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Construction of a Realistic, Whole-body, Three-dimensional Equine Skeletal Model Using Computed Tomography Data

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TITLE:

Construction of a Realistic, Whole-body, Three-dimensional Equine Skeletal Model Using Computed Tomography Data

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3D whole-body equine skeleton, animal model, animation, CT data, motion, posture

SUMMARY:

The purpose of this protocol is to describe the method of creation of a realistic, whole-body, skeletal model of a horse that can be used for functional anatomical and biomechanical modeling to characterize whole-body mechanics.

ABSTRACT:

Therapies based upon whole-body biomechanical assessments are successful for injury prevention and rehabilitation in human athletes. Similar approaches have rarely been used to study equine athletic injury. This study investigates how mechanical stress causing degenerative osteoarthritis originates from chronic postural dysfunction, which, because the primary dysfunction is often distant from the site of tissue injury, is best identified through modeling whole-body biomechanics. To characterize whole-body equine kinematics, a realistic skeletal model of a horse was created from equine computed tomography (CT) data that can be used for functional anatomical and biomechanical modeling. Equine CT data were reconstructed into individual three-dimensional (3D) data sets (i.e., bones) using 3D visualization software and assembled into a complete 3D skeletal model. The model was then rigged and animated using 3D animation and modeling software. The resulting 3D skeletal model can be used to characterize equine postures associated with degenerative tissue changes as well as to identify postures that reduce mechanical stress at the sites of tissue injury. In addition, when animated into 4D, the model can be used to demonstrate unhealthy and healthy skeletal movements and can be used to develop preventative and rehabilitative individualized therapies for horses with degenerative lamenesses. Although the model will soon be available for download, it is

currently in a format that requires access to the 3D animation and modeling software, which has quite a learning curve for new users. This protocol will guide users in (1) developing such a model for any organism of interest and (2) using this specific equine model for their own research questions.

INTRODUCTION:

Chronic lameness in horses is often associated with progressive degenerative tissue lesions similar to those of osteoarthritis (OA), a major public health problem in humans[1-9]. In human medicine, because therapeutic approaches focused on treating specific lesions (e.g., pharmacotherapy and direct chondral repair) have mostly failed, pathomechanical forces are now recognized as the root cause of tissue damage in OA. Aberrant or pathomechanical forces impact both bone and cartilage cells directly, inducing the release of inflammatory mediators and progressive tissue degeneration[9]. These observations indicate that unless the causative mechanical forces are corrected, many chronic degenerative bone and joint diseases will continue to progress. Hence, the therapeutic focus in human medicine is shifting to approaches that "unload" the affected joints through targeted exercise[10,11]. However, this shift has not yet been made in equine medicine, partly because models for equine motion that can be adapted to show an individual's movements are needed.

Comprehensive, whole-body biomechanical analysis is common in designing training programs to optimize athletic performance and facilitate injury recovery in human athletes[11] (see also e.g., the journal "Sports Biomechanics"), but is less commonly done for equine athletes (but see[12]). Thus, the overarching goal here is to establish pathomechanical models of equine lameness that can be used to develop individualized preventative and rehabilitative therapies to improve the health of equine athletes. Such pathomechanical models can characterize differences in the functional anatomy of regions (i.e., the spine) that are not as easily discernible to the naked eye as others (i.e., the lower limb). To achieve this goal, the first objective was to develop an anatomically accurate, manipulatable whole-body, equine, skeletal model that can be used as a template by researchers interested in functional anatomical, kinematic, and kinetic analyses. To be useful to equine clinicians and researchers, this model must (1) be biologically realistic to enable accurate anatomical positioning, (2) allow for easy and accurate adjustments for modeling various postures of healthy and non-healthy horses, (3) be able to be animated to study the effects of various gaits, and (4) facilitate repeatable re-creations of positions and movements.

A 3D graphic, equine, whole-body, skeletal model was built from CT data in which the positions of bones relative to each other could be manipulated and then animated to match movements from pictures or videos of a horse in motion, thus creating a 4D equine skeletal model. Depending upon what best fits the question to be addressed, the model can be used in 2D, 3D, and 4D versions or in various combinations to illustrate and characterize the pathomechanical effects of specific positions or postures. Because of its basic and flexible design, the model serves as a template that can be modified by researchers to reflect their specific questions and data parameters. Such parameters include, for example, anatomical information based on sex and animal size, 3D motion analysis data, soft tissue force estimations, and inertial properties.

Thus, the model allows for more detailed analysis of specific areas or joints, while also providing the basis to set up experiments that are unable to be performed on living horses. Due to practical limitations related to specimen availability (e.g., the ribs cut) and the scanner, the whole-body equine model is the result of merging data from three equine specimens. Thus, the model is not a perfect representation of a single individual, but has been standardized to represent individual variability more broadly. In short, it is a template to be used and modified to suit the needs of researchers. CT scans of the trunk, head and neck, and limbs were acquired from two equine specimens of approximately the same size with a 64-slice CT scanner using a bone algorithm, pitch of 0.9, 1 mm slice. CT scans of a set of ribs were acquired with a 64-slice CT scanner using a bone algorithm, pitch of 0.9, 0.64 mm slices.

Anatomic integrity of the bony joints (e.g., within the limb) was maintained. The soft tissues available in the CT scans were also used to confirm the placement of the bones. As some whole ribs and the proximal portions of all the ribs were available and scanned on the thorax specimen, the separately scanned ribs could be accurately sized and placed within the whole-body skeletal model. The resulting CT Digital Imaging and Communications in Medicine (DICOM) data were imported into the 3D visualization software (see the **Table of Materials**), and individual bones were segmented into individual data sets (i.e., bone meshes). The individual 3D bone meshes were then imported into the 3D animation and modeling software (**Table of Materials**) where they were sized, if necessary, and assembled into a complete equine skeleton in preparation for rigging—a graphic method of connecting the bone meshes so that their movements are linked (**Figure 1**).

PROTOCOL:

1. Forelimb rigging

1.1 Place graphic joints inside the forelimb in all areas of movement.

NOTE: The resulting joint placement is a joint chain from the scapula to the distal end of the coffin bone (**Figure 2A**). In the area of the carpal bones, 3 joints in close proximity are used to increase the bending radius.

1.1.1 Press the **F3** key to enable the **Rigging Menu** set. In the menus, select **Skeleton | Create Joints** to select the **Create Joints** tool.

1.1.2 In the **View** panel of the software, click in the approximate areas of the joints found in **Figure 2A** in the order of 1 to 10, and press the **ENTER** key.

1.1.3 Adjust the position of the joints by clicking on the desired joint and using the **Move Tool** and pressing the **W** key to translate the joint into the desired position. Alternatively, adjust a joint by clicking on the desired joint and altering the **Translate X, Translate Y, and Translate Z** values found in the **Channel Box/Layer Editor** panel.

1.2 Create 5 separate Inverse Kinematic handles (IK handles) (joints will be referred to by the numbers found in **Figure 2A**).

1.2.1 In the menus, select **Skeleton | Create IK Handle** to select the **Create IK Handle** tool. Using the **Create IK Handle** tool, select joint 1, then joint 3; name this IK handle **Front Leg IK** in the **Outliner** panel. Using the **Create IK Handle** tool, select joint 3, then joint 7; name this IK handle **Front Lower IK**.

1.2.2. Using the **Create IK Handle** tool, select joint 7, then joint 8; name this IK handle **Front Toe 1 IK** in the **Outliner** panel. Using the **Create IK Handle** tool, select joint 8, then joint 9; name this IK handle **Front Toe 2 IK** in the **Outliner** panel. Using the **Create IK Handle** tool, select joint 9, then joint 10; name this IK handle **Front Toe 3 IK** in the **Outliner** panel.

1.3 Create forelimb controls

1.3.1 Create a Non-Uniform Rational B-Splines (NURBS) circle by using the **Circle** tool in the menu **Create | NURBS Primitives | Circle**.

1.3.2 Create two NURBS Circles encircling joint 3 and joint 10, and name them **Front Ctrl** and **Front Lower Ctrl**, respectively, in the **Outliner** panel.

1.3.3 Create a NURBS Circle; select the circle, and in the **Channel Box/Layer Editor** Panel, change the **Rotate Z** value to 90. Using the **Move** tool, place it at the tip of joint 10, and name it **Front Flick Ctrl** in the **Outliner** panel.

1.4 Group **Front Toe 1 IK**, **Front Toe 2 IK**, and **Front Toe 3 IK** by selecting all three and pressing the **CTRL + G** keys. Name this group **Front Toe Group** in the **Outliner** panel. Parent the IK handles and **Front Toe Group** to the controls.

NOTE: It is important to select in the exact order described below to ensure a proper parent tree.

1.4.1 Select **Front Leg IK**, then **Front Ctrl** in the **Outliner** panel, and press the **P** key.

1.4.2 Select **Front Lower Ctrl**, then **Front Ctrl** in the **Outliner** panel, and press the **P** key.

1.4.3 Select **Front Lower IK**, then **Front Lower Ctrl** in the **Outliner** panel, and press the **P** key.

1.4.4 Select **Front Flick Ctrl**, then **Front Lower Ctrl** in the **Outliner** panel and press the **P** key.

1.4.5 Select **Front Toe Group**, then **Front Flick Ctrl** in the **Outliner** panel, and press the **P** key.

1.5 Use the **Bind Skin** tool to bind the bone meshes, except sesamoid, including navicular bones to the most proximal joint. Ensure that each bone mesh is only bound to one joint.

1.5.1 Click on the bone mesh, **Shift + click** on the most proximal joint, and select the **Bind Skin** tool under **Skin | Bind Skin**.

1.6. Rigging sesamoid bones and the navicular bone

1.6.1 Create a joint, place it in the middle of sesamoid bone, and press the **Enter** key. In the **View** panel, select the sesamoid bone mesh, and **Shift + click** the joint in the middle of the bone. Use the **Bind Skin** tool to bind the mesh to the joint.

NOTE: The sesamoid bone can now be manipulated using the **Move** and **Rotate** tools for adjustment when changing the leg position.

1.6.2. In the **View** panel, select the joint in the sesamoid bone, **Shift + click** the nearest joint in the forelimb, and press the **P** key.

NOTE: This parents the joint in the sesamoid bone to the forelimb.

1.6.3. Repeat steps 1.6.1 to 1.6.2 for other sesamoid bones and the navicular bone.

1.7. Repeat steps 1.1 through 1.6 for the other forelimb.

NOTE: The joint at the scapula can be selected and translated in all 3 directions (6 degrees of freedom) using the **Move** tool.

2. Hindlimb rigging

2.1. Place joints inside the hindlimb in all areas of movement to obtain a joint chain from the head of the demur to the distal end of the coffin bone (**Figure 2B**).

2.2. Create 5 separate IK handles (joints will be referred to the numbers found in **Figure 2B**).

2.2.1. Using the **Create IK handle** tool, select joint 11, then joint 12; name this IK handle **Hind IK** in the **Outliner** panel. Using the **Create IK handle** tool, select joint 12, then joint 14; name this IK handle **Hind Lower IK** in the **Outliner** panel.

2.2.2. Using the **Create IK handle** tool, select joint 14, then joint 15; name this IK handle **Hind Toe 1 IK** in the **Outliner** panel. Using the **Create IK handle** tool, select joint 15, then joint 16; name this IK handle **Hind Toe 2 IK** in the **Outliner** panel.

2.2.3. Using the **Create IK handle** tool, select joint 16, then joint 17; name this IK handle **Hind Toe 3 IK** in the **Outliner** panel.

2.3. Create hindlimb controls

2.3.1. Create two NURBS Circles named **Hind Ctrl** and **Hind Lower Ctrl** encircling joint 12 and joint 17, respectively.

2.3.2. Create a NURBS Circle named **Hind Flick Ctrl**. Make this circle vertical, and place it at the tip of joint 10.

2.4. Group **Hind Toe 1 IK**, **Hind Toe 2 IK**, and **Hind Toe 3 IK** by selecting all three and pressing **CTRL + G**. Name this group **Hind Toe Group**.

2.5. Parent the IK handles and **Hind Toe Group** to the controls. Be sure to select in the exact order described below to ensure a proper parent tree.

2.5.1. Select **Hind IK**, then **Hind Ctrl**, and press the **P** key.

2.5.2. Select **Hind Lower Ctrl**, then **Hind Ctrl**, and press the **P** key.

2.5.3. Select **Hind Lower IK**, then **Hind Lower Ctrl**, and press the **P** key.

2.5.4. Select **Hind Flick Ctrl**, then **Hind Lower Ctrl**, and press the **P** key.

2.5.5. Select **Hind Toe Group**, then **Hind Flick Ctrl**, and press the **P** key.

2.6. Use the **Bind Skin** tool to bind the bone meshes to the most proximal joint. Ensure that each bone mesh is bound to only one joint.

2.6.1. Click on the bone mesh, **Shift + click** the most proximal joint, and select the **Bind Skin** tool under **Skin | Bind Skin.**

2.7. Rigging patella, sesamoid bones, and navicular bone

2.7.1. Create a joint, place it in the middle of the patella, and press the **Enter** key. In the **View** panel, select the patella mesh, and **Shift + click** on the joint in the patella. Use the **Bind Skin** tool to bind the mesh to the joint.

NOTE: The patella can now be manipulated using the **Move** and **Rotate** tools for adjustment when changing the leg position.

2.7.2. In the **View** panel, select the joint in the patella, **Shift + click** on the nearest joint in the forelimb, and press the **P** key to parent the joint in the patella to the forelimb.

2.7.3. Repeat steps 2.7.1 and 2.7.2 for the sesamoid bones and the navicular bone.

2.8. Repeat steps 2.1 through 2.7 for the other hindlimb.

3. Ribbon spine rigging

3.1. Create a NURBS Plane with altered options with the length roughly equal to the length of the spine with 1 U-patch and # V-patches, where # is the number of thoracic and lumbar vertebrae.

NOTE: For this paper, the length is 20 with 22 V patches.

3.1.1. Select the square found next to the **Create Plane** tool under **Create | NURBS Primitives | Plane**.

3.2. Rebuild the plane with altered options.

3.2.1. Press the **F2** key to enter the modeling menu set. Select the plane in the view panel, and select the **Rebuild** tool settings by selecting the square next to the **Rebuild** tool under **Surfaces | Rebuild.** Use the following options: number of spans U = 1; number of spans V = # (22 in this case); select "1 Linear" for both the Degree U and Degree V options; keep the other settings to default; and press the **Rebuild** button.

3.3. Create nhairs with altered options.

3.3.1. Press the **F5** key to enter the FX menu set. Use the **Create Hair** tool with altered options by selecting the square next to **nHair | Create Hairs**. Use the following options: output set to NURBS curves; U count =1; V count = # (22 in this case); keep the other options to default; and press the **Create Hairs** button.

3.4. Delete the following in the outliner panel: **nucleus1**, **hairSystem1OutputCurves** group, and **hairSystem1**. Fully expand the group labeled **hairSystem1Follicles**, and delete all the items labeled with **curve__.**

NOTE: The result should leave a group labeled **hairSystem1Follicles** that contains a list of items labeled **nurbsPlane_Follicle____.**

3.5. Select the plane, and move and orient it so that it is roughly overlapping with the spine by using the **Move** tool and **Rotate** tool. Select the plane, hold the right mouse button, and select **Control Vertex** to make all the vertices of the plane visible.

3.6. Move the vertices to orient the follicles to be between the vertebrae at the height where the spinal cord would be. Create # number of separate joints (22 in this case) at any place in the **View** panel as the position of these joints will be corrected in later steps.

3.7. Parent a joint with a **nurbsPlane_Follicle____** so that each has a single joint under its tree.

3.7.1. In the **Outliner** panel, select a joint created in step 3.6, then **Ctrl + click** on a **nurbsPlane_Follicle____,** and press the **P** key. Repeat 3.7.1 with the other joints created in step 3.6 and the other **nurbsPlane_Follicle____** objects.

3.8. In the **Outliner** panel, **Ctrl + select** all the joints; in the **Chanel Box/Layer Box** panel, set the **Translate X**, **Y**, and **Z** to **0**. Duplicate all the joints by **Ctrl + selecting** all the joints in the **Outliner** panel and pressing the **Ctrl + D** keys. Un-parent all the duplicate joints by **Ctrl + selecting** all the duplicate joints in the **Outliner** panel and pressing the **Shift + P** keys

3.9. Bind the joints under **nurbsPlane_Follicle____** with their respective vertebra mesh.

3.9.1. Press the **F3** key to enter the **Rigging** menu set. Click on the original joint (not the duplicate joint) under **nurbsPlane_Follicle____**, **Shift + click** on the respective vertebra mesh, and then use the **Bind Skin** tool under **Skin | Bind Skin.** Repeat these actions in step 3.9.1 for each joint and vertebrae mesh.

3.10. **CTRL + click** all duplicate joints and the plane, and use the **Bind Skin** tool to bind all the duplicate joints to the plane.

NOTE: The duplicate joints can now be manipulated to control the vertebrae.

3.11 Repeat steps 3.1 through 3.10 for the cervical and caudal vertebrae.

4. Rib and sternum rigging

4.1. Place separate joints at the rib head, at the proximal end of the costal cartilage, and at the distal end of the costal cartilage. Parent the joint at the proximal end of the costal cartilage to the joint at its rib head.

4.2. Parent the joint at the distal end of the costal cartilage to the closest joint at the proximal end of the costal cartilage. Parent the joint at the rib head to the spine joint that controls the vertebrae caudal to the rib.

4.3. In the **Rigging** menu set under the **Skin** tab, use the **Bind Skin** tool to bind the rib to the joint at its head and the costal cartilage to both the joints at its proximal end and the distal end.

4.4. Repeat steps 4.1 through 4.3 for each rib.

4.5. Place separate joints at the most cranial end of each sternal segment. Parent each sternal segment joint to the spinal joint most dorsal to each sternal segment joint. In the **Rigging** menu set under the **Skin** tab, use the **Bind Skin** tool to bind the sternal segment to its joint.

5. **Positioning and animation**

5.1 Select a frame in the timeline.

5.2 Position the model and controls. Import an image to use as a reference by creating a **Free Image Plane**.

NOTE: The images from Muybridge[15] of the horse at the walk were used as proof of concept.

5.2.1 While the **Free Image Plane** is selected, select the image file under the **Attribute Editor** tab and under the **Image Plane Attributes** dropdown menu.

5.3 Select all controls and the spine control joints, and press the **S** key to save them as a key frame.

5.4 Along different frames along the timeline, move and rotate the controls and spine control joints, and press **S**.

NOTE: Repositioning controls and spine control joints and saving them as key frames along different points of the timeline creates an animation. There need not be a key frame set along each frame of the timeline; only critical positions or timings need to be key-framed. The 3D animation and modeling software will interpolate between the key-framed positions of each control and spine control joint, creating a smooth animation.

REPRESENTATIVE RESULTS:

The result of the method was a 3D full equine skeletal model inside the 3D animation and modeling software that allows for accurate anatomical positioning and movement simulations. The model itself has a graphic rigging system delegated to the forelimbs, hindlimbs, spine, neck, and ribcage. The 3D model could be placed into different postures (**Figure 3** and **Figure 4**) by multiple individuals. The movements of the 4D model (in motion) have been compared to videos from the side, back, and front, as well as with overhead drone footage to more accurately depict the motion of the spine and video of horses at the walk (**Video**), canter, and trot to create animations of those gaits.

FIGURE AND TABLE LEGENDS:

Figure 1: The 3D equine model can be moved into various postures and animated to demonstrate whole-body movements in various gaits in the 3D animation and modeling software. (**A, C**) Graphic rigging systems for the horse. The graphic ribbon spine that enables natural movement of the bony spine is illustrated by the green plane. The controls used to move the various graphic rigs and the attached bone meshes are illustrated by the yellow ovals and cross arrows on the model. (**A**) Standing position. (**C**) Rearing position. (**B**, **D**) The model with the bone meshes attached to the graphic rigging system. The positions of the controls change the position of the skeleton of the horse. (**B**) Standing horse. (**D**) Rearing horse.

Figure 2: The rigging of each limb with joints allows for positioning and the creation of movement. (**A**) Forelimb with graphic joints indicated with numbers 1–10. (**B**) Hindlimb with graphic joints indicated with numbers 11–17.

Figure 3: The 3D equine model was matched to classic Muybridge[15] photos as proof of concept and to create the first animations. (**A**) Muybridge photographs of a horse at the walk. (**B**) The 3D equine model superimposed over the photographs to be used as key frames in the animation. (**C**) The 3D equine model.

Figure 4: The 3D equine model can be moved into various postures (e.g., the transversal rotation of the spine demonstrated here) to understand the relationship of such postures to pathomechanical force regimes and the resulting degeneration of the affected skeletal elements, joints, and soft tissues. (**A**) A graphic 2D representation of a normal posture of a horse (with rider) using graphically manipulated photographs of an equine skeleton compared to a still image of the 3D equine model with the head and cervical vertebrae hidden to enable the visualization of the thorax. (**B**) A graphic 2D representation of a horse (with rider) with a transversal rotation of the spine using graphically manipulated photographs of an equine skeleton compared to a still image of the 3D equine model with the head and cervical vertebrae hidden to enable the visualization of the thorax. Note here the effect of the transversal rotation on the skeleton and the limbs of the body. The depicted position would overload the left forelimb, which was supported by the compression and cracking of the left front hoof wall in the living horse.

Video. The 4D Horse. Key positions of the skeleton, as matched to the Muybridge[15] photos of the horse, have been interpolated to create an animation of the horse at a walk. The movement can be seen from the front, side, top, and back.

DISCUSSION:

This protocol demonstrates how to create a 3D whole-body skeletal model of an organism and demonstrates how to use the whole-body equine skeletal model described in this paper. The model is currently in a format that requires a specific 3D animation and modeling software, which has quite a learning curve for new users. However, a version of this software is freely available for those who are affiliated with a university. Although modeling whole-body posture and movement is used to assess human athletes and to identify causes of mechanically induced chronic injuries[11], it is less commonly done with equine athletes. To use this approach for the assessment of the potential causes of equine athletic injuries and performance issues, a realistic whole-body skeletal equine model was created from CT data using the 3D visualization software and 3D animation and modeling software. This model is different from other equine models that are either artistic graphic recreations of the skeleton (https://www.youtube.com/watch?v=YncZtLaZ6kQ) or that only depict the limbs.[16-19] In this whole-body model, forelimbs, hindlimbs, spine, and ribcage were all rigged and had controls attached that allow for easy manipulation of the model for realistic and accurate positioning and animation.

The protocol used to rig the model allows for repeatability and future alterations to suit the needs of the specific horse being rigged, enabling individualized analysis. Thus, the equine model is a tool to be used by researchers as they analyze movement. However, it is not an automated program that provides answers without the input of parameters specific to the animal being modeled and the question being addressed, as the accuracy of the model is directly related to the strength of a particular analysis. The ability to input parameters also allows the model to be continually updated with data from future research studies. Additionally, this graphic rigging protocol can be applied and/or adjusted to reflect the anatomical differences between individuals. It can also be adapted to effectively model other animals. The 3D equine model can be easily manipulated and positioned to simulate positions and movements. This is especially evident with the limbs as their movements are relatively simple to see and model.

Graphic joint positioning in the model was determined by a similar approach to that used in other studies[13,14]. The bone meshes were placed in a neutral position. Graphic joints were positioned so that the bones were able to rotate freely without causing any collision with other bone meshes. In the digits, the graphic joint was placed at the point where a sphere coincided with the surfaces of movement. The graphic joint of the scapula was placed in the approximate center of the scapula blade. This positioning of the graphic joint allows it to be moved in 6 degrees of freedom to orient the scapula into the desired position. Unlike the limbs, the movement of the spine is not easily seen, is more complex than often realized, and thus is more difficult to model. Although the model has the flexibility to be used to investigate movements and issues at specific spinal joints, it also needed to be able to represent the often hard-to-distinguish movements of the whole spine. The use of the “ribbon spine” allows for more realistic movement of the spine during animations.

This is important as the spine in horses, as has been found in humans, is often the origin of issues that are potentially related to aberrant biomechanical movements and injury to the limbs. A strength of this model is the ability to accurately demonstrate spine positions, like transversal vertebral rotations[20] (**Figure 4**). How these postures impact the limbs in three dimensions during various gaits can be determined by using the model in combination with kinematic and force analysis (e.g., pressure plate studies to confirm increased loading of the limbs and static force analysis). Soft tissue musculofascial components are currently being added to the whole-body skeletal model. Future goals are to expand the use of the model in 3D biomechanical analysis for studies of equine lameness. Such expansion would include using the model to complete 3D force analyses that compare healthy and unhealthy postures and registering the model with 3D data points collected in motion capture studies to provide a more effective visual representation of movement.

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The authors have declared no conflicts of interest.

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Figure 1

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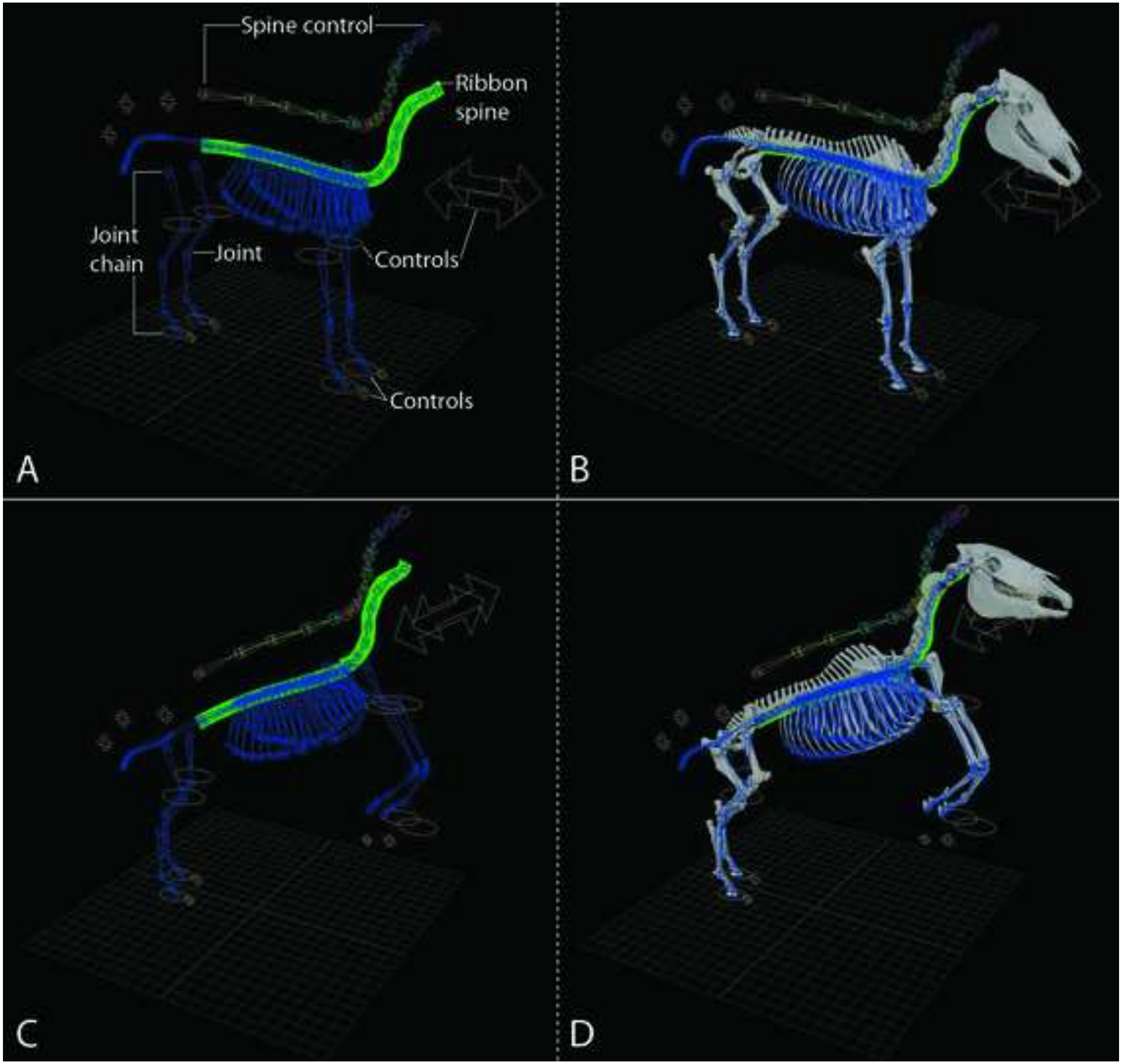


Figure 2

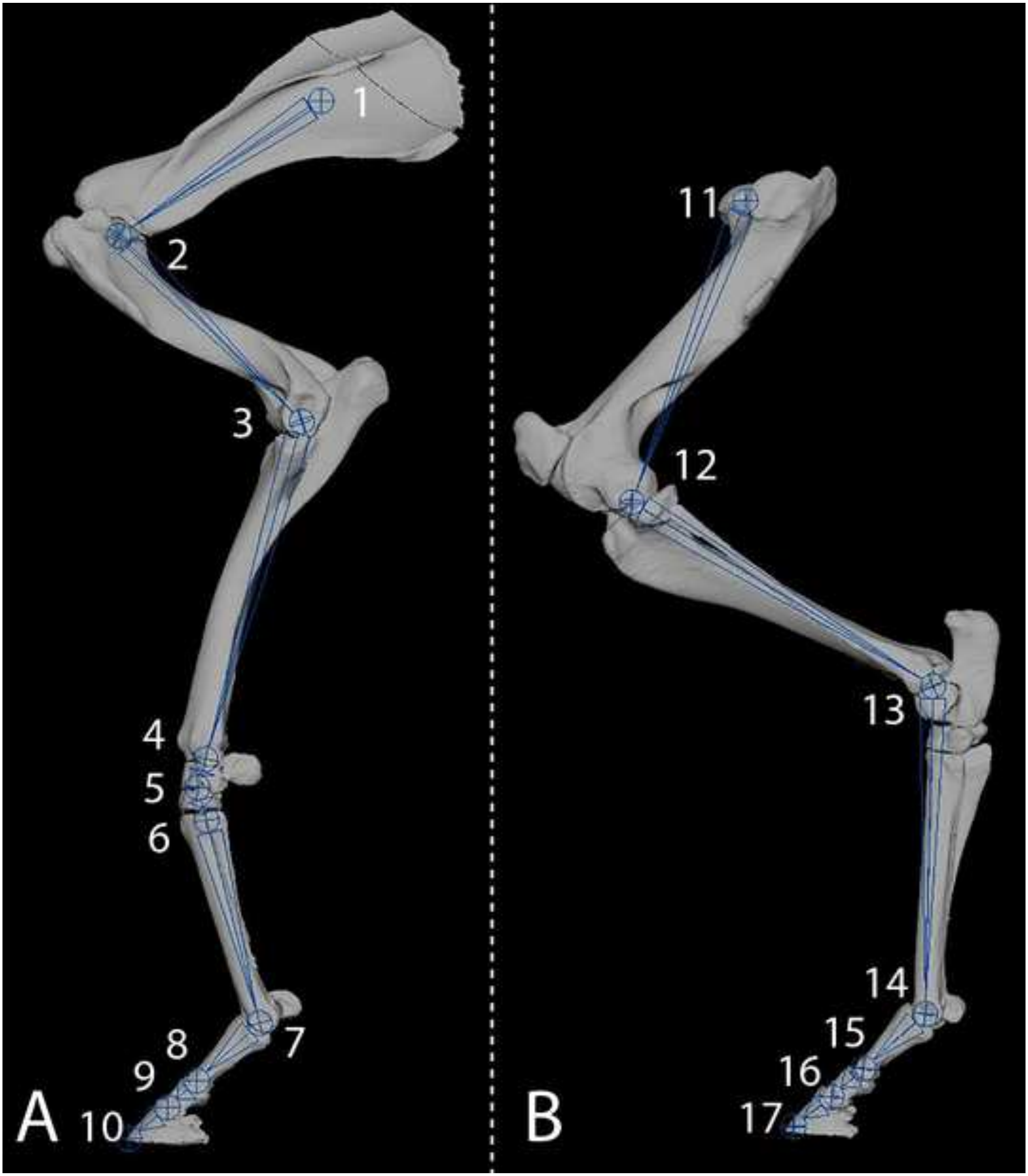
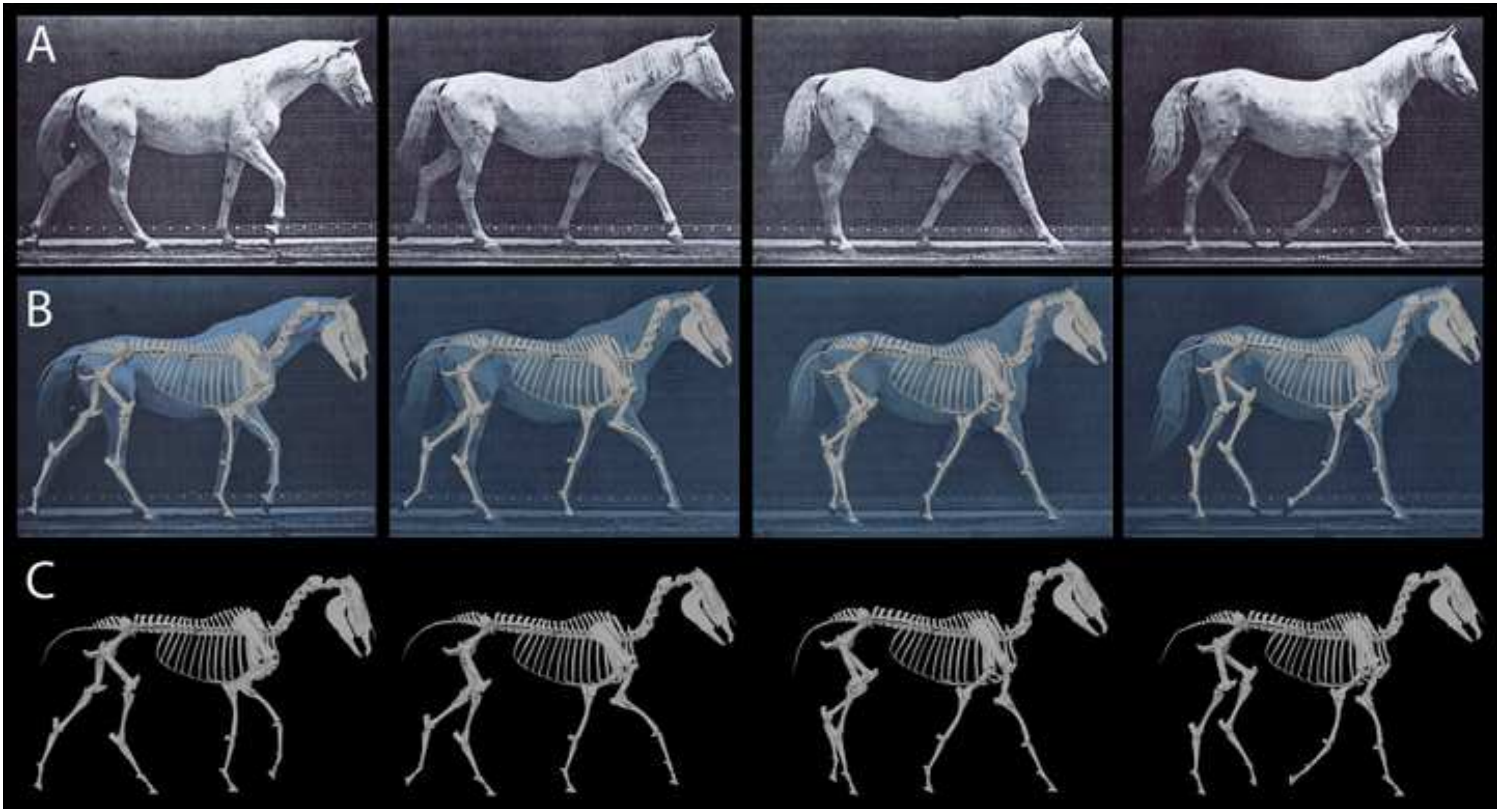
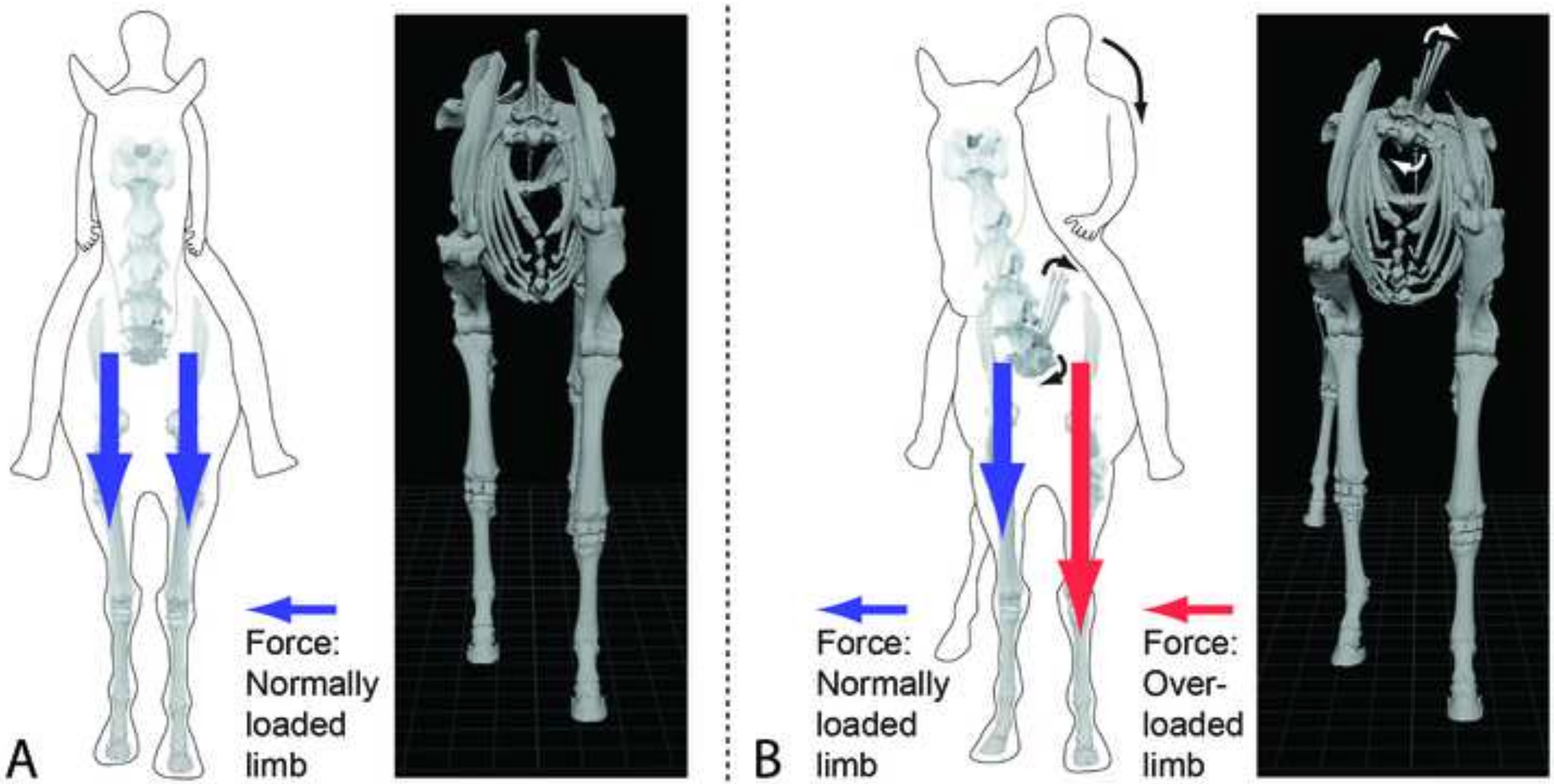


Figure 3




A
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Name of Material/Equipment	Company	Catalog Number	Comments/Description
Avizo	VSG, Visualization Science Group, Inc., Burlington, MA	N/A	cited in text as "3D visualization software"
Maya	Autodesk, Inc., San Rafael, CA	N/A	cited in text as "3D animation and modeling software"; Free student version

School of Veterinary Medicine

Comparative Biomedical Sciences

Vidhya Iyer, Ph.D.
Review Editor
JoVE

22 January 2021

Dear Dr. Iyer,

Thank you very much for allowing us to submit a revised version of our manuscript now titled "**Construction of a Realistic, Whole-Body Three-Dimensional Equine Skeletal Model Using CT Data**" to *JoVE*.

We are again grateful to the reviewers for their insightful and useful suggestions, which have helped us to improve the manuscript. In this revision we have made substantive changes in response to the reviewers' comments. All aspects of the review have been modified to address both the requested editorial changes and the concerns of the reviewers. The specific responses are on the following pages with reviewers comments in regular font and answers indented. in bold and italicized.

The purpose of this protocol is to guide users in (1) developing such a model for any organism of interest and (2) using this specific equine model for their own research questions.

Aspects of this research have been published as abstracts from meetings of the American Association for Anatomy and at the annual Science of Motion Symposia.

With many thanks for your consideration and best regards,

Michelle L. Osborn (**mosborn@lsu.edu**), 225-578-9470, fax: 225-578-9895 (corresponding author)

Alexander K.K. Lee (**alee76@lsu.edu**)

Elizabeth W. Uhl (**euhl@uga.edu**)

Please note that the reviewers raised some significant concerns regarding your method and your manuscript. Please revise the manuscript to thoroughly address these concerns. Additionally, please describe the changes that have been made or provide explanations if the comment is not addressed in a rebuttal letter. We may send the revised manuscript and the rebuttal letter back to peer review.

Editorial comments:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

The manuscript has been proofread.

2. JoVE cannot publish manuscripts containing commercial language. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials: e.g., Avizo, Autodesk Maya etc. We must maintain our scientific integrity and prevent the subsequent video from becoming a commercial advertisement.

The specific commercial product names have been removed throughout the manuscript and are listed only in the Table of Materials.

3. Please define all abbreviations before use (IK).

We have done so. For IK, please see Lines 140.

4. Please note that your protocol will be used to generate the script for the video and must contain everything that you would like shown in the video. Please add more details to your protocol steps. Please ensure you answer the "how" question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action.

The requested specific details have been added throughout the protocol.

5. Line 95: Please specify how are the IK handles created.

We have done so. Please see Lines 140-158.

6. Line 109: Please define the term "NURBS"

We have done so. Please see Lines 162.

7. Please add more specific details (e.g., button clicks for software actions, numerical values for settings, etc.) to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. If button clicks/menu selections are identified (e.g., quotes or cursive text has been used), change them to bold text. Use either | or > between the clicks/selections. Example: File > Options > Advanced or File | Options | Advanced

The requested specific details have been added throughout the protocol.

8. Please highlight up to 3 pages 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. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.

The protocol steps are highlighted. We cut some of what we would like to record to accommodate the increased word count.

9. Please ensure that the references appear as the following: [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. Source. Volume (Issue), FirstPage – LastPage (YEAR).] For more than 6 authors, list only the first author then et al.

The references have been modified to follow the specific example given here.

10. Please remove trademark (™) and registered (®) symbols from the Table of Equipment and Materials.

The Table has been updated with these changes.

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

This is a visually compelling and clear presentation of how to build a 3D model of a horse.

Major Concerns:

Scientifically it is not made clear how the joint locations are determined. They seem to be fit by eye rather than determined functionally (e.g. cadaver/in vivo analyses) or via more objective geometric measures (e.g. see Panagiotopoulou et al, 2016, PeerJ; which also used a Maya "digital puppet").

2 new references, including the suggested Panagiotopoulou paper, were added to describe similar methods on how joint positions were determined. Please see Lines 119-125. Details have also been added to the protocol with specifics about changing joint locations. Please note that the Panagiotopoulou paper reports data from the XROMM system in combination with Maya. The XROMM technology has been incredible at helping us understand movements in animals. However, the field of view of the resultant models is relatively small. As is the challenge with doing whole-body studies, to our knowledge there are currently no horse-size scanners that can incorporate full-body data holistically. It will all have to be done section by section. As these technologies do become available, our model will be ready and waiting to be a part of it.

Without access to the 3D geometry files and Maya files, the utility of this paper is very limited. It would be far more useful to readers if the science was 100% open and files shared to enable replication of the workflow. As the claim is made that this enables "accurate anatomical positioning and movement simulations" the issues of accuracy and replicability should be better addressed.

All the files on the model will be made available. We have already discussed this with our universities and plans have been made to release the model. The purpose of this paper is to let people know how the model was created, show what can be done with it, and how to use it. It is meant to be a tool for clinicians and researchers and thus is a

template that can be continually modified (please see Lines 85-90 in the revised manuscript). As such it can be updated to increase its accuracy to model a specific position or movement. For example, if they have anatomic or video data to make a joint position more accurate they can change the model to reflect it.

We do disagree, however, that the utility is limited if you don't have the specific files. The rigging described can be lightly modified to be used within any whole-body CT data. For example, using a similar protocol with just a few tweaks, we rigged a full human CT skeleton to be used as the rider.

The model is said to be dynamic, which means kinematic and kinetic (e.g. forces). But no forces are integrated into the model; including not masses, centers of mass or inertial tensors of body segments needed for such analyses. This needs to be addressed, or the paper restricted purely to discussing kinematics.

Thank you for this feedback. When we selected the term "dynamic", we were using the more general definition of dynamic (constant change, activity, or progress) as opposed to the physics field of dynamics focused on forces and movements. However, we realize that this term imbued certain characteristics on the model that we did not intend. So, we have removed the term from the title and throughout the paper and have specified the real purpose of the model: a whole-body realistic equine skeleton that can be moved and positioned and used for further analyses by researchers.

Many of the mentioned parameters vary depending upon the question being asked and the position being analyzed. However they can be added and the model modified to answer specific research questions. For example, we have used <u>static force analysis in the model</u> to determine if a hyperextended position of the foreleg often observed in horses with navicular disease (a common form of osteoarthritis) increased the forces on the navicular apparatus compared to the normal, more vertical position. Use of the model allowed force data from a variety of studies to be incorporated into the analysis. As proof of concept, we have demonstrated how it can be used with data collected from movement studies. We used a straight-forward method here (matching to still frames) but we are working on a project that incorporates 3D motion analysis data from surface markers with the model for a movement study. As we are finding this useful in our own research into understanding the complicated relationship of posture and movement in horses, we believe others will also benefit from having it. Indeed, when we have presented this at various meetings, people have asked when they would be able to get the model.

How was the scapular origin decided? Scapular rotation is important but tricky. Does it only rotate or translate too (as the real scapula does)? Are all joints 6 degrees of freedom?

We completely agree. All joints are given the range of motion that they are capable of producing, with constraints applied only to keep biologically un-realistic movements from happening (e.g., the scapula rotating medially to cross the ribs and enter the thorax, or the elbow to be bent backwards). The location of the joint in the scapula and how it has 6 degrees of freedom in this method is described in the protocol (Lines 123-125).

Sesamoids seem to be immobile and this should be noted as the patella and digital sesamoids not only move but may be major locations of pathology.

We agree with the importance of the sesamoids with regards to movement and pathologies. In the submitted video, the patellae were left stable because we were focusing on other movements that could be effectively matched with the 2D images. However, all of the sesamoids are moveable when the information of their movement is added in (e.g., from data collected from a surface marker). We added two sections in the protocol for sesamoid bone (general) and patella (specific) rigging to address your comment.

Statements like the model has the "ability to accurately demonstrate spine positions" are big claims- this implies that quantitative measurements of in vivo/ex vivo motion match those of the model, ideally with statistical tests. But here there is no evidence of this; it seems "accurate" is being used to mean "looks good". This should be clarified or amended-- as per the journal's criterion "efficacy of the protocol must be demonstrated".

The model does have the ability to accurately demonstrate spine positions when correct data is added into it. Often spinal movement is left out of models or it is considered to act as stiff rod when the animal moves. We have rigged this model to be able to (1) create a natural movement, in line with what studies suggest it actually does, using the ribbon spine method reported in the protocol, while also (2) maintaining freedom at each joint for specific manipulations that may become known as research continues.

It is currently not possible to add all the specific details on joint movements that would cover every possible position for all the joints in the body, as the data to do this does not currently exist. This paper is meant to present this model as a tool to facilitate the practical application of new data on joint movements as it is acquired. A great strength of the model is that it can be specifically modified depending upon the questions being asked. If very fine movements are being targeted the specific

parameters of interest can be added as needed based upon data collected from the individuals being modeled. Depending upon exactly what is being analyzed this data can come from measurements taken from the live animals and/or from 2D or 3D imaging.

There is a lot of individual variation in joint mobility. Joint mobility also varies with body position. This variability is difficult to capture using quantitative measurements, since not all possible combinations of body positions can be measured and even those that can be determined can practically only be measured on a limited number of animals. There are also additional problems in measuring joint function using cadavers because of both the disruption induced by the dissection needed and tissue changes after death. However, since this model can be modified it can reflect the variability of an individual by using images and anatomic markers to position the bones and joints. This is especially important in assessment of clinical patients that have pathology-induced limitations in their joint movements.

In addition, the ability to model the whole body of specific individuals can identify potential root causes of their mechanical dysfunction and tissue damage (i.e.: poor overall body balance).

Clear outline of the steps used to make the Video would be a benefit. It is unclear how 3D motions can be made from 2D lateral view images without substantial guesswork involved? The video looks great but this may mislead readers that it is more than a best guess at detailed motions.

Similar to how motion in animals is depicted by flipping a sequential series of still photographs, sequential 2D images were used to align the model and put it in motion. Because the positions of the various body parts have to remain stable in the lateral views their positions are thus also set in other views, which produces a 3D moving image. The only guesswork is on components that cannot be seen in the lateral 2D images. If these components are of interest, other views can be used.

The limits set up in the rigging of the model were based upon the range of motion for the postures depicted in the sequential photographs as well as anatomic measurements. This resulted in a more realistic motion than that produced by joint parameters measured out of context of the whole-body posture. We plan to further refine the accuracy of motion through both anatomic measurements and the depiction of joint function in the context of specific body postures.

The animation is described in section 5 of the Protocol and the Note (Lines 438-442) gives more information about the animation.

Minor Concerns:
The first paragraph too quickly switches to a focus on human OA; surely there is plenty of OA research on horses that is already doing very similar things such as unloading joints through targeted exercise etc.? (And we likely know a lot about the fundamental mechanisms from model organism studies, e.g. mice-- some citations here could help readers?)

> ***There is actually very little literature on individualized whole-body physical therapy for chronic arthritis in horses, which was a big factor in our developing this model; we did, however, add one source. Corrective shoeing is the main mechanically based therapy used in equine medicine, which has either led to or has been led by an intense focus on the foot. However, at best corrective shoeing can only deflect pathomechanical forces impacting the foot (i.e.: those impacting it from above due to movement in poor body balance) and usually provides only temporary relief.***
>
> ***The sad fact is that, in our experience, horses that potentially could have been rehabilitated through individually targeted physical therapy are being condemned as unsound and euthanized.***
>
> ***Because of this, our goal is to promote the development of individualized, physical therapy for horses. This therapy must be based upon assessment of whole-body function, which is an approach that has been overwhelmingly successful in the treatment human athletes, but has yet to be applied to horses. The power of our model is in how it can be used to assess whole-body function, as it can be manipulated to model an individual's posture in motion.***
>
> ***In terms of citations, we have focused on those documenting the successes of therapies based on correcting pathomechanical loading of the joints in humans. These therapies are based upon a whole-body functional perspective and correlate best with how the model can be used. We feel it is beyond the scope of this article to get into mechanistic studies of laboratory animals, important as they are for understanding the pathogenesis of mechanical tissue damage, when so much of the big picture of where the forces originate is being missed because assessment of whole-body function is not being done for horses.***

line 67- "anatomical and force analysis" recommend changing to "anatomical, kinematic and kinetic analyses".

> ***Thank you for this suggestion; we have made the change (Line 74).***

There have been quite a few 3D horse (fore)limb modelling studies already. At least a short literature review of what exists is warranted-- e.g. Brown, Harrison/Stover, Panagiotopoulou, O'Hare, others have done such models (although some just focused on distal limb). Line 363 claims "unique from other equine models that are artistic graphic recreations" but this does not seem true vs. these published studies, in terms of limbs or regions of limbs.

> ***All of these 3D models are of the distal limb because of the intense therapeutic focus on shoeing. Our model is unique because it is of the whole-body, and it is not designed by a graphic artist but is directly based upon actual CT data. We have emphasized the "whole-body" throughout the revision of the manuscript to make this clearer. However, we have cited the suggested literature and one suggested by another reviewer to show that there are CT models of specific areas available; we did not intend to argue otherwise. But none of these models or any others we have seen are equivalent to our whole-body model. It is also a unique feature and big strength of the model that it can be continually updated as needed by the users.***

11 references for a broad area of research seems too limited.

> ***This paper describes how a model was constructed and how it can be used in research and for assessment of clinical cases, but is not meant to be a review paper. The references we cited were to justify the approach and the methodology used.***

For CT scans resolution and scan settings should be provided. What is the subject? Male/female, age, mass, breed, etc.? Single or multiple individuals? This is important information.

> ***We've added the following paragraph (Lines 93-104):***
> ***"Due to practical limitations related to specimen availability (e.g., the ribs cut) and the scanner , the whole-body equine model is the result of merging data from three equine specimens. The model thus, is not a perfect representation of a single individual, but has been standardized to more broadly represent individual variability. In short, it is a template to be used and modified to suit the needs of researchers. CT scans of the trunk, head and neck, and limbs were acquired from two equine specimens of approximately the same size with a 64-Slice CT scanner using a bone algorithm, pitch of 0.9, 1 mm slice. CT scans of a set of ribs were acquired with a 64-Slice CT scanner using a bone algorithm, pitch of 0.9, .64 mm slices. Anatomic integrity of the bony joints (e.g., within the limb) was maintained. The soft tissues available in the CT scans were also used to confirm the placement of the bones. Since some whole ribs and the proximal portions of all the ribs were available and scanned on the thorax specimen, the separately scanned ribs could be accurately sized and placed within the whole-body skeletal model. The resulting CT DICOM data were imported into the 3D***

visualization software and individual bones were segmented into individual data sets (i.e., bone meshes). The individual 3D bone meshes were then imported into the 3D animation and modeling software where they were sized, if necessary, and assembled into a complete equine skeleton in preparation for rigging, a graphic method of connecting the bone meshes so that their movements are linked (Figure 1).”

Because this model is flexible and can be modified, parameters related to age, sex and breed as well as individual conformation, for example a club foot, can be added as needed by the users.

Reviewer #2:

Manuscript Summary:

A method for using Autodesk Maya to assemble a rigid body simulation of equine bone positions is presented. I think the model has some useful attributes in terms of understanding macro-scale bone movement during equine locomotion. The method for creating the model in Maya is sufficiently described. My only concern with this paper is that the authors claim that this can be used to understand loading of the bones (and implicitly cartilage) and that this model is unique in its ability to do so.

Major Concerns:

The loading of bone/musculoskeletal tissues is a function of not just the kinematic pose but also the specific muscle and soft tissue (e.g. ligaments) forces that the joint is subjected to. It's possible to have different loading scenarios with the same kinematic pose. As the authors state in the last paragraph of the discussion, the actual loading of the joint requires inclusion of 3D force analyses. This is not a trivial calculation and requires knowledge of inertial/mechanical parameters per bone segment as well as estimates of maximum force capacity by muscles. The inclusion of motion capture data with external force data and the development of a full 3d musculoskeletal model of the equine forelimb has been published by Harrison.

Harrison, S.M., Whitton, R.C., Kawcak, C.E., Stover, S.M. and Pandy, M.G., 2010. Relationship between muscle forces, joint loading and utilization of elastic strain energy in equine locomotion. Journal of Experimental Biology, 213(23), pp.3998-4009. I think it warrants inclusion in the discussion that these models exist - but admittedly can be difficult to use without experience. Checking the simtk website, an equine model does exist that is openly available (https://simtk.org/projects/horse).

The limitation of the cited example and other models is that they are of the limbs and do not consider the effects of whole-body posture. They are also mostly confined to analysis of the normal rather than the pathological gait. Our model allows a <u>comparative</u> analysis of the effects of posture in both normal (i.e.: not lame) versus lame animals. It can also be used to model a horse before and after successful physical rehabilitation to help specifically identify pathological postural positions. We agree that the 3D force analysis is difficult and time consuming but is necessary to truly understand the effects of posture and movement on the skeletomuscular system. This can be done statically and dynamically, depending on the research question and parameters used.

However, while force analysis is important, it is not the only factor in joint damage. Repetitive abnormal motion without dramatic increases in force also induces pathology. Our model can be used to assess both factors because it can be used for comparative (sound versus lame, before and after physical therapy) studies. Its use is dependent on the specific inputted data, a fact that we have done more to highlight in the revision.

Perhaps the intention here is to highlight the spinal kinematics (as included in the discussion) and this does seem more novel. But again, I would suggest that this is a kinematic pose estimation only that may be suggestive of changes in joint loading. However, the case study presented with the horse in different positions and the inclusion of an interpretation of forces is overstated as these are not known/estimated using kinematic data alone. Further, the model has not been validated for accuracy (line 383) as this would require some sort of validation via fluoroscopy or in comparison to motion capture data.

It is not the purpose of this paper to provide definitive data on any specific position, but rather to describe how this model was made, how to use it, and its potential applications for research and in the clinics.

While absolute force data is important, evidence of compressive tissue damage in places that are predicted through the use of our model is arguably definitive. The horse used in the example had evidence that pathological compressive forces had impacted the foot shown as being overloaded by the transversal vertebral rotation. Furthermore, when the transversal rotation was corrected through physical therapy, the hoof pathology was resolved.

Unfortunately the technology does not exist in a format that can be used on the whole body of horses for validation purposes. We hope that our model, which provides a unique whole-body functional perspective, will facilitate validation studies by identifying targets for these types of studies.

It's also not clear to me what degree of accuracy is required to assess clinically significant aberrations in movement. How many degrees of malrotation would one expect and can this be measured/detected by aligning the Maya model to 2d pictures?

The model as shown has the flexibility to be very closely aligned and its accuracy depends on the images, either 2D or 3D that are used to align it. Motion analysis data (i.e., points in three-dimensional space) could also be used.

It should be noted that joint dysfunction and damage is often due to abnormal loading forces that were generated at another site in the body. The unique power of this model is not in the ability to measure the specifics of the joint dysfunction (although it

has the capacity to do this), but in its ability to identify whole-body postural abnormalities that are the <u>root cause</u> of the joint overloading and resulting dysfunction.

In summary, I find the kinematic pose estimation useful - and I agree that it can provide some insights into different pathologies provided the kinematic differences are large enough to be measured on the 2d images that are used to pose the model (if I have misunderstood how the poses are estimated, this should be clarified). However, I think the leap forward that this then can be used to for joint loading analyses (and is unique (line 363)) is overstated and should be reconsidered.

Although 2D images were used in the examples shown, 3D data can also be used to align the model, so it has the potential to be very closely aligned to the whole-body postures of individual horses. We are not arguing that other CT models exist (we agree that there are many of the distal limbs as all of the reviewers' examples show), but rather that our whole-body model based on CT data is unique.

In terms of assessing joint loading forces, this model is unique in that it allows comparative analysis of the effects of whole-body posture on the forces impacting a specific limb or joint. Such comparative analysis of the forces is critical to answer the question of whether a particular posture within a movement increases the mechanical forces on the specific joint that has been damaged.

Comparative analysis can be done through static force analysis, which is powerful because it allows force data from a variety of studies to be incorporated into the analysis of a specific posture. It is the ability to incorporate the effects of variations in body posture into the force analysis of joints that makes this model unique. However, each posture of interest must be separately analyzed using all the available information, including from other studies. The model will only be as correct as the data that is input by skilled researchers.

The ability to input data from other studies is a unique strength of this model as this capacity allows it to be continually updated by the users.