1 **Online Resource 1 for:**

2 Aging and the effects of a half marathon on Achilles tendon force-elongation

3 relationship.

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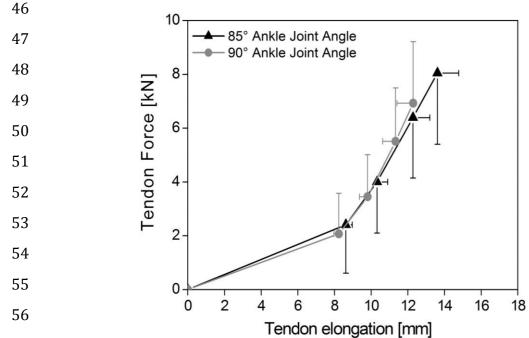
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14 **Methodological Considerations and Pilot Data**

15 Before conducting the current study we performed several pilot studies in order to test 16 the accuracy of the current method to examine the mechanical properties of the AT. 17 While a 90 degree knee position, with more secure fixation of the limb, may result in 18 much less ankle joint angular rotation than straight knee set ups (average changes of 19 14-18 degrees at the ankle joint have been reported when using a fully extended knee; 20 see e.g.: Arampatzis et al., 2005; Karamanidis et al., 2005), examining the force-21 elongation relationship of the tendon in this way could potentially place the GM in a 22 less favorable position on the force-length relationship, when compared to a fully 23 extended knee. This might be problematic, since we calculated the net joint moment 24 at the ankle joint, but measured the elongation of the GM tendon. Moreover, the AT 25 cannot be considered as a single tendon that extends uniformly during plantarflexor 26 contractions, which might lead to an erroneous calculation of the stiffness in absolute 27 terms. In order to address this drawback we conducted a pilot study using a more 28 dorsiflexed ankle joint position (85 degrees ankle joint ankle). As a result we lengthened the entire TS MTU and in particular, tensed the GM tendon, causing a 29 30 rightwards shift in the force-length relationship of the contractile element of the 31 gastrocnemii. This increased its force potential and hence the contribution of the GM 32 to the net joint moment (Arampatzis et al., 2006). In this pilot study, with 10 healthy

33 subjects, we compared an ankle angle of 90 degrees to the dorsiflexed ankle joint 34 angle of 85 degrees. An ankle angle of 90 degrees resulted in a significantly (P <35 0.05) lower ankle joint moment (90 degrees: 277 ± 35 N·m vs. 85 degrees: 322 ± 46 36 N·m; mean and SD) and maximal tendon elongation (90 degrees: 12.3 ± 2.3 mm vs. 37 85 degrees: 13.6 ± 2.8 mm) compared to a dorsiflexed ankle joint angle of 85 degrees. 38 However, there was no significant difference in tendon stiffness between the two setups (85 degrees: $1219 \pm 461 \text{ N} \cdot \text{mm}^{-1} \text{ vs. } 90 \text{ degrees: } 1210 \pm 283 \text{ N} \cdot \text{mm}^{-1}$; see Fig. 39 40 S1). Therefore, while a flexed knee joint reduces triceps surae force generation 41 capacity, and the contribution of the gastrocnemii to this force potential (decreasing 42 total tendon lengthening) and, in addition, might place the GM tendon in more slack 43 position, it appears not to lead to changes in the force-length relationship of the 44 tendon during loading.





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Fig S1 Force-length relationship (mean and SD) of the tendon measured with an ankle joint angle of 90 degrees (foot perpendicular to the shank) and a more dorsiflexed angle of 85 degrees, each with a knee joint angle of 90 degrees (thigh perpendicular to shank), for 10 healthy young subjects. No significant differences in force-elongation relationship of the tendon were found when comparing the ankle joint angles (tendon stiffness at 85 degrees: 1219 ± 461 Nmm⁻¹ vs. tendon stiffness at 90 degrees: 1210 ± 283 Nmm⁻¹).

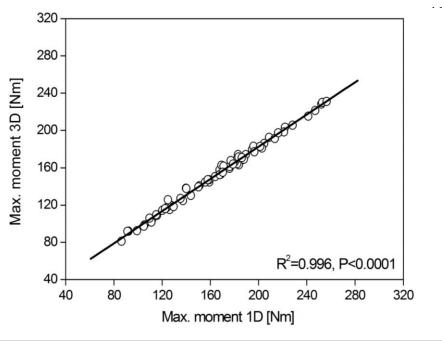
64 In a second pilot study, with nine healthy subjects, we compared ankle joint angle 65 changes during isometric contractions estimated using different calculation methods. 66 The ankle joint angle changes in the sagittal plane (2D analysis), calculated by inverse 67 kinematics using reflective markers fixed at the lower extremity of the subjects 68 acquired by a motion capture system (120Hz, Qualisys, Gothenburg, Sweden) 69 together with a force plate (1080 Hz, 400 x 600 mm, Bertec, Columbus OH, USA), 70 were compared to the method used in the current study that estimated ankle joint 71 angle changes based on data from a potentiometer located under the heel (current 72 method). The potentiometer used in the current study measured any heel elevation and, 73 thereby, calculated changes in the ankle joint angle from rest until maximal (Fig. 1 of 74 the original manuscript) plantarflexion moment via the inverse tangent of the ratio of 75 the heel lift to the distance between the head of the fifth metatarsal bone and the 76 potentiometer axis. Our pilot study found this to be in accordance with the motion 77 capture results, with an absolute difference of less than 1.1 degrees in ankle joint 78 angle changes during maximal isometric plantarflexion contractions (from rest to the 79 maximal ankle joint moment; current method: 3.8 ± 1.1 degrees; 2D analysis: $4.9 \pm$ 80 1.4 degrees).

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82 In order to investigate the influence of ankle joint-dynamometer axis misalignment 83 during contractions, which is not accounted for in the current setup, we examined the 84 maximal anterior displacement of the ankle joint axis in our pilot subjects during 85 maximal isometric plantarflexion contractions. The maximal anterior shift of the 86 ankle joint axis during contraction was on average 3.4 ± 2.1 mm, thereby leading to 87 an overestimation of our calculated joint moments. However, relating this anterior shift to the moment arm of the ground reaction force (GRF) acting about the ankle 88 89 joint, the relative change was approximately 1.7 %. Hence, this drawback has only a 90 negligible effect on our joint moment calculations. Furthermore, the resultant ankle 91 joint moments in the current study were calculated using the vertical component of 92 the GRF, neglecting any force vector direction changes during contraction. Using our

93 pilot data, we compared the maximal ankle joint moments calculated by the vertical 94 component of the GRF to the moments calculated using all three dimensions of the 95 GRF. The maximal ankle joint moment calculated using the vertical component was 96 on average 8% higher compared to the 3D calculations (157.8 \pm 47.7 N·m vs. 146.3 \pm 97 40.9 N·m). However, there was a significant correlation in maximal ankle joint moment between the two methods ($R^2 = 0.996$, P < 0.01; see Fig S2) showing that the 98 99 relative difference between methods was constant, and that calculating the ankle joint 100 moments during isometric contractions using only the vertical component of the GRF 101 is a valid method.

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Fig S2 Relationship between the maximal ankle joint moments measured during a isometric voluntary plantarflexion contraction calculated by talking into account solely the vertical component of the ground reaction force (1D) and by considering the 3D vector of the of the ground reaction force (3D).

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One might argue the fact that we calculated the secant stiffness and not the tangential stiffness of the tendon. Currently the tendon stiffness is determined by the divided change in force by change in elongation from 30-80% of MVC and, thus, only two data points are used to examine the tendon stiffness. However, using the current 124 method, we have a clear steady state in the ultrasound image at target force making 125 digitalization easier than in dynamic contractions, when the tendon deforms quite 126 rapidly during MVC which is in particular problematic when using ultrasound 127 sampling frequencies lower than 30 Hz. By using a constant force held by the subjects 128 for a given time, we may be better able to exclude potential measurement errors due 129 to the ultrasound sampling frequency or small time delays of synchronization between 130 ultrasound and force data when performing ramp contractions. In addition, we were 131 able to negate the effect of loading rate dependency on tendon strain, because the 132 object of interest (in this case a tendon) is given enough time to respond to the applied 133 force (Oberländer et al. 2015). Moreover, before applying the current method we 134 extensively tested this method and compared the calculation of the secant tendon 135 stiffness during sustained contractions with the tangential tendon stiffness during slow 136 and fast isometric ramp contraction and hence using a set of data points digitized over 137 the entire path of the length changes. Although, the stiffness of the tendon is slightly 138 affected by the contraction type, potentially due to the viscoelastic properties of the 139 tendon, we found a similar day-to-day reliability in tendon stiffness between slow 140 ramp contractions and our sustained contraction method, when a skilled and 141 experienced investigator tracked the data points used in the current study (Oberländer 142 et al. 2015).

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