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Constant force muscle stretching induces greater acute deformations and changes in passive mechanical properties compared to constant length stretching

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ABSTRACT

Stretching is applied to lengthen shortened muscles in pathological conditions such as joint contractures. We investigated (i) the acute effects of different types of stretching, i.e. constant length (CL) and constant force (CF) stretching, on acute deformations and changes in passive mechanical properties of medial gastrocnemius muscle (MG) and (ii) the association of acute muscle–tendon deformations or changes in mechanical properties with the impulse or maximal strain of stretching. Forty-eight hindlimbs from 13 male and 12 female Wistar rats (13 weeks old, respectively 424.6 ± 35.5 and 261.8 ± 15.6 g) were divided into six groups ($n = 8$ each). The MG was initially stretched to a length at which the force was 75%, 95%, or 115% of the force corresponding to estimated maximal dorsiflexion and held at either CF or CL for 30 min. Before and after the stretching protocol, the MG peak force and peak stiffness were assessed by lengthening the passive muscle to the length corresponding to maximal ankle dorsiflexion. Also, the muscle belly length and tendon length were measured. CF stretching affected peak force, peak stiffness, muscle belly length, and tendon length more than CL stretching ($p < 0.01$). Impulse was associated only with the decrease in peak force, while maximal strain was associated with the decrease in peak force, peak stiffness, and the increase in muscle belly length. We conclude that CF stretching results in greater acute deformations and changes in mechanical properties than CL stretching, which appears to be dependent predominantly on the differences in imposed maximal strain.

1. Introduction

Contractures are joint dysfunctions in which muscles spanning the joint are pathologically shortened. Clinically, they are characterized by a forced joint position, decreased joint range of motion (ROM), and increased joint stiffness (Nuckolls et al., 2020). Contractures can occur in a wide range of joints and are caused by various chronic (e.g. Duchene, brain injury) or acute (e.g. fractures) conditions (Fergusson et al., 2007). They can lead to a significant burden on patients due to decreased independence in activities of daily living and a decreased quality of life (Nuckolls et al, 2020). Muscle stretching is frequently used to treat contractures through modalities like casting, orthoses, automated stretching devices or physical therapy (Craig et al., 2016; Kalkman et al., 2018; Katalinic et al., 2017; Lecharte et al., 2020; Magnusson, 1998). Stretching in patients with contractures has been shown to acutely increase joint ROM, decrease joint stiffness (Gao et al., 2011; Rao et al., 2021;) and increase muscle belly length (Theis et al., 2013). Studies in rodents have also shown that stretching acutely decreases peak passive muscle force (Ishikura et al., 2015). These acute deformations and changes in mechanical properties elicit tissue adaptation if stretching is applied long-term, as shown in humans by the increase in joint ROM and fascicle length, and the decrease in joint and muscle stiffness (Costa et al., 2012; Kalkman et al., 2019; Selles et al., 2005; Theis et al., 2015) and in rodents by the increase of joint ROM (Usuba et al., 2007), muscle mass and sarcomere number in series (De Jaeger et al., 2015).

Muscles can be stretched by imposing a constant length (CL) or a constant force (CF). Both types of stretching have been used clinically and in human and animal studies, but the mechanical characteristics are different due to the non-linear behavior of viscoelastic tissues (Fung,

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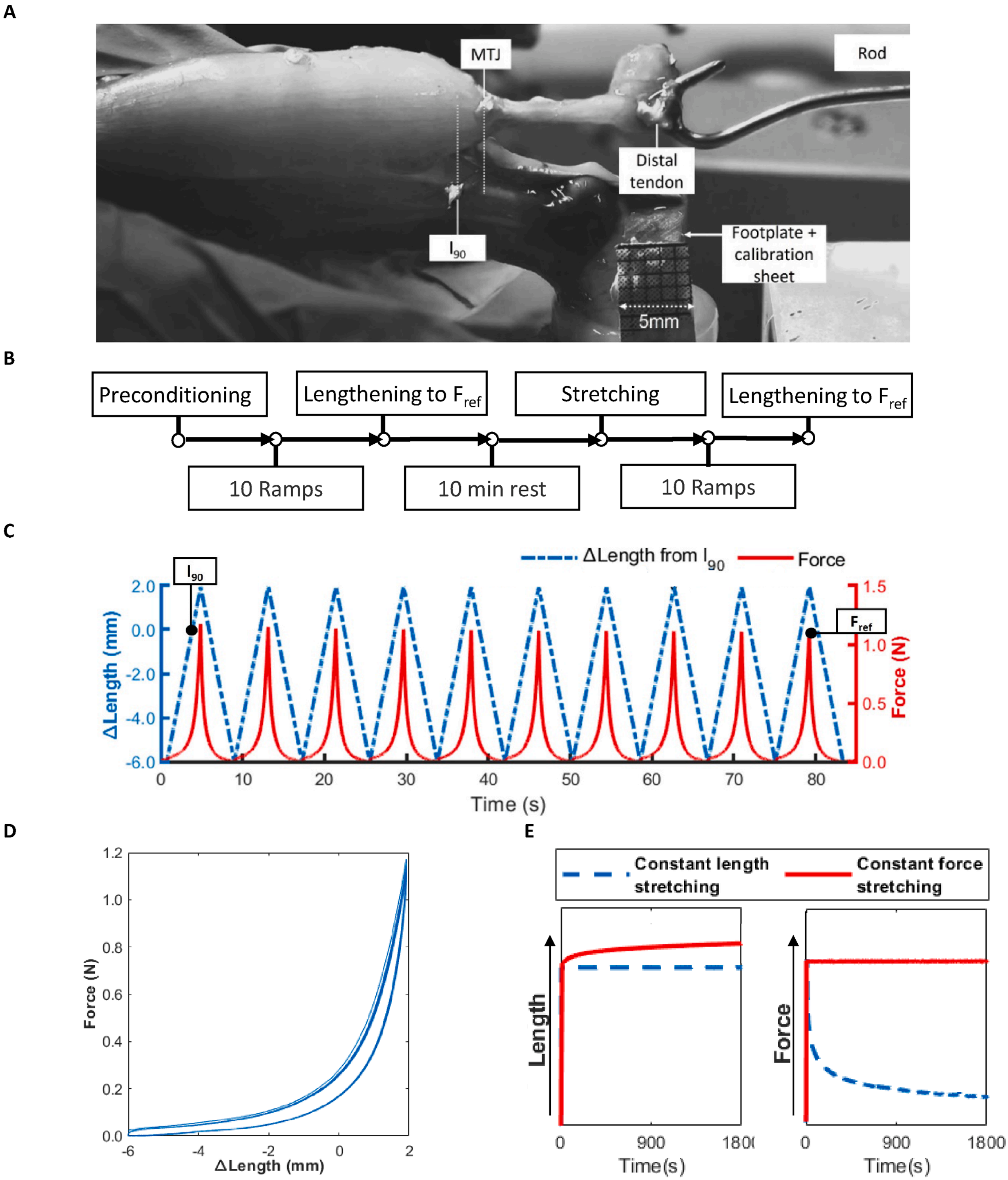

Fig. 1. **(A)** A videoframe of the medial side of a medial gastrocnemius (MG) the instant it was lengthened 2 mm past its length at 90° ankle flexion. The muscle–tendon junction (MTJ) marker and the reference marker on the flexor hallucis longus, which represented the horizontal position of the MG MTJ at 90°ankle flexion (l_{90}) are shown and the distance is indicated by the vertical white dotted lines. **(B)** Timeline of the experimental protocol. **(C)** Example of Δlength data with l_{90} as reference length and filtered force data (10 Hz low-pass) of ten lengthening ramps of the MTU from 6 mm under to 2 mm over l_{90}. The peak force of the 10th ramp prior to the stretching protocol was defined as the reference force (F_{ref}) and used to determine the stretching intensity. Similar ramps were performed after the stretching protocol. **(D)** Typical length–force loops generated from the length and force data of the 10 ramps in B. The slope (i.e. derivative of force over length) indicates MTU stiffness. **(E)** Length (left) and force (right) data for constant length (blue dashed) and constant force (red) stretching. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1

Absolute medial gastrocnemius muscle and tendon mass and length per stretching group with the knee joint in maximal extension and the ankle joint at 90° (l_{90}).

		Stretching group					
		75% Constant length	**95% Constant length**	**115% Constant length**	**75% Constant force**	**95% Constant force**	**115% Constant force**
Muscle	**Mass (mg)**	1000 ± 255	1012 ± 216	1004 ± 242	1022 ± 267	947 ± 179	1020 ± 243
	Length (mm)	353 ± 27	362 ± 29	352 ± 30	358 ± 34	358 ± 20	365 ± 32
Tendon	**Mass (mg)**	12.4 ± 2.63	10.5 ± 2.93	11.6 ± 1.72	11.8 ± 3.28	11.2 ± 4.49	10.8 ± 2.93
	Length (mm)	92 ± 12	90 ± 20	95 ± 8	92 ± 7	96 ± 6	90 ± 12

Means ± SD are shown per group ($n = 8$), group means were not significantly different.

Table 2

Pre and post stretch muscle tendon unit (MTU) passive peak force and peak stiffness and muscle and tendon lengths at reference force (F_{ref}) for each stretching group.

		Stretching group					
		75% Constant length	**95% Constant length**	**115% Constant length**	**75% Constant force**	**95% Constant force**	**115% Constant force**
MTU peak force (N)	**Pre**	1.3 ± 0.6	1.2 ± 0.6	1.2 ± 0.7	1.2 ± 0.5	1.1 ± 0.3	0.9 ± 0.3
	post	1.1 ± 0.5***	1.0 ± 0.4**	0.9 ± 0.5**	0.8 ± 0.3***	0.7 ± 0.2***	0.6 ± 0.2***
MTU peak stiffness (N/mm)	**Pre**	1.0 ± 0.4	0.9 ± 0.4	0.9 ± 0.5	0.9 ± 0.3	0.9 ± 0.2	0.7 ± 0.2
	post	0.9 ± 0.4***	0.8 ± 0.4**	0.7 ± 0.4**	0.7 ± 0.3***	0.5 ± 0.2***	0.4 ± 0.2***
Muscle length F_{ref} (mm)	**Pre**	36.4 ± 3.0	38.0 ± 3.2	38.1 ± 3.1	37.6 ± 2.9	37.4 ± 2.3	37.0 ± 2.5
	post	36.6 ± 3.0***	38.2 ± 3.3***	38.3 ± 3.1**	37.8 ± 3.0***	37.7 ± 2.3***	37.4 ± 2.6***
Tendon length F_{ref} (mm)	**Pre**	10.0 ± 2.1	9.1 ± 1.6	8.6 ± 1.1	9.4 ± 1.3	9.89 ± 0.8	10.4 ± 1.2
	post	10.1 ± 2.1***	9.2 ± 1.7**	8.7 ± 1.2**	9.6 ± 1.3***	10.1 ± 0.8***	10.6 ± 1.2***

Means ± SD are shown per group ($n = 8$). Significant difference between pre and post stretching data, ** $p < 0.01$, *** $p < 0.001$.

1967). Stretching with a CL causes force in the stretched structures to decrease over time (i.e. stress relaxation). Alternatively stretching with a CF results in a slowly increasing length over time (i.e. creep). There is some evidence in humans that a single session of CF stretching acutely increases ROM and decreases peak torque more than CL stretching (Konrad et al., 2017; Yeh et al., 2007). It has not yet been investigated if acute deformations and passive mechanics of the muscle–tendon unit (MTU) are affected differently by each of the stretch types.

Besides stretch type, two factors have been proposed (Fukaya et al., 2021; Kruse et al., 2021) to explain and be associated with the effects of muscle stretching: 1) the impulse (i.e. volume) of the stretch, as assessed by the force times the duration of stretching, and 2) the maximal MTU strain of stretching. How these factors affect the deformations and changes in mechanical properties in response to stretching and if they are dependent on the type of stretch has not been investigated.

The first aim of this study was to investigate the acute effects of CF and CL stretching on deformations and the changes in passive mechanical properties of rat medial gastrocnemius muscle (MG). The second aim was to assess the association of acute muscle–tendon deformations or changes in mechanical properties with the impulse or maximal strain of stretching. For this purpose, we used an animal model in which the MG was stretched in situ for 30 min imposing either a CL or a CF at various initial stretch intensities.

2. Methods

2.1. Study design & animals

Data were obtained from both hindlimbs of 25 Wistar rats (12 females: 261.8 ± 15.6 g, 13 males: 424.6 ± 35.5 g), 24 left and 24 right legs were equally divided by stratified random sampling among six groups (three initial stretch intensities for both CF and CL, see below) with research randomizer V4.0 (Urbaniak & Plous 2013). Due to experimental failure, one experiment was repeated in a male rat. Surgical and experimental procedures were approved by the Central Authority for Animal Experiments of the Dutch Government (Permit Number: FBW- AVD11200202114471) and were performed at the Vrije Universiteit Amsterdam according to the guidelines and regulations concerning animal welfare and experimentation set forth by the Dutch law on Animal Research in full agreement with the Directive 2010/63/EU with local approval by and under supervision of the local Animal Welfare Body. Animals were anesthetized according to standard procedures in our laboratory (Maas et al., 2001) by an intraperitoneal injection of urethane solution (1.2 ml/100 g body mass, 12.5% urethane solution). Extra doses (0.2 > 0.5 ml) were administered if necessary, i.e. until full suppression of withdrawal reflexes to a pain stimulus (pinching the front paw). Animals were placed on a heating pad to maintain a body temperature of 37° and subcutaneous injections (1 ml) of NaCl solution (0,9%) were administered every 1–2 h to prevent dehydration. Heart rate, body temperature and withdrawal reflexes were monitored every 10–20 min throughout the experiment. At the end of the experiment, animals were killed with intracardially injected pentobarbital sodium (Euthasol 20%) and a double-sided pneumothorax.

2.2. Surgical procedures

Hindlimbs and lower back were shaved. The posterior crural compartment was exposed by removing the skin and biceps femoris muscle. The MG was carefully separated from the lateral gastrocnemius (LG) and soleus (SOL) muscles. Markers were placed at the distal muscle–tendon junction (MTJ) of MG and the distal end of the tendon (Fig. 1A). LG, SOL and plantaris sub-tendons (Finni et al., 2018) were cut at the MTJ. The sciatic nerve was exposed and the sural, peroneal, tibial, SOL and LG nerve branches were severed. The femur was exposed and rigidly secured with a metal clamp, the foot was attached to a plastic plate with tie wraps. With the knee joint in maximal extension and the ankle in 90°, a reference marker was placed on the tendon of the flexor hallucis longus at the same horizontal position as the MTJ of MG. These markers were used to define the reference MTU length (l_{90}) during the experiment. The calcaneus was severed from the rest of the foot and connected to a stainless-steel rod with sewing thread. Lastly, a bipolar cuff electrode was placed on the MG nerve branch.

Table 3
Final regression models resulting from the linear mixed models (LMM) that tested the effect of fixed categorical predictors type and stretch intensity on peak force decrease (ΔPF), peak stiffness decrease (ΔPS), changes in muscle length (ΔML) and tendon length (ΔTL), the impulse (J) and maximal strain of stretching (ε_{max}). LMMs were performed and reported per stretch type in case of a significant interaction. In case of no interaction LLMs were performed and reported without interaction. Constant length stretching and 75% stretch intensity were set as reference. 95% stretch intensity was set as reference for 115%-95% data.

Peak force decrease (ΔPF)	**Estimate**	**95% CI**	**t**	**p**
constant length stretching				
Intercept	21.26	19.50, 23.02	23.72	<0.001
Intensity: 95%-75%	2.51	−1.79, 6.81	1.14	0.266
Intensity: 115%-75%	8.95	4.65, 13.26	4.08	<0.001
Intensity: 115%-95%	6.44	2.14, 10.75	2.93	0.008
constant force stretching				
Intercept	34.47	32.68, 36.70	34.15	<0.001
Intensity: 95%-75%	8.72	3.95, 13.50	3.58	0.002
Intensity: 115%-75%	10.31	5.55, 15.10	4.24	<0.001
Intensity: 115%-95%	1.60	−2.60, 5.79	0.747	0.466
Peak stiffness decrease (ΔPS)				
constant length stretching				
Intercept	13.83	11.69, 15.97	12.68	<0.001
Intensity: 95%-75%	1.87	−3.36, 7.11	0.70	0.491
Intensity: 115%-75%	10.47	5.24, 15.71	3.92	<0.001
Intensity: 115%-95%	8.60	3.36, 13.83	3.22	0.004
constant force stretching				
Intercept	33.00	30.43, 35.50	25.58	<0.001
Intensity: 95%-75%	10.20	4.09, 16.30	3.27	0.004
Intensity: 115%-75%	15.10	9.02, 21.30	4.85	<0.001
Intensity: 115%-95%	4.92	−0.82, 10.67	1.68	0.112
Muscle length change (ΔML)				
constant length stretching				
Intercept	0.50	0.43, 0.56	14.51	<0.001
Intensity: 95%-75%	0.07	−0.10, 0.23	−0.82	0.421
Intensity: 115%-75%	0.13	−0.03, 0.29	1.55	0.136
Intensity: 115%-95%	0.06	0.08, −0.10	0.73	0.474
constant force stretching				
Intercept	0.89	0.78, 1.00	15.78	<0.001
Intensity: 95%-75%	0.23	−0.04, 0.50	1.66	0.111
Intensity: 115%-75%	0.46	0.19, 0.73	3.32	0.003
Intensity: 115%-95%	0.23	−0.04, 0.50	1.66	0.112
Tendon length change (ΔTL)				
Intercept	1.50	1.25, 1.75	11.67	<0.001
Stretch type difference	0.72	0.22, 1.22	2.81	0.007
Intensity: 95%-75%	0.54	−0.08, 1.16	1.72	0.093
Intensity: 115%-75%	0.44	−0.17, 1.06	1.41	0.165
Intensity: 115%-95%	−0.10	−0.71, 0.52	−0.31	0.761
Impulse of stretching (J)				
Intercept	1420	1278.8, 1561.0	19.70	<0.001
Stretch type difference	742	472.4, 1012.0	5.39	<0.001
Intensity: 95%-75%	224	−114.8, 562.0	1.30	0.203
Intensity: 115%-75%	304	−29.7, 637.0	1.79	0.081
Intensity: 115%-95%	80	−253.0, 413.0	0.47	0.641
Maximal strain of stretching (ε_{max})				
Intercept	0.0546	0.0532, 0.0560	75.64	<0.001
Stretch type difference	0.0263	0.0238, 0.0287	21.12	<0.001
Intensity: 95%-75%	0.0084	0.0052, 0.0116	5.11	<0.001
Intensity: 115%-75%	0.0152	0.0121, 0.0182	9.72	<0.001
Intensity: 115%-95%	0.0068	0.0038, 0.0099	4.36	<0.001

Estimates, confidence intervals (CI), t- and p-values are shown per final regression model.

2.3. *Experimental procedures*

The rod connected to the calcaneus was attached to the arm of a servomotor (AURORA Scientific dual motor 309C, Ontario Canada). A camera (Panasonic, HC-V720, 30 Hz, 1920x1080, 20.4MP) was placed perpendicular to the line of pull of MG to capture the displacement of the markers during the measurements. Before acquiring data, pre-conditioning contractions were performed to remove any slack in the connection between MG and the servomotor without exposing the muscle to high lengths. The MG nerve was stimulated supramaximally for 300 ms (0.03–2 mA, 120 Hz, pulse width: 100 µs) to elicit a tetanic contraction (Roszek et al., 1994). Hereafter the MG nerve branch was severed. The following lengths were measured at l_{90} by calipers: 1) MTU ($_{MTU}l_{90}$), from the posterior surface of the proximal part of the medial condyle (i.e. origin of MG) to the distal tendon marker, 2) distal tendon, from the MTJ to the distal tendon marker. MG muscle belly length at l_{90} was calculated by subtracting MG tendon length at l_{90} from $_{MTU}l_{90}$.

2.4. *Experimental protocol*

The protocol consisted of the following steps (Fig. 1B): pre-conditioning contractions, 10 stretch–shortening ramps, lengthening to reference force (F_{ref}), 10 min rest, 30 min stretching, 10 stretch–shortening ramps, and lengthening to F_{ref}.

For pre-conditioning, the MG nerve was stimulated supramaximally at MTU lengths between eight and four mm under l_{90}, until the force output stabilized between four and six N (≈40% of maximal active force exertion, derived from pilot data). For the ramps, the MG was lengthened and shortened 10 times by 8 mm (2 mm/s), from 6 mm below to 2 mm over l_{90} (Fig. 1C). This length (l_{90} + 2 mm) corresponds approximately to the MTU length at maximal ankle dorsiflexion with the knee in maximal extension. This length was assessed in pilot experiments by placing another reference marker on the flexor hallucis longus tendon at the same horizontal position as the MTJ during maximal dorsiflexion, whereafter the distance between the two reference markers was measured. For lengthening to F_{ref}, the peak force of the 10th ramp prior to stretching was assessed. MG was lengthened (2 mm/s) to F_{ref} once while videos were recorded for assessment of muscle and tendon lengths. During the 10 min rest, MG was shortened to 6 mm under l_{90}. Stretching was performed for 30 min at an initial stretch intensity of 75%, 95% or 115% of F_{ref}. For stretching, passive MG was lengthened until the force measured matched the force calculated for the selected intensity. Then, either the force was held constant (CF stretching) or the MTU length was kept constant (CL stretching) for a duration of 30 min (Fig. 1E). At the end of the experiment, the muscle belly and distal tendon were extracted and weighed. Force and displacement data were stored on a computer using Spike2 V7.18 software (CED, Cambridge, UK) at a sampling rate of 200 Hz.

2.5. *Data analysis*

Force and displacement data were analyzed with a custom script in MATLAB (R2020a). All force and displacement data were filtered with a 10 Hz lowpass, 2nd-order Butterworth filter. Peak force was identified for each force curve for each ramp. Length-force loops were generated from the pre- and post-stretching ramps (Fig. 1 D). Stiffness was calculated as the numerical derivative (finite difference) of force as a function of length from each ramp. Peak stiffness was identified as the maximum stiffness of each ramp. The 10 pre- and post-stretching peak force and peak stiffness values of each ramp were averaged. The impulse (J) of stretching, defined in Eq. (1) was calculated through numerical, trapezoidal integration.

$$J = \int_{t_{start}}^{t_{end}} F dt \tag{1}$$

Where F indicates the force and t_{start} indicates the start and t_{end} the end of stretching. The maximal strain of stretching (ε_{max}) was calculated according to Eq. (2).

$$\varepsilon_{max} = \frac{{}_{MTU}l_{max} - {}_{MTU}l_{90}}{{}_{MTU}l_{90}} \tag{2}$$

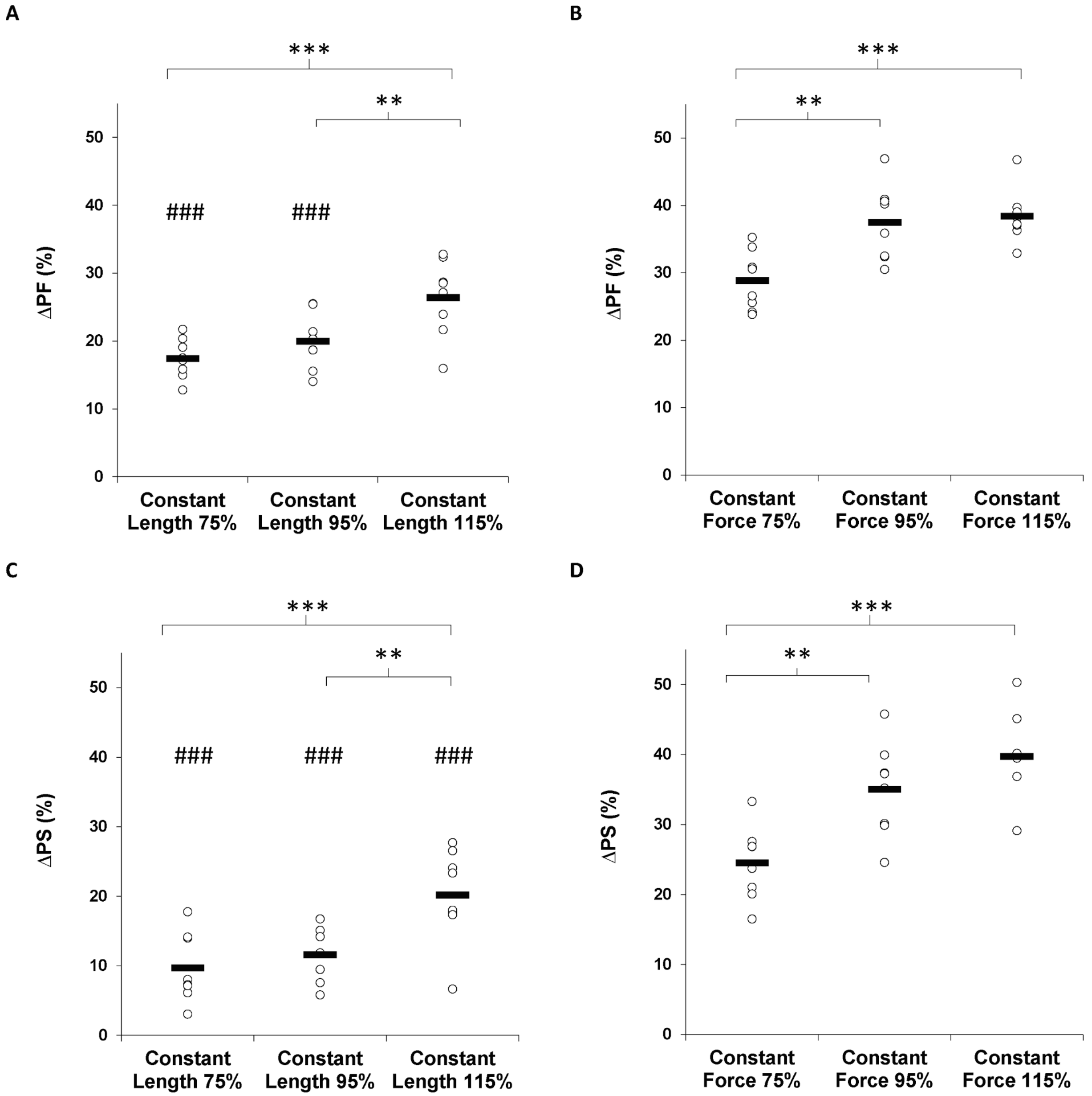

Fig. 2. Individual (○) and mean (▬) values of the decrease (%) in MTU peak force (ΔPF) **(A & B)** and peak stiffness (ΔPS) **(C & D)** after 30 min of constant length stretching **(A & C)** or constant force stretching **(B & D)** at 75%, 95% or 115% of reference force. Significant differences between stretch intensities ** $p < 0.01$, *** $p < 0.001$ as reported in Table 3. Significant differences between stretch types, $^{\#\#\#}$ $p < 0.001$ for each stretch intensity. For details of statistical results, see Table 3/appendix.

Where ${}_{MTU}l_{max}$ refers to the maximal MTU length during stretching, which was assessed from the displacement data recorded during stretching and MTU length measured at l_{90} (${}_{mtu}l_{90}$).

Before and after stretching, the following parameters were calculated from the video and displacement data at F_{ref}: 1) length of distal tendon: the mean of three distance measurements between the MTJ and distal tendon end markers; using a videoframe and FIJI software (National Institutes of Health, Bethesda, MD, USA), 2) MTU length: the sum of ${}_{MTU}l_{90}$ and the MTU lengthening beyond l_{90} at F_{ref} (the proximal origin of the MG was not visible in the video; hence, MTU length was assessed using the displacement beyond l_{90}), and 3) length of muscle belly: by subtracting tendon length from the MTU length. Changes in peak force (ΔPF), peak stiffness (ΔPS), muscle belly length (ΔML) and tendon length (ΔTL) at F_{ref} were expressed in percentages of pre-stretching values.

2.6. *Statistical analysis*

All statistical analyses were performed in Jamovi (V2.3.2.0). Data are presented as mean ± standard deviation (SD). One-way ANOVAs were performed with group (6 levels) as factor, to assess if prior to the stretch protocol there were differences between groups in muscle belly mass, tendon mass, muscle belly length, tendon length, peak force and peak stiffness. For each group, to test if the stretching protocol resulted in significant changes in peak force, peak stiffness, muscle belly length and tendon length paired samples t-tests were performed. The assumption of normality was tested with Shapiro-Wilk tests; the assumption was not met if $p < 0.05$. Homogeneity of variance was tested with Levene's tests; the assumption was not met if $p < 0.05$. A Kruskal-Wallis or Wilcoxon rank test was performed in case of violation of the assumption.

Regression analyses using linear mixed models (LMM) were performed with a random intercept for animal ($n = 25$), to control for

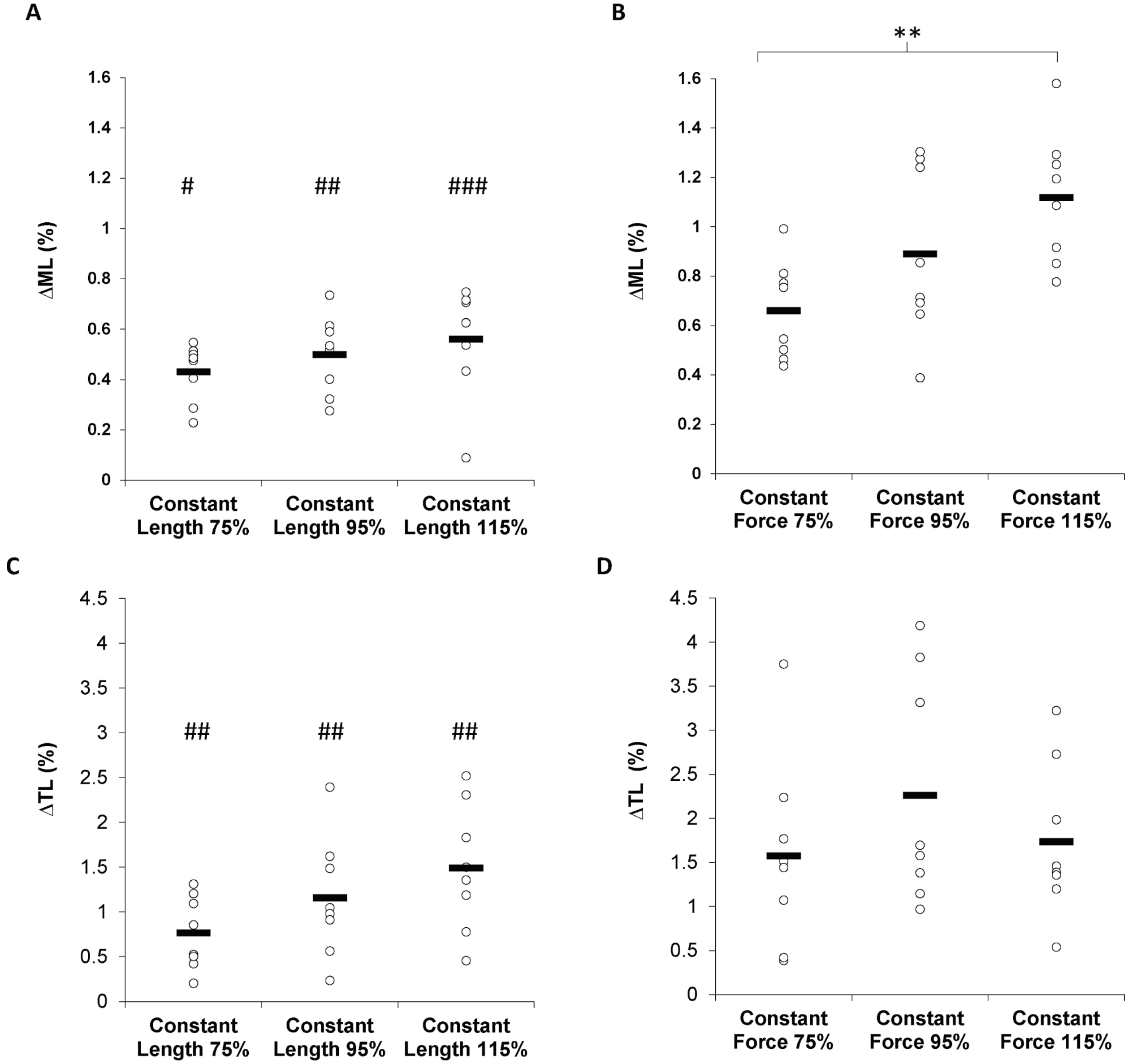

Fig. 3. Individual (○) and mean (▬) values of changes in muscle length (ΔML) **(A & B)** and tendon length (ΔTL) **(C & D)** after 30 min of constant length stretching **(A & C)** or constant force stretching **(B & D)** at a 75%, 95% or 115% reference force. Significant differences between stretch intensities ** $p < 0.01$ for each stretch type. Significant differences between stretch types $^{\#}$ $p < 0.05$, $^{\#\#}$ $p < 0.01$, $^{\#\#\#}$ $p < 0.001$ for each stretch intensity. For details of statistical results, see Table 3/appendix.

related observations within individual animals. Stretch type (2 levels, with CL as reference) and stretch intensity (3 levels, with 75% as reference) were set as fixed categorical predictor variables along with their interaction to investigate their effects on each outcome parameter separately: for acute deformations (ΔML, ΔTL), acute changes in passive mechanical MTU properties (ΔPF, ΔPS) and stretch characteristics (J and the ε_{max}). In case of a significant interaction effect, LMMs were estimated for each stretch intensity separately with stretch type as factor and for each type separately with stretch intensity as factor (the latter were reported as final regression models). In case of no significant interaction effect, the LMMs were estimated without the interaction as final regression models. The resulting models were repeated with the stretch intensity category of 95% as reference, to be able to also report the difference between 95% and 115%, because comparisons can only be made to the reference with the LMM approach.

To investigate whether deformations and mechanical changes due to stretching are associated with the J or ε_{max} and whether these associations are affected by stretch type, LMMs were estimated with J or ε_{max} as continuous predictor variables and stretch type as categorial predictor variable along with their interaction. In case of a significant interaction effect, LMMs were estimated for each stretch type separately. In case of no interaction, the interaction was removed from the LMM. Final model estimates of regression coefficients, their 95% confidence intervals (95% CI), t- and p-values were reported. The level of significance was set at $p < 0.05$.

3. Results

3.1. Baseline comparisons

There were no statistically significant differences between the six groups in muscle belly mass ($p = 0.952$) and length ($p = 0.883$), tendon mass ($p = 0.822$) and length ($p = 0.707$) (Table 1), neither in pre-stretching peak force ($p = 0.506$) and stiffness ($p = 0.637$) (Table 2). Muscle belly mass and length data violated the assumption of normality; Kruskal-Wallis tests were performed for these parameters.

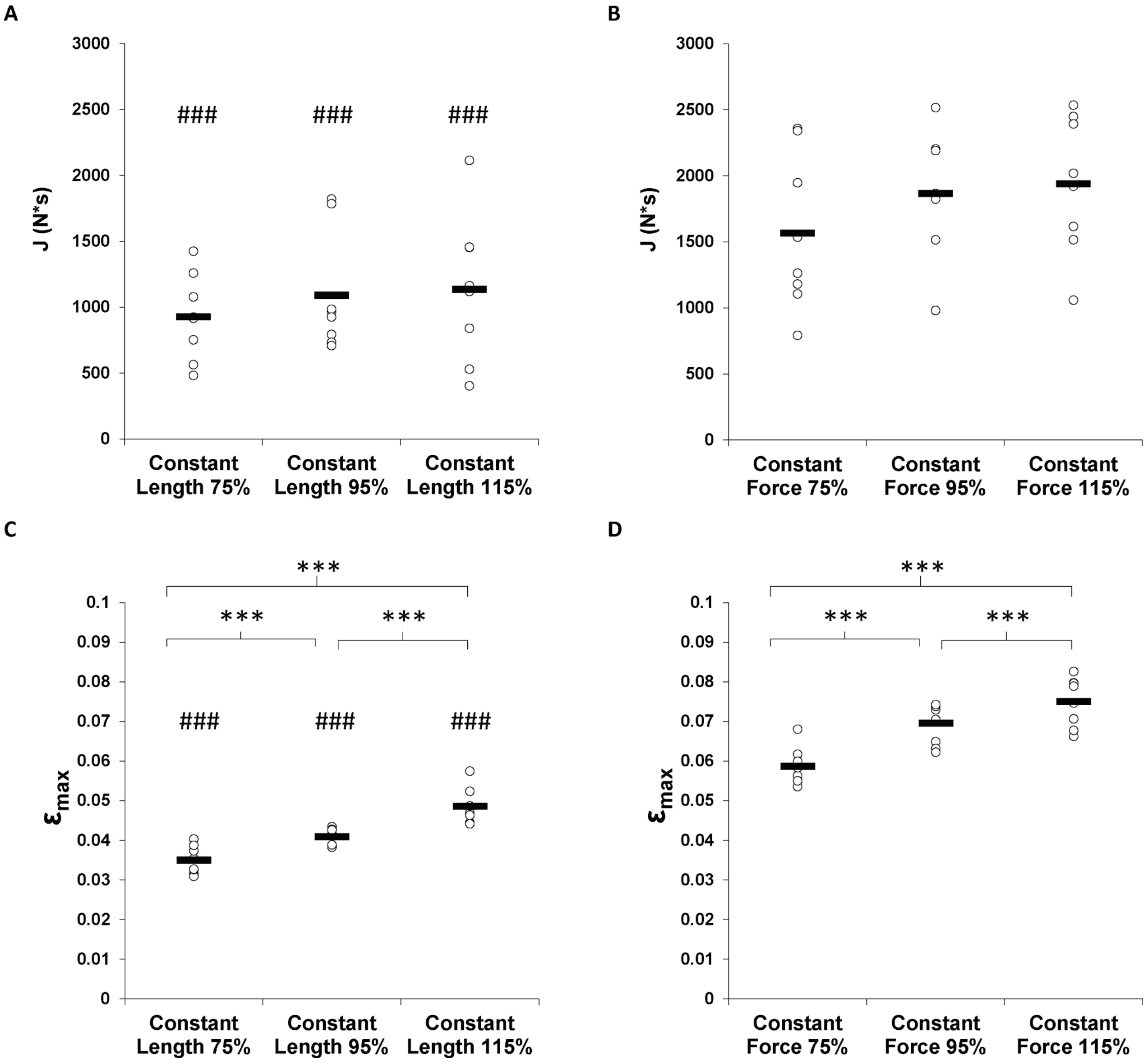

Fig. 4. Individual (○) and mean (▬) values of stretch impulse (J), **(A & B)** and maximal strain (ε_{max}) during stretching **(C & D)** for constant length **(A & C)** and constant force stretching **(B & D)** at 75% &, 95% and 115% of reference force. The impulse of stretching was calculated as the integral of the force over time. Significant differences between stretch intensities *** $p < 0.001$ and between stretch types $^{\#\#\#}$ $p < 0.001$ for each stretch intensity. For details of statistical results, see Table 3.

3.2. Differences between stretch types and initial stretch intensities

Paired samples t-tests showed there was a significant difference between pre and post-data in all groups for peak force, peak stiffness, muscle length and tendon length (Table 2). Muscle belly length data of the CL 115% group violated normality; a Wilcoxon rank test was performed for this group.

All results described below regard changes in response to the stretching protocol (i.e. pre-post differences). For the decrease in peak force (ΔPF) and the decrease in peak stiffness (ΔPS), a significant interaction between stretch type and stretch intensity was found ($p = 0.032$ and $p = 0.030$, respectively). Hence, separate LMMs were estimated for each stretch type (Table 3) and for each stretch intensity (Supplementary material). ΔPF in response to CF stretching was significantly higher than following CL stretching for the 75% ($p < 0.001$) and 95% ($p < 0.001$), but not for the 115% ($p = 0.973$) stretch intensity (Fig. 2A & B, Supplementary material). ΔPS after CF stretching was significantly higher than following CL stretching for all stretch intensities ($p < 0.001$). Within each stretch type, significant differences between the stretch intensities were found (Fig. 2, Table 3).

For changes in muscle belly length (ΔML), a significant interaction effect between stretch type and stretch intensity was found ($p = 0.048$). Hence, separate LMMs were estimated for each stretch type (Table 3) and for each intensity (Supplementary material). For all stretch intensities, CF stretching caused significantly larger ΔML than CL stretching (75%: $p = 0.014$, 95%: $p = 0.008$, 115%: $p < 0.001$) (Fig. 3A & B, Supplementary material). For CF stretching, differences between stretch intensities were found between 75% and 115%, but not for CL stretching (Fig. 3B, Table 3).

For changes in tendon length (ΔTL), no significant interaction effect was indicated ($p = 0.372$). CF stretching caused significantly larger ΔTL than CL stretching ($p = 0.007$). No significant differences between stretch intensities were found (Fig. 3C & D, Table 3).

For J, no significant interaction effect was indicated ($p = 0.616$). J was significantly higher for CF stretching than for CL stretching. No significant differences between stretch intensities were indicated

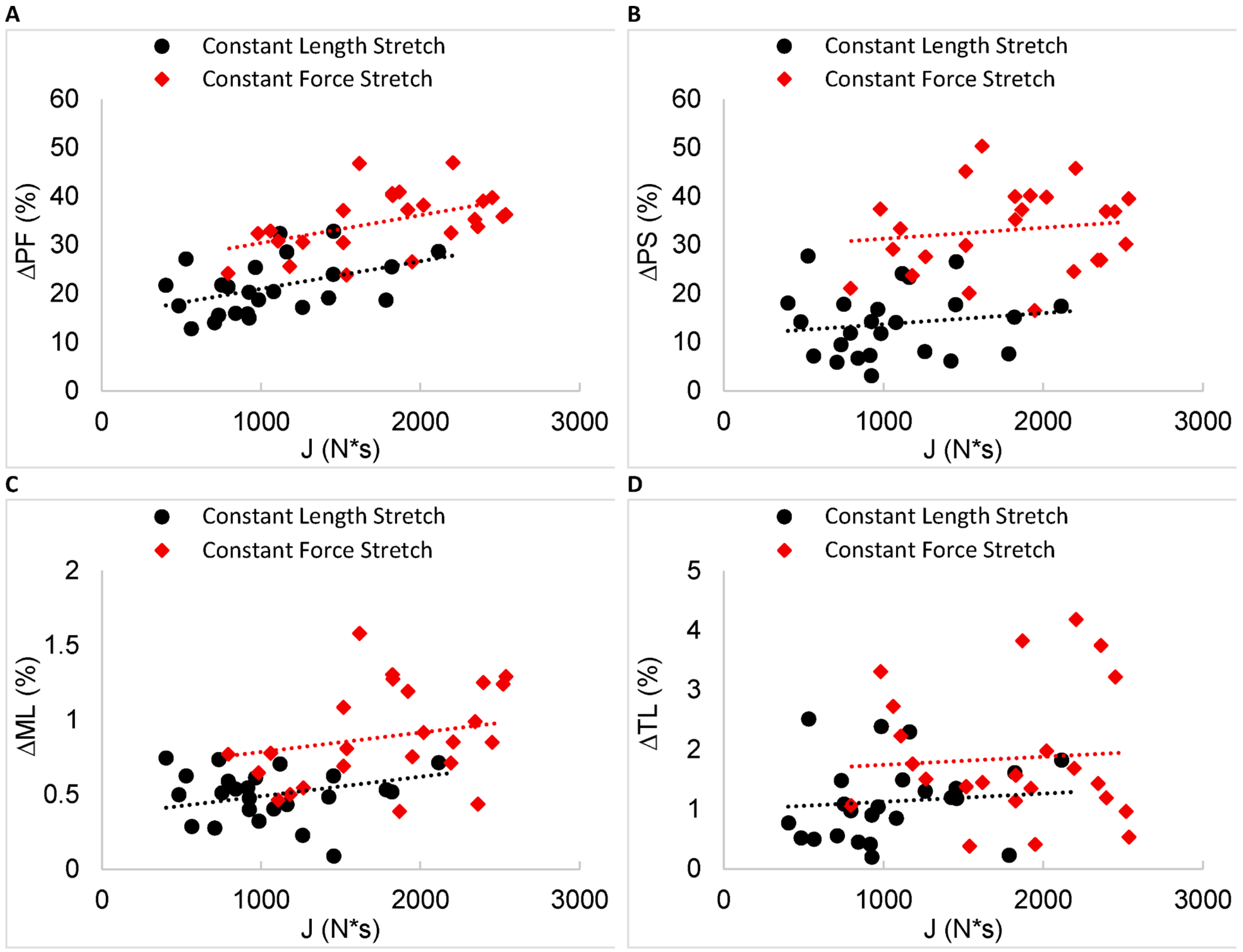

Fig. 5. Scatter plots of the relationship between stretch impulse (J) and peak force decrease (ΔPF) **(A)**, peak stiffness decrease (ΔPS) **(B)**, changes in muscle length (ΔML) **(C)** and tendon length (ΔTL) **(D)** per stretch type. Individual data points are displayed for constant length **(• black)** and constant force **(◆ red)** stretching. The dotted lines represent the final regression models for the constant length **(black)** and constant force stretching **(red)** (Table 4). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 4
Final regression models resulting from linear mixed models (LMM) that tested the effects of categorical predictor stretch type and continuous predictors impulse (J) (left) and maximal strain (ε_{max}) (right) on peak force decrease, peak stiffness decrease, changes in muscle length and tendon length. Constant length stretching was set as reference.

	Impulse (J) and type				**Maximal strain (ε_{max}) and type**			
Peak force decrease	**Estimate**	**95% CI**	**t**	**p**	**Estimate**	**95% CI**	**t**	**p**
Intercept	20.031	15.264, 24.798	8.24	<0.001	−2.225	−11.09, 6.62	−0.491	0.625
Slope	0.006	0.004, 0.009	3.49	0.001	554.516	393.88, 715.15	5.766	<0.001
Stretch type difference:	9.446	5.596, 13.295	4.81	<0.001	−0.922	−5.78 3.94	0.372	0.712
Peak stiffness decrease								
Intercept	20.208	13.238, 27.176	5.68	<0.001	−13.97	−26.42, -1.51	−2.198	0.033
Slope	0.002	−0.002, 0.007	0.97	0.340	684.48	459.23, 909.72	5.956	<0.001
Stretch type difference:	17.560	11.932, 23.188	6.12	<0.001	1.11	−5.711 7.92	0.319	0.751
Changes in muscle length								
Intercept	0.508	0.283, 0.733	4.42	<0.001	−0.106	−0.596, 0.384	−0.43	0.673
Slope	1.30e-4	−2.02e, 2.80e-4	1.70	0.097	14.622	5.744, 23.501	3.23	0.002
Stretch type difference:	0.297	0.115, 0.479	3.20	0.003	0.010	−0.259, 0.278	0.07	0.944
Changes in tendon length								
Intercept	1.303	0.498, 2.110	3.17	0.003	−0.2136	−2.030, 1.604	−0.23	0.819
Slope	1.36e-4	−4.00e − 4, 6.73e-4	0.50	0.621	31.289	−1.650, 64.231	1.862	0.069
Stretch type difference:	0.620	−0.030, 1.270	1.87	0.068	−0.100	−1.100 0.897	0.197	0.845

Estimates, 95% confidence interval (95% CI), t- and p-values.

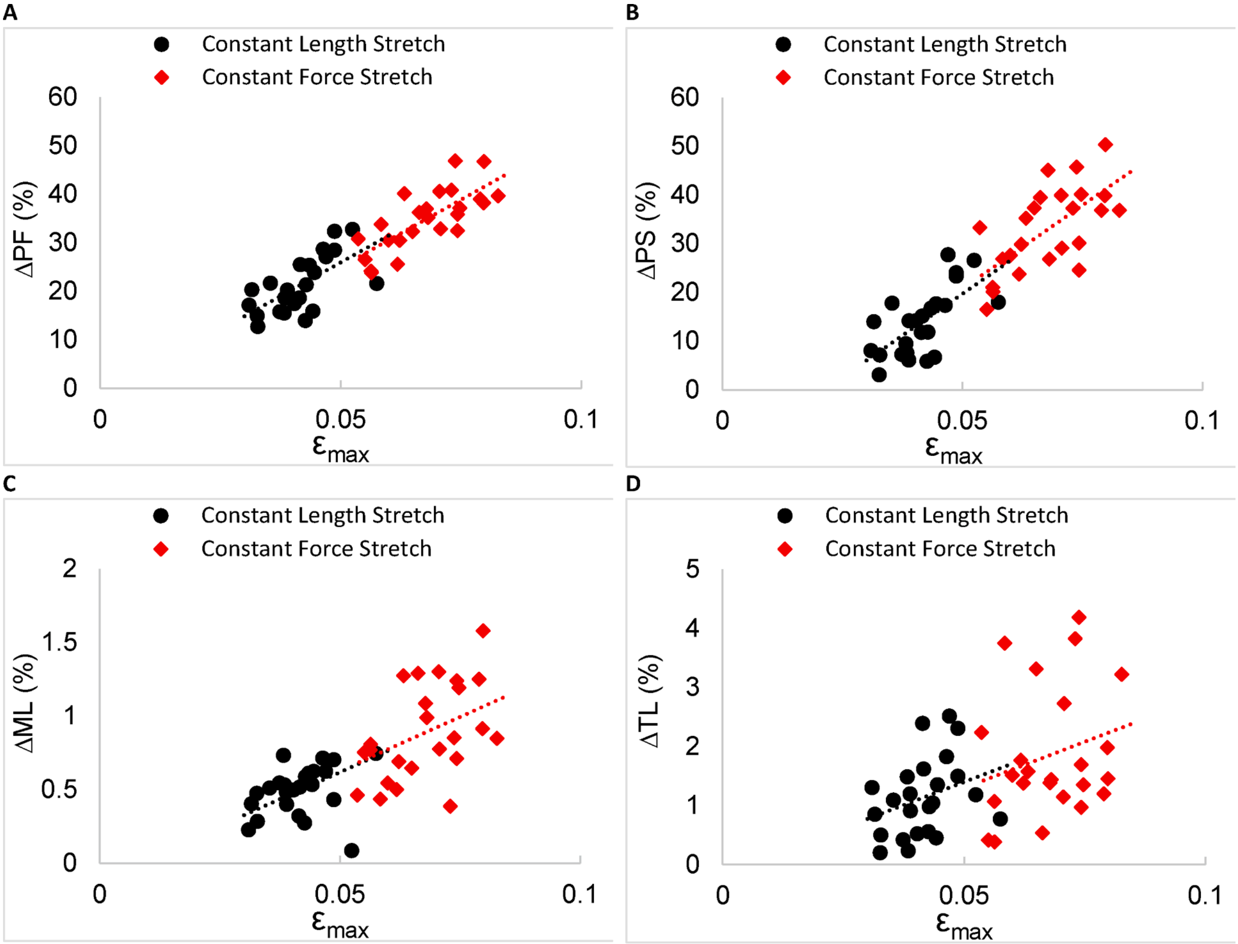

Fig. 6. Scatter plots of the relationship between the maximal strain (ε_{max}) of the stretch condition and peak force decrease (ΔPF) **(A)**, peak stiffness decrease (ΔPS) **(B)**, changes in muscle length (ΔML) **(C)** and tendon length (ΔML) **(D)** per stretch type. Individual data points are displayed for constant length (● **black**) and constant force (◆ **red**) stretching. The dotted lines represent constant length **(black)** and constant force stretching **(red)** from the final regression model (Table 4). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Fig. 4A & B, Table 3).

For ε_{max}, no significant interaction effect was indicated ($p = 0.075$). ε_{max} was significantly higher for CF stretching than for CL stretching ($p < 0.001$). In addition, ε_{max} was significantly higher for each stretch type with higher stretch intensities (Fig. 4C & D, Table 3).

3.3. Associations of impulse and maximal strain of stretching with outcome parameters

For ΔPF, ΔPS, ΔML and ΔTL, no significant interaction effects were found between J and stretch type ($p = 0.901$, $p = 0.664$, $p = 0.127$ and $p = 0.612$ respectively). In final regression models, only for ΔPF, a significant association with J was found, in all outcomes except ΔTL a significant effect of stretch type was found (Fig. 5, Table 4).

For ΔPF, ΔPS, ΔML and ΔTL, no significant interactions were observed between ε_{max} and stretch type ($p = 0.929$, $p = 0.859$, $p = 0.250$ and $p = 0.871$ respectively). In final regression models, ε_{max} was significantly associated with ΔPF, ΔPS and ΔML but not with ΔTL. In all outcomes, no effect of stretch type was found (Fig. 6, Table 4).

4. Discussion

The main findings of this study are 1) that 30 min of stretching a passive muscle at a constant force resulted in greater acute deformations and changes in passive mechanical characteristics of MG muscle than stretching at a constant length; and 2) that the maximal strain of stretching (ε_{max}) was associated with the decrease in passive peak force, peak stiffness and the increase in muscle belly length, independent of stretch type.

Our finding that at comparable initial stretching intensities, CF stretching resulted in greater changes in MG than CL stretching is consistent with prior observations. Acute changes in passive mechanical properties and ROM of the knee and ankle joint were higher after CF stretching than CL stretching of the hamstrings (Cabido et al., 2014) and of plantar flexors in healthy adults (Konrad et al., 2017), and of stroke affected plantar flexors (Yeh et al., 2007). The increase of ROM indicates that the MTU was lengthened, but muscle belly and tendon lengths were not assessed. In these studies on human subjects, neural components may also have played a role. Despite the absence of a neural component in our study, we also found that CF stretching caused significantly greater effects than CL stretching.

CF and CL stretching at high initial intensities elicited larger acute changes in the mechanical properties of MG muscle than at lower initial intensities. These results are in agreement with previous studies reporting acute effects of CL stretching (Freitas et al., 2015) and CF stretching (Oba et al., 2018) of human calf muscles, also when the duration of high-intensity CL stretching was shorter (i.e. 2–4 times) than that of low-intensity CL stretching (Freitas et al., 2016; Fukaya et al., 2020). In addition, maximal strain increased with higher intensities, but the effect of intensity on stretch impulse was not significant. Nevertheless, an association between stretch impulse and decrease in peak force was found. It should be noted that also a significant effect of stretch type was found, indicating that the effects of the same impulse differed between CF and CL stretching. This suggests that the effects of impulse are not independent of the effects of stretch type. Previously, only a weak relation between impulse and the decrease in ankle joint stiffness following acute CL stretching in humans was found (Fukaya et al., 2021). In line with the effects of intensity, maximal strain was associated with acute changes in muscle belly length and mechanics, independent of stretch type. This suggests that maximal strain is an important factor for the acute effects of stretching.

Our findings were obtained in a muscle that was maximally dissected from surrounding structures and, hence, not fully representative of muscle stretching in vivo. Connective tissue linkages between muscle bellies (Maas, 2019) and the subtendons of the Achilles tendon (Maas & Finni, 2018) may affect how strains and stresses are distributed between and within the triceps surae muscles. However, we expect that the effects of these linkages were similar in all imposed stretching conditions and, thus, would not alter the observed differences between CL and CF stretching. It should also be noted that only the acute effects of stretching were investigated. Whether this also translates to tissue adaptations after long-term stretching is currently unknown. The maximal strain of stretching has been proposed as a key stimulus for adaptations in muscle length (Kruse et al., 2021). Based on our results, CF stretching is hypothesized to result in greater adaptations than CL stretching. However, 4 weeks of CF stretching with a low torque in rats with a knee joint contracture was found to result in a greater restoration of knee ROM than with a high torque and, presumably, also a higher maximal strain (Usuba et al., 2007). Thus, the long-term effects of stretch are not necessarily the same as the acute effects.

5. Conclusions

We showed that constant force stretching causes greater acute deformations and changes in passive mechanical muscle characteristics than constant length stretching. This can be explained by differences in maximal strain during stretching, of which the amplitude varies with stretch type (CF versus CL) and stretch intensity. Whether these results also translate to long-term effects, requires further studies. During low-intensity CF stretching similar maximal strains can be attained as during high-intensity CL stretching. Because stretching at high intensities is less comfortable than at low intensities, this may be relevant when applying stretching for the treatment of joint contractures.

CRediT authorship contribution statement

G. Geusebroek: Writing – original draft, Visualiza-tion, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **J.H. van Dieën:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **M.J.M. Hoozemans:** Methodology, Writing – review & editing. **W. Noort:** Methodology, Investigation. **H. Houdijk:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **H. Maas:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jbiomech.2023.111594.

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