Supplementary Materials & Methods

Estimation of tendon slack length from experimental measures

Tendon slack length was estimated from experimental measures of muscle and tendon morphology as follows. First, the maximum isometric force along the tendon was calculated from the maximum force along the fiber and the pennation angle at optimal fascicle length, θ_{OFL}, according to the equation:

$$F_{maxT} = F_{max} \cos\theta_{OFL}. \quad \text{(S1)}$$

The passive force of the muscle exerted on the tendon in the experimentally measured posture was found from the normalized passive muscle force as a function of normalized fiber length curve, $f_{np}(nFl)$ [104,105]. This was first scaled, for each muscle, by the maximum isometric force along the tendon, F_{maxT},

$$f_{muscP} = F_{maxT} f_{np}(nFl). \quad \text{(S2)}$$

By normalizing the experimentally measured average fiber length by the muscle's optimal fascicle length, we could calculate the normalized fiber length of the muscle in the fixed posture, *nFl*, allowing us to solve equation (S2) for the passive force, f_{muscP}, each muscle exerted on the tendon in the experimental posture. Since the three heads of the gastrocnemius attach to the Achilles tendon, the passive force of each muscle was calculated separately and summed. As the gastrocnemius intermedia head makes up ~10% of the total gastrocnemius muscle by volume, the passive contribution of this muscle was not experimentally determined for each bird but was estimated from values previously collected [61]. The passive force exerted by the muscle must be balanced by an equal tendon force, thus, the summed passive muscle forces equal the passive force the tendon experienced in the experimental posture.

The MTU lengths, L_{MTU}, were measured on the fixed limbs by digitizing the three-dimensional paths of the MG and LG from their origins on the tibiotarsus and femur, respectively, to the insertion of the Achilles tendon on the hypotarsus. This approach inherently includes the aponeurosis in the overall tendon length. Digitizing was done using a digitizing arm (Microscribe 3DX, Immersion, San Jose, CA). The MTU path was described by 11 points. The linear distances along the MTU path were summed to obtain an overall MTU length. This experimentally measured MTU length, L_{MTU}, is the sum of the measured fiber length, L_M, the tendon's slack length, L_T, and length change in the tendon (tendon stretch) due to passive muscle fiber force. The length change in the tendon due to passive muscle fiber force can be described as the tendon strain, ε_T, times its tendon slack length, L_{T0}.

$$L_{MTU} = L_M + L_T + L_T\varepsilon_T \quad \text{(S3)}$$

The strain in the tendon due to the passive muscle fiber force, ε_T, was calculated using the experimentally measured tendon force-displacement curve. The tendon force-displacement curve was normalized by tendon length to generate a force-strain curve.

$$f_T = g(\varepsilon_T). \quad \text{(S4)}$$

The strain at which the tendon force is equal to the passive fiber force can then be found from the inverse of equation (S4) and the passive muscle force, f_{MP} .

$$\varepsilon_T = g^{-1}(f_{MP}). \tag{S5}$$

The tendon slack length, for each muscle, then, can be calculated from equations (S4) and (S5).

$$L_{T0} = \frac{L_{MTU} - L_M}{(1 + \varepsilon_T)} \tag{S6}$$

Development of subject specific models

To perform system level analyses, we modified the generic OpenSim guinea fowl model [61] to generate subject-specific models for each individual. First, the generic model was scaled to match the measured bone lengths and body mass for each bird and saved as distinct models. In each subject specific model, the generic LG and MG maximum isometric force, pennation angle, optimal fascicle length and tendon slack length were modified to match the experimentally measured and calculated properties.

The moment arms of the LG and MG acting at the ankle was fit to experimental values by adjusting the of the size and orientation of the cylindrical wrapping surface for the Achilles at the ankle. During the trial-and-error fitting process, the radius, translation, and rotation of the wrap surface was modified, and the resulting moment arm was compared to the experimentally collected data at 31-34 points across the experimental range with a mean moment arm normalized root mean square of the error (Figure S1A) of 0.009 ± 0.007.

Additionally, the tendon force-strain curve was updated to match experimentally collected force-strain values. Because OpenSim scales the tendon force-strain curve by the maximum isometric force of the muscle, each tendon force-strain curve was normalized by the maximum isometric force capacity of the LG or MG, respectively. The parameters of the Millard muscle model's tendon force-strain curve were iteratively varied for both the LG and MG and compared to the experimental curve for each individual, resulting in an average root mean square error (normalized by tendon force) over 26-31 points for each tendon force-length curve (Figure S1B) of 0.061+0.025.

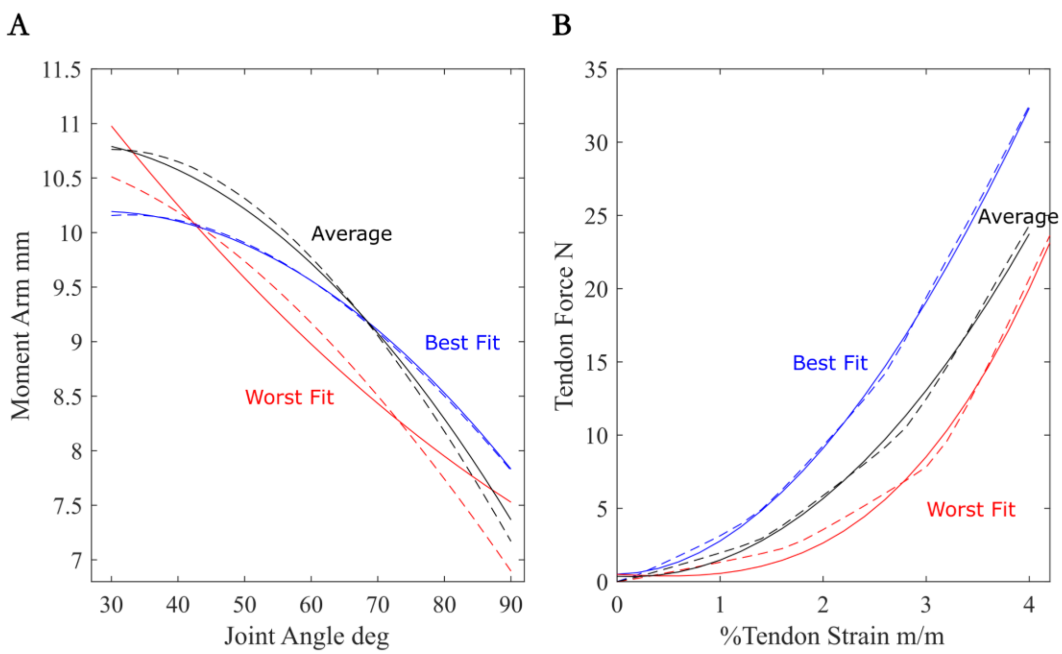

Fig. S1. Example comparisons between experimental (solid lines) and modeled (dashed lines) moment arms (A) and tendon force-strain curves (B). In each plot, experimental and modeled curves are displayed for three animals, showing the best, the average and the worst fit across individuals.