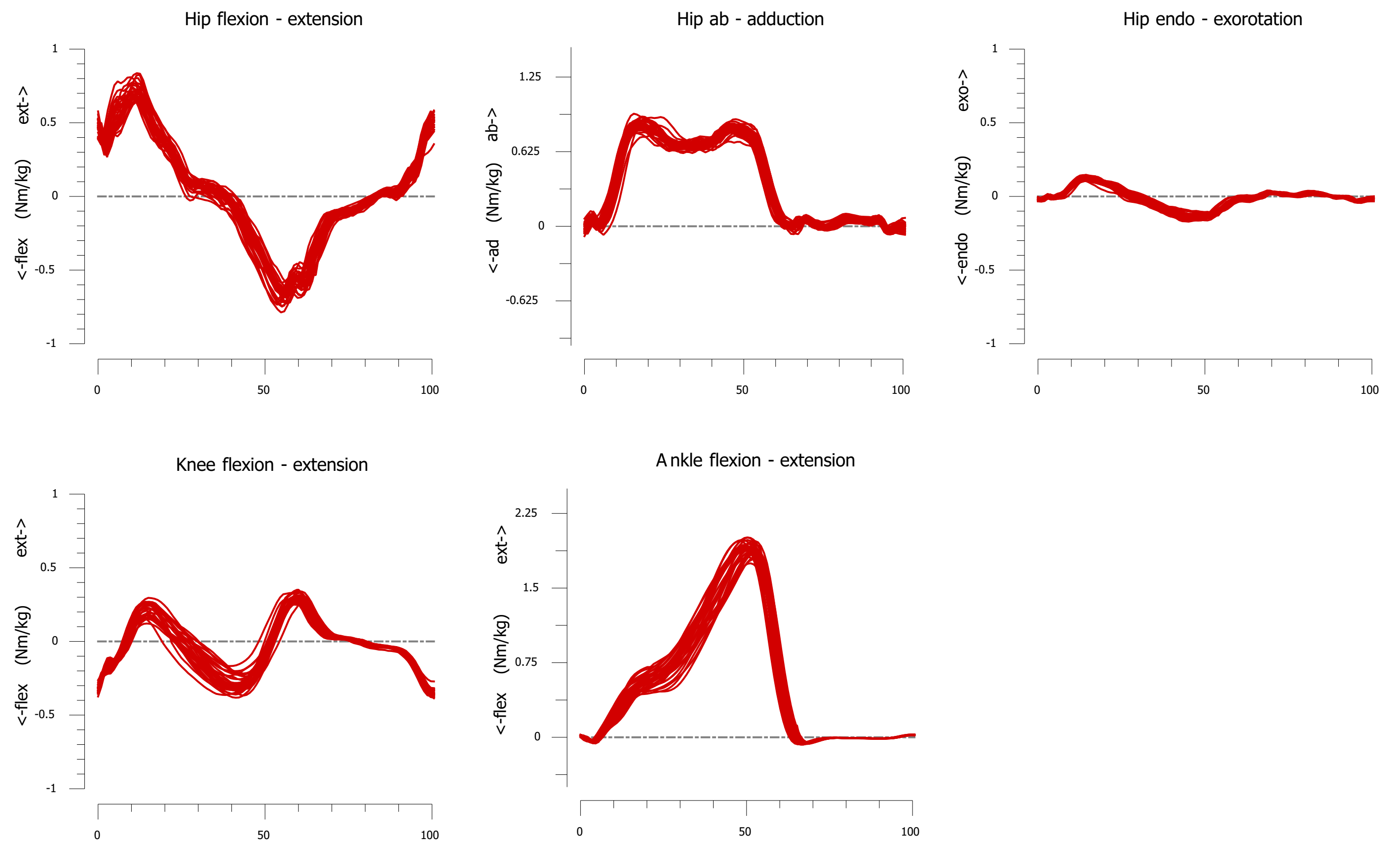
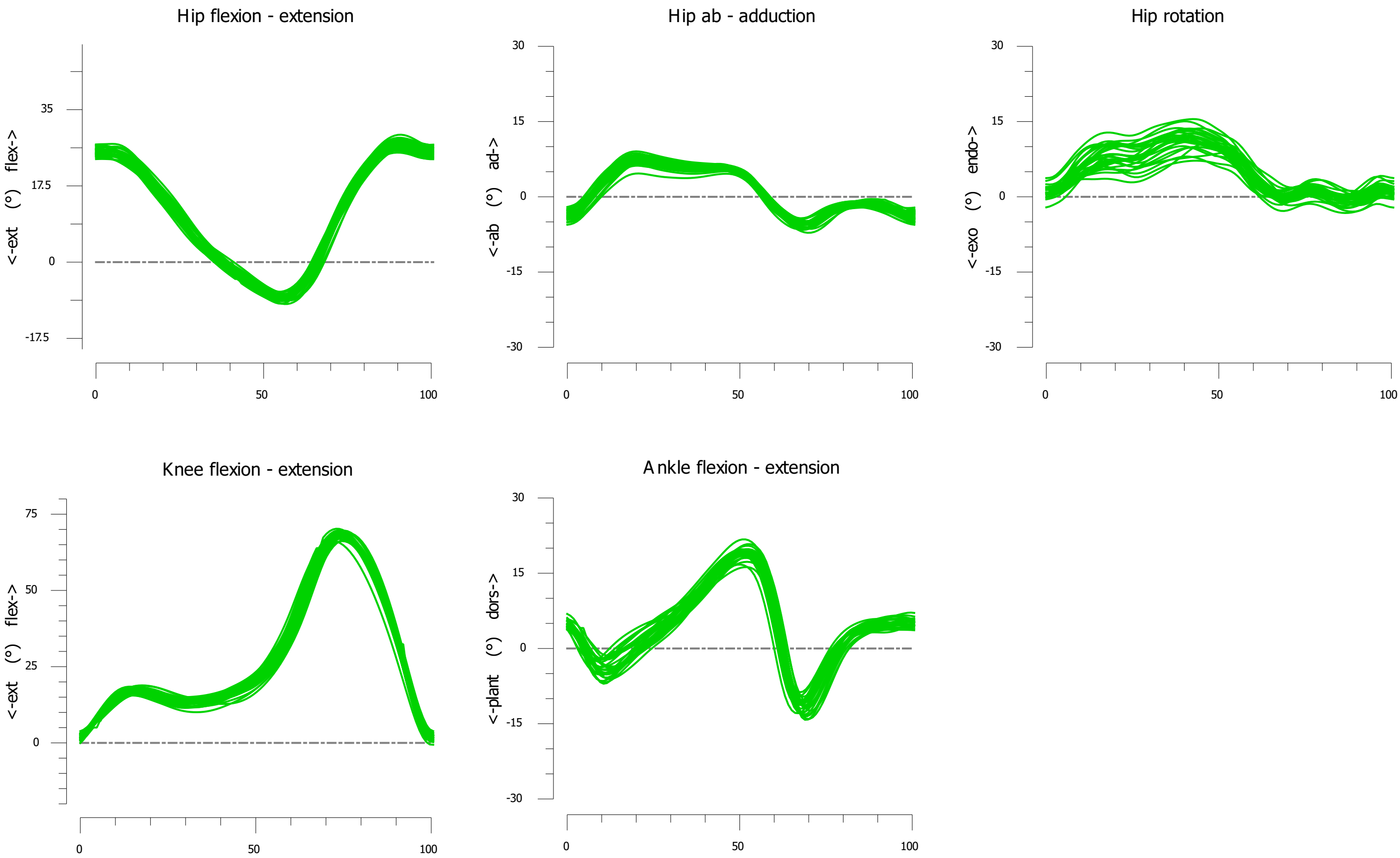


Figure **Right leg:**

Spatial-temporal parameters

Mean stance time: 0.70 s **Mean swing time:** 0.37 s

Kinematics - Joint angles



Kinetics - Joint moments

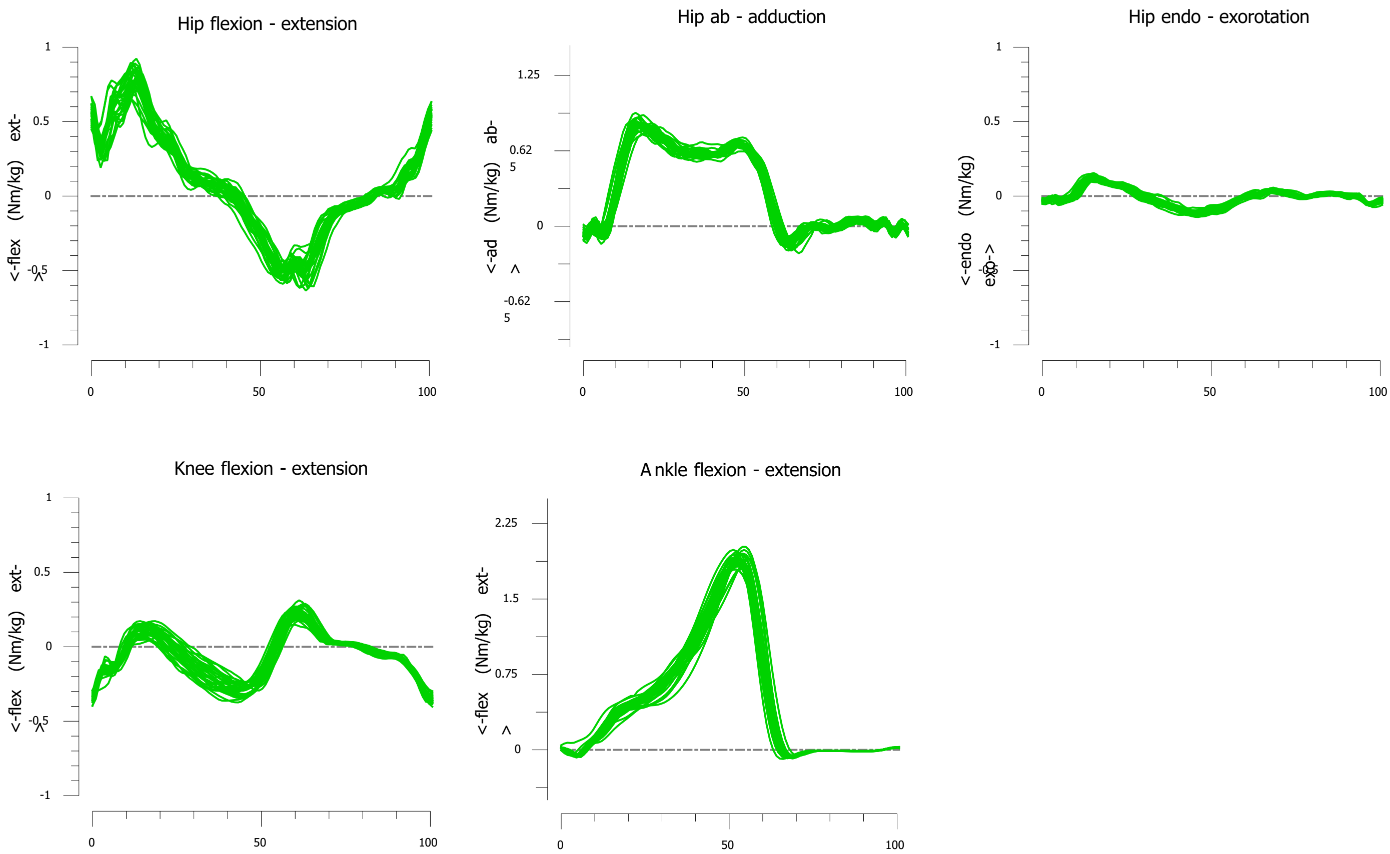


Table 1: Markers used in the Human Body Model (HBM)

Label	Anatomical location	Description
T10	T10	On the 10th thoracic vertebrae
SACR	Sacrum bone	On the sacral bone
NAVE	**Navel**	**On the navel**
XYPH	**Xiphoid process**	**Xiphiod procces of the sternum**
STRN	Sternum	On the jugular notch of the sternum
LASIS	**Pelvic bone left front**	**Left anterior superior iliac spine**
RASIS	**Pelvic bone right front**	**Right anterior superior iliac spine**
LPSIS	Pelvic bone left back	Left posterior superior iliac spine
RPSIS	Pelvic bone right back	Right posterior superior iliac spine
LGTRO	**Left greater trochanter of the femur**	**On the center of the left greater trochanter**
FLTHI	Left thigh	On 1/3 on the line between the LGTRO and LLEK
LLEK	**Left lateral epicondyle of the knee**	**On the lateral side of the joint axis**
LATI	Left anterior of the tibia	On 2/3 on the line between the LLEK and LLM
LLM	**Left lateral malleolus of the ankle**	**The center of left lateral malleolus**
LHEE	**Left heel**	**Center of the heel at the same height as the toe**
LTOE	**Left toe**	**Tip of big toe**
LMT5	**Left 5th meta tarsal**	**Caput of the 5th meta tarsal bone, on joint line midfoot/toes**
RGTRO	**Right greater trochanter of the femur**	**On the center of the right greater trochanter**
FRTHI	Right thigh	On 2/3 on the line between the RGTRO and RLEK
RLEK	**Right lateral epicondyle of the knee**	**On the lateral side of the joint axis**
RATI	Right anterior of tibia	On 1/3 on the line between the RLEK and RLM
RLM	**Right lateral malleolus of the ankle**	**The center of right lateral malleolus**
RHEE	**Right heel**	**Center of the heel at the same height as toe**
RTOE	**Right toe**	**Tip of big toe**
RMT5	**Right 5th meta tarsal**	**Caput of the 5th meta tarsal bone, on joint line midfoot/toes**

Table 1: The mean and standard deviation of the spatial-temporal, kinematics, kinetic gait parameters for the 30 participants.

Variable name	Side	Mean	Standard Deviation
Spatial temporal			
Walking speed (m/s)		1.37	0.22
Step length (m)	left	0.72	0.07
	Right	0.73	0.07
Stride time (s)	left	1.07	0.10
	Right	1.07	0.10
Stance time (s)	left	0.70	0.08
	Right	0.70	0.08
Swing time (s)	left	0.37	0.03
	Right	0.37	0.03
Kinematic			
Hip Flex (deg)	Left	30.05	9.08
	Right	29.92	8.79
Hip Ext (deg)	Left	-13.26	7.75
	Right	-13.36	7.68
Hip Abd (deg)	Left	-7.27	3.00
	Right	**-7.72**	**3.17**
Hip Add (deg)	Left	8.66	4.22
	Right	7.81	3.72
Hip Int Rot (deg)	Left	5.38	6.95
	Right	**6.82**	**6.42**
Hip Ext Rot (deg)	Left	-9.04	7.03
	Right	-5.77	5.97
Knee Flex (deg)	Left	67.46	5.16
	Right	**68.47**	**4.75**
Knee Ext (deg)	Left	-0.43	2.26
	Right	-0.29	2.01
Ankle Flex (deg)	Left	-17.20	6.94
	Right	**-14.91**	**6.47**
Ankle Ext (deg)	Left	18.13	5.92
	Right	19.36	6.54
Kinetic			
Peak Hip Ext (Nm/kg)	Left	0.82	0.21
	Right	0.80	0.24
Peak Hip Abd (Nm/kg)	Left	0.91	0.15
	Right	0.92	0.11
Peak Hip Int Rot (Nm/kg)	Left	0.26	0.13
	Right	0.26	0.14
Peak Knee Ext (Nm/kg)	Left	0.38	0.06
	Right	0.39	0.06
Peak Ankle Flex (Nm/kg)	Left	1.85	0.21
	Right	1.86	0.22

Table 2: The mean and standard deviation of the Maximum Voluntary Isometric Contraction for knee joint using the muscle strength testing equipment for the 30 participants.

Variable name	Side	Mean	Standard Deviation
Knee Ext	**left**	527.17	136.42
	Right	550.60	132.55
Knee Flex	**left**	191.60	38.53
	Right	203.87	47.67

Name of Material/ Equipment	Company
701 Small lever	Baltimore Therapeutic Equipment Company (BTE)
D-Flow Software - Vresion 3.26	Motekforce Link
Gait Offline Analysis (GOAT) - Version 2.3	Motekforce Link
Gait Real-time Analysis Interactive Lab (GRAIL)	Motekforce Link
Leg Pad for 701	Baltimore Therapeutic Equipment Company (BTE)
Positioning Chair	Baltimore Therapeutic Equipment Company (BTE)
Primus RS	Baltimore Therapeutic Equipment Company (BTE)

Catalog Number
Not Available - Online link provided in description
Not Available - Online link provided in description
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Comments/Description

The unique attachment designed for the Primus RS to measure Knee Extension/Flexion - https://store.btetech.com/collections/primus/products/701-small-lever

Software used to control GRAIL system - https://summitmedsci.co.uk/products/motek-dflow-hbm-software/

Software used for the analysis of the gait parameters - https://www.motekmedical.com/product/grail/

GRAIL system measures and quantifies gait biomechanics by using a virtual reality based self-paced (SP) treadmill - https://www.motekmedical.com/product/grail/

The unique attachment designed for the Primus RS to measure Knee Extension/Flexion - https://store.btetech.com/collections/primus/products/701-802-leg-pad

Participant Positioning Chair is designed for assessment and treatment of the lower exteremeties. The chair is designed for multiple positions. https://www.btetech.com/product/primus/

Primus RS equipment captures and reports real time objective data in Isotonic, Isometric, and Isokinetic resistance modes - https://www.btetech.com/wp-content/uploads/BTE-Rehabilitation-Equipment-PrimusRS-Brochure-1.pdf

Lower limb biomechanical analysis of healthy participants.

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2. Please address all the specific comments marked in the manuscript.	Thank you for your valuable feedback and comments.	We agree with all comments and have answered and amended as requested using track changes in the manuscript.
3. For steps in the protocol, please ensure that you describe how the action is performed. Please include discrete actions, button clicks in the software, the knob turns, command lines, etc. This is important because the script for the video is directly derived from the protocol text.	Thank you for this comment and we agree	This comment is addressed throughout the protocol
4. Please ensure the Representative Results is described in the context of the technique you have described and how it is in line with the title, e.g., how do these results show the technique, suggestions about how to analyze the outcome, etc. The paragraph text should refer to all of the figures. Data from both successful and sub-optimal experiments can be included.	Thank you for this comment and we agree. We have added information to establish the effectiveness of our protocol for assessing gait and muscle strength.	Done, lines 182 – 183 and 264-270
5. There is a 10-page limit for the Protocol section of the manuscript, but there is a 2.75-page limit for filmable content. Please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol.	Thank you for this comment	Essential steps of the protocol for the video are highlighted.

Manuscript Editorial Comments

Comment	Response	Action
Please check if it is ok for you to publish standard access article. Please use American English throughout. Please make the title concise maybe: Lower Limb Biomechanical Analysis of Healthy Participants.	Thank you	Yes, standard access is fine. American English used throughout. Title changed, line 2
Please provide at least 6 keywords or phrases.	Thank you for this comment	Done, line 19
Please expand this part with citations.	Thank you for this comment	Done, line 58, reference number 6
Please expand this part. Where are the markers placed in your study and how?	Thank you for this comment and we agree. We have added a table with concise placement of the markers.	Done, lines 87 – 90
What is the delay time between acclimatization and experimental step?	Thank you for this comment. There will be no delay time. We have made this clear in the protocol.	Done, line 112.
How is this done? Please include button clicks in the software, knob turns etc. e.g., Once the participant is walking on the treadmill, click "Start" in the associated software to start recording.	Thank you for this comment. Further information is added.	Done, line 117 - 121
How is this done? Please include button clicks, command lines etc.	Thank you for this comment. Further information is added.	Done, lines 124 - 126
Please include how is this done.	Thank you for this comment. Further information is added.	Done, line 129 - 130
What is the output format? Please include the script generated if any as supplementary files or provide a reference.	Thank you for this comment. Further information is added.	Done, line 127. Matlab code file added as a supplementary file.
Commercial term BTE is visible in the equipment, please do not show any commercial terms in the	Thank you for this comment. We have now removed the term BTE from Figure 3.	Done, Figure 3.

figure as well.		
Attached where? How is this done? Please mark this in the figure	Thank you for this comment. Further information is added.	Done, line 141
How is this done?		
How? Do you do this manually, do you perform any button clicks in the associated software?	Thank you for this comment. Further information is added.	Done, lines 147 - 149
Please describe how did you conclude the effectiveness of the technique and conclusion derived- using data presented here.	Thank you for this comment. We have added information to establish the effectiveness of our protocol for assessing gait and muscle strength.	Done, lines 182 – 183 and 264 - 270
This is not result and can be moved to the protocol instead.	Thank you for this comment and we agree. This section has been added to the protocol along with further steps for further clarification.	Done, lines 97 – 105, and 117 – 130
Each Figure/Table Legend should include a title and a short description of the data presented in the Figure and relevant symbols.	Thank you for this comment. Further information is added.	Done, lines 193 - 222
Please indicate what red and green signify?	Thank you for this comment. Further information is added.	Done, lines 197 - 201
Please move this to the reference section and use in text citation.	Thank you for this comment	Done, lines 89 – 90, reference number 8

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