## **Review article**

# Acute and Long-Term Effects of Static Stretching on Muscle-Tendon Unit Stiffness: A Systematic Review and Meta-Analysis

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#### Abstract

Static stretching can increase the range of motion of a joint. Muscle-tendon unit stiffness (MTS) is potentially one of the main factors that influences the change in the range of motion after static stretching. However, to date, the effects of acute and long-term static stretching on MTS are not well understood. The purpose of this meta-analysis was to investigate the effects of acute and longterm static stretching training on MTS, in young healthy participants. PubMed, Web of Science, and EBSCO published before January 6, 2023, were searched and finally, 17 papers were included in the meta-analysis. Main meta-analysis was performed with a random-effect model and subgroup analyses, which included comparisons of sex (male vs. mixed sex and female) and muscle (hamstrings vs. plantar flexors) were also performed. Furthermore, a meta-regression was conducted to examine the effect of total stretching duration on MTS. For acute static stretching, the result of the meta-analysis showed a moderate decrease in MTS (effect size = -0.772, Z = -2.374, 95% confidence interval = -1.409 - -0.325, p = 0.018, I<sup>2</sup> = 79.098). For long-term static stretching, there is no significant change in MTS (effect size = -0.608, Z = -1.761, 95% CI = -1.284 – 0.069, p = 0.078,  $I^2$  = 83.061). Subgroup analyses revealed no significant differences between sex (long-term, p = 0.209) or muscle (acute, p = 0.295; long-term, p = 0.427). Moreover, there was a significant relationship between total stretching duration and MTS in acute static stretching (p = 0.011, R2 = 0.28), but not in long-term stretching  $(p = 0.085, R^2 < 0.01)$ . Whilst MTS decreased after acute static stretching, only a tendency of a decrease was seen after long-term stretching.

**Key words:** Stiffness, torque-angle curve, passive movement, hamstrings, plantar flexors.

## Introduction

Static stretching is widely used by athletes, and for health promotion for various people with the goal of increasing the range of motion (ROM) (Small et al., 2008; Costa and Vieira, 2008; Behm et al., 2016; 2021). Change in ROM is attributed to changes in mainly the passive properties of the muscle-tendon unit and stretching tolerance (Magnusson, 1998; Freitas et al., 2018; Takeuchi and Nakamura, 2020a; Takeuchi et al., 2021b; c). Muscle-tendon unit stiffness (MTS) is used to assess the changes in the elastic property of the muscle-tendon unit after static stretching (Magnusson et al., 1996a; b; Mizuno et al., 2013; Freitas et al., 2018; Takeuchi and Nakamura, 2020a; Fukaya et al., 2021; Takeuchi et al., 2021c; 2022b). MTS is calculated from a slope of the torque-angle curve during passive joint movement (Magnusson et al., 1996c; Nordez et al., 2006; Andrade et al., 2020) and there is a significant relationship between changes in ROM and MTS after static stretching (Guissard and Duchateau, 2004).

For long-term stretching, Freitas et al. (2018) conducted a meta-analysis examining the effects of long-term stretching training including all types of stretching (static, dynamic, and proprioceptive neuromuscular facilitation stretching) regardless of the health status of the participants (young adults, old adults, and patients with osteoarthritis), and found that stretching for 8 weeks or less did not change MTS. However, a recent meta-analysis reported that longterm static stretching for an average of 5.8 weeks significantly decreased muscle stiffness of healthy young participants which was assessed by using ultrasonography (Takeuchi et al., 2023). MTS reflects the extensibility of all components of the muscle-tendon unit and is influenced by several factors, including muscles (Nakamura et al., 2013; Ichihashi et al., 2016; Konrad et al, 2017), tendons (Kubo et al., 2002a; b), nerves (Blazevich et al., 2014; Andrade et al., 2020), and connective tissue (Nakamura et al., 2011). On the other hand, muscle stiffness is evaluated using ultrasonography equipment and is a measure that reflects muscle extensibility (Takeuchi et al., 2023). It is not clear whether the differences in the results of these meta-analvses (Freitas et al., 2018; Takeuchi et al., 2023) are due to differences in outcomes (MTS and muscle stiffness), differences in stretching types (static stretching alone and all types of stretching), or characteristics of participants (age and health status). Therefore, the effect of long-term static stretching on MTS in healthy young participants needs to be examined.

Numerous studies have reported a significant decrease in MTS after acute static stretching (Mizuno et al., 2013; Hatano et al., 2019; Takeuchi and Nakamura, 2020a; Takeuchi et al., 2021c). However, no meta-analysis of the acute effect of static stretching on MTS has been conducted, and there is a need to summarize all the available evidence. Therefore, the purpose of this study was to systemically review the papers and analyze the acute and longterm effects of static stretching on MTS in healthy young participants including some potential moderating variables (sex, muscle, and total stretching duration).

## Methods

This review was conducted according to PRISMA guidelines for a systematic review with meta-analysis (Moher et al., 2009).

## Search strategy

The electronic literature was searched in PubMed, Web of Science, and EBSCO. The search was conducted on January 6, 2023, and papers published before that date were included in this study. The search code for all three databases was ((Flexib\*) OR (Stiff\*) OR (Extensib\*) OR (Passive tension)) AND (Stretch\*) AND ((Muscle) OR (Tendon)) AND ((intervention) OR (effects)).

#### **Study selection**

All procedures for this study selection were performed by four independent reviewers (KT, MS, TF, and TM). First, the titles and abstracts of all papers were reviewed to assess suitability. Those not consistent with the purpose of the study were excluded with reference to the inclusion and exclusion criteria. Following the initial screening process, the full texts were assessed. This process was done by two researchers, with any disagreements resolved by the remaining researcher.

#### Inclusion and exclusion criteria

This study included peer-reviewed original studies reported in English. The studies were included when they were either randomized or non-randomized trials with static stretching interventions in healthy young (<40 years) human participants. We excluded studies that investigated combined interventions (e.g., static stretching with resistance training), lacked a control group, or had another treatment as a control group (e.g., dynamic stretching). Moreover, we excluded review papers, case reports, special communications, letters to the editor, invited commentaries, conference papers, or theses.

## **Extraction of the data**

The following data were extracted from the included papers: (1) the characteristics of the paper (authors, years of publication, and sample size); (2) characteristics of participants (sex and age); (3) characteristics of stretching (targeted muscle, duration, frequency, and intensity), and (4) calculation method of MTU stiffness. For MTU stiffness, mean and standard deviation values before and after interventions were extracted. If MTU stiffness was measured more than once in different time series, the MTU stiffness after the last intervention was adopted. When the required data was not described in the included papers, the authors of the papers were contacted via e-mail or similar channels (e.g., ResearchGate) to provide information.

## Statistics and data synthesis

The statistical analysis was performed according to previous studies (Konrad et al., 2021a, 2022). The meta-analysis was performed using Comprehensive Meta-Analysis software (Biostat Inc, Englewood, NJ, USA), based on the recommendation of Borenstein et al. (2009). By applying a random-effect meta-analysis, the effect size in terms of the standardized mean difference was assessed. If more than one effect size was reported in one study, the mean of all measurements within one study was used for the metaanalysis and defined as combined (Borenstein et al., 2009; Konrad et al., 2022). Although there is no general rule (Borenstein et al., 2009), subgroup analyses were performed when there were  $\geq 3$  studies included in each subgroup (Konrad et al., 2022). Consequently, subgroup analysis for sex (male vs. mixed sex and female) of long-term static stretching and muscle (hamstrings vs. plantar flexors) of both acute and long-term static stretching were conducted. Q-statistics were applied to determine differences between the effect sizes of the subgroups (Borenstein et al., 2009). In addition, a meta-regression was conducted to examine the association between changes in MTU stiffness and total stretching duration. The effects of a standardized mean difference of < 0.2 were considered trivial, 0.2 - 0.6 were considered small, 0.6 - 1.2 were considered moderate, 1.2 - 2.0 were considered large, 2.0 - 4.0 were considered very large, and >4.0 were considered extremely large (Hopkins et al., 2009). I<sup>2</sup> statistics were calculated to assess the heterogeneity among the included studies, and thresholds of 25%, 50%, and 75% were defined as having a low, moderate, and high level of heterogeneity, respectively (Higgins et al., 2003; Behm et al., 2021; Konrad et al., 2021b; Konrad et al., 2022). An alpha level of 0.05 was defined for the statistical significance of all the tests.

### Risk of bias assessment and methodological quality

The methodological qualities of included papers were assessed by using the Physiotherapy Evidence Database (PEDro) scale. The PEDro scale consists of 11 methodological criteria, and two independent assessors scored each item with a 0 or 1 point. Higher scores on the PEDro scale indicated a better methodological quality of a study. When there were differences in scores between assessors, the mismatched scores were resolved between the assessors. Moreover, statistics of Egger's regression intercept test and visual inspection of the funnel plot were applied to detect possible publication bias.

## Results

## **Results of the Search**

In total, 7708 papers were identified from a database search (Figure 1). After the removal of any duplicates, 4541 papers were screened, and 23 papers were identified as eligible for this systematic review and meta-analysis. However, 6 papers were excluded because we did not obtain the MTU stiffness data from authors of these papers (Halbertsmaet al., 1996; Magnusson et al., 1996b; Reid and McNair, 2004; Herda et al., 2009; 2010; Blazevich et al., 2014). Overall, 26 effect sizes of 17 papers were included in this systematic review and meta-analysis (14 effect sizes of 7 papers for acute effects, and 12 effect sizes of 10 papers for long-term effects) (Table 1) (Kubo et al., 2002a; Guissard and Duchateau, 2004; LaRoche and Connolly, 2006;

Gajdosik et al., 2007; Ryan et al., 2008; Marshall et al., 2011; Konrad and Tilp, 2014a; Akagi and Takahashi, 2014; Rodrigues et al., 2017; Konrad et al., 2017; 2019; Palmer et al., 2019; Brusco et al., 2019; Nakao et al., 2021; Oba et al., 2021b; Longo et al., 2021; Hatano et al., 2022). On average, the included papers had a total stretching duration of 248.6 seconds (acute static stretching, range: 120 - 480 seconds) and 8856.0 seconds (long-term static stretching, range: 3600 - 27000 seconds) and an intervention term of 5.6 weeks (long-term static stretching, range: 2.9 - 12 weeks).

## **Risk of Bias Assessment and Methodological Quality**

Figure 2 shows a funnel plot of the 17 papers included in the meta-analysis. A visual inspection of the funnel plot and Egger's regression intercept test indicated a significant reporting bias for acute (intercept = -9.52, p = 0.018) and long-term stretching (intercept = -5.65, p = 0.018). To assess the methodological quality of each paper, the PEDro scale was used (Table 2). The agreement of the PEDro scale between assessors was 95.7% (179 of 187 points). The average and standard deviations of the PEDro scale was  $5.83 \pm 0.71$  (range between 5 and 8 points), which indicated a low risk of bias (Maher et al., 2003; Moran et al., 2021; Konrad et al., 2022).

## Acute effect on MTS

Acute static stretching training had a significant moderate effect on MTS (effect size = -0.772, Z = -2.374, 95% confidence interval (CI) = -1.409 - 0.325, p = 0.018) (Figure 3); however, high heterogeneity was observed (I<sup>2</sup> = 79.098). Subgroup analysis revealed that there was no significant difference between the muscles (plantar flexors vs. hamstrings) (p = 0.295) (Table 3). The meta-regressions revealed that total stretching duration was associated with the magnitude of the effect size for MTS (p = 0.011, coefficient = -0.0061 95% CI = -0.0109 - 0.0014, R<sup>2</sup> = 0.28) (Figure 4).

## Long-term effect on MTS

Meta-analysis revealed that long-term static stretching training had no significant effect on MTS (effect size = -0.608, Z = -1.761, 95% CI = -1.284 - 0.069, p = 0.078, I<sup>2</sup> = 83.061). Subgroup analysis revealed that there were no significant differences in muscle (plantar flexors vs. hamstrings) (p = 0.427) or sex (mixed sex and female vs. male) (p = 0.209) (Table 3). The meta-regressions revealed that total stretching duration was not associated with the magnitude of the effect size for MTS (p = 0.085, coefficient = -0.0001 95% CI = -0.0002 - 0.0000, R<sup>2</sup> < 0.01) (Figure 4).



Figure 1. PRISMA flowchart.

## Table 1. Characteristics of the included studies.

|                            |  | Interve | ntion         | -Calculated method of MTS                           |   |  |  |
|----------------------------|--|---------|---------------|---|---|--|--|
| Study                      | Participants   | Muscle  | Term          | Total stretching du-<br>ration                      | Stretching<br>intensity   | from torque-angle curve  |  |
| Konrad et<br>al. (2017)    | 79 men $(23.3 \pm 2.5)$<br>years) and 43 women<br>$(23.4 \pm 3.7)$ years); IG:<br>n = 23, CG: $n = 21$                       | PF      | Acute         | 120 sec (30 sec * 4<br>sets)                        | ROM maximum   | Between 0 and 100%ROM  |  |
| Konrad et al. (2019)       | 7 men $(27.5 \pm 8.3)$<br>years) and 7 women<br>$(24.9 \pm 3.1)$ years)  | PF      | Acute         | 300 sec (60 sec * 5<br>sets)                        | Until a maximum tolerable stretch   | Between last 10 deg of ROM maximum   |  |
| Oba et al.<br>(2021)       | 14 men (22.9 ± 1.0<br>years)   | PF      | Acute         | 300 sec (60 sec * 5<br>sets)                        | (a) 100%, (b) 75%,<br>and (c) 50% of the<br>maximal passive<br>resistive torque                         | Between 15-25 deg of ankle<br>DF   |  |
| Ryan et al.<br>(2008)      | 7 men (24 $\pm$ 4 years) and<br>5 women (21 $\pm$ 1 years)   | PF      | Acute         | 480 sec (30 sec * 16<br>sets)                       | Point of discomfort   | At (a) 1, (b) 5, (c) 9, and (d)<br>13 deg of ankle DF (fourth-or-<br>der polynomial regression)  |  |
| Hatano et al. (2022)       | 8 men $(21.3 \pm 0.7)$<br>years) and 8 women $(20.9 \pm 0.8)$ years)   | Ham     | Acute         | 300 sec (300 sec * 1<br>set)                        | 100%ROM,<br>110%ROM,<br>120%ROM   | Between 50 and 100%ROM   |  |
| Palmer et al. (2019)       | 13 women ( $21 \pm 2$ years)   | Ham     | Acute         | 120 sec (30 sec * 4<br>sets)                        | Point of discomfort   | Between 90 and 100%ROM<br>(fourth-order polynomial re-<br>gression)  |  |
| Rodrigues<br>et al. (2017) | 12 men (22.2 $\pm$ 0.5 years)  | Ham     | Acute         | 30 sec * 4 sets                                     | 90%ROM  | Between last third of ROM maximum  |  |
| Akagi et al.<br>(2014)     | 19 men (23.7 ± 2.3 years)  | PF      | Long-<br>term | 10800 sec (360 sec *<br>6 times/week * 5<br>weeks)  | 3 deg lower than the ROM  | Between 15 and 25 deg of an-<br>kle DF   |  |
| Longo et al. (2021)        | 18 men and 12 women<br>(22.7 $\pm$ 1.8 years); IG:<br>n = 15 (22.3 $\pm$ 0.8<br>years), CG: n = 15<br>(23.4 $\pm$ 0.8 years) | PF      | Long-<br>term | 27000 sec (450 sec *<br>5 times/week * 12<br>weeks) | Maximally tolera-<br>ble stretch within<br>the pain limit   | At 20 deg of ankle DF (best polynomial regression)   |  |
| Konrad et<br>al. (2014)    | 35 men $(23.9 \pm 2.9)$<br>years) and 14 women<br>$(22.5 \pm 2.5)$ years); IG:<br>n = 19, CG: n = 15                         | PF      | Long-<br>term | 3600 sec (120 sec * 5<br>times/week * 6<br>weeks)   | Point of discomfort   | Between 0 and 100%ROM  |  |
| LaRoche et al. (2006)      | 29 men (31.6 ± 15.2 years)   | Ham     | Long-<br>term | 3600 sec (300 sec * 3<br>times/week * 4<br>weeks)   | Point of mild dis-<br>comfort   | Between 50 and 85 deg of knee extension  |  |
| Gajdosik et<br>al. (2007)  | 10 women; IG: $n = 6$<br>(23 ± 4 years), CG: $n =$<br>4 (21 ± 1 years)   | PF      | Long-<br>term | 4500 sec (150 sec * 5<br>times/week * 6<br>weeks)   | Not reported  | Between (a) 0 and 100%ROM<br>and (b) last 10 deg of ankle<br>DF (third order polynomial<br>regression)   |  |
| Brusco et al.<br>(2019)    | 13 men (23.6 ± 3.9<br>years)   | Ham     | Long-<br>term | 5760 sec (480 sec * 2<br>times/week * 6<br>weeks)   | Maximum tolerable<br>stretch amplitude<br>irrespective of the<br>timing or magni-<br>tude of pain onset | Between last third angles rela-<br>tive to (a) the ROM maximum<br>reached before and after train-<br>ing and (b) the same angle<br>reached before training |  |
| Marshall et al. (2011)     | 14 men and 8 women<br>(22.7 ±3.8 years)  | Ham     | Long-<br>term | 7200 sec (360 sec * 5<br>times/week * 4<br>weeks)   | Not reported  | Between 20-50 deg of knee extension  |  |
| Kudo et al.<br>(2002)      | 8 men (24.6 $\pm$ 1.8 years)   | PF      | Long-<br>term | 4500 sec (225 sec * 7<br>times/week * 2.9<br>weeks) | 35 deg of ankle<br>dorsiflexion   | Between 15 and 25 deg of ankle DF  |  |
| Guissard et al. (2004)     | 8 men and 4 women<br>(21- 35 years)  | PF      | Long-<br>term | 18000 sec (600 sec *<br>5 times/week * 6<br>weeks)  | Maximum dorsi-<br>flexion tolerated by<br>the participants  | Between 15 and 25 deg of ankle DF  |  |
| Nakao et al.<br>(2021)     | 30 men (22.7 ± 2.2<br>years); IG: n = 15 (22.5<br>± 2.9 years), CG: ne =<br>15 (22.9 ± 1.2 years)                            | Ham     | Long-<br>term | 3600 sec (300 sec * 3<br>times/week * 4<br>weeks)   | Point just before<br>pain   | Between 50 and 100%ROM   |  |

IG, Intervention group; CG, Control group; Ham, hamstrings; PF, plantar flexors; ROM; range of motion; DF, dorsiflexion; deg, degree; MTS, muscle-tendon unit stiffness.



Figure 2. Funnel plot analysis for acute (A) and long-term (B) stretching.

## Table 2. PEDro scale.

| Study            | Random<br>allocation | Concealed allocation | Groups<br>similar<br>at<br>baseline | Assessor<br>blinding | Subject<br>blinding | Therapist<br>blinding | Less<br>than<br>15%<br>dropouts | Intention-<br>to-treat<br>analysis | Between-<br>group<br>statistical<br>comparisons | Point<br>estimates<br>and<br>variability | Eligibility<br>criteria<br>specified * | Total |
|------------------|----------------------|----------------------|-------------------------------------|----------------------|---------------------|-----------------------|---------------------------------|------------------------------------|---|--|--|-------|
| Akagi (2014)     | 1                    | 0                    | 1                                   | 0                    | 0                   | 0                     | 1                               | 1                                  | 1   | 1  | 1                                      | 6     |
| Longo (2021)     | 1                    | 0                    | 1                                   | 0                    | 0                   | 0                     | 1                               | 1                                  | 1   | 1  | 1                                      | 6     |
| Konrad (2014)    | 1                    | 1                    | 1                                   | 0                    | 0                   | 0                     | 0                               | 1                                  | 1   | 1  | 1                                      | 6     |
| Konrad (2017)    | 1                    | 1                    | 0                                   | 0                    | 0                   | 0                     | 0                               | 1                                  | 1   | 1  | 0                                      | 5     |
| Konrad (2019)    | 1                    | 0                    | 1                                   | 0                    | 0                   | 0                     | 1                               | 1                                  | 1   | 1  | 1                                      | 6     |
| LaRoche (2006)   | 1                    | 0                    | 1                                   | 0                    | 0                   | 0                     | 1                               | 1                                  | 1   | 1  | 1                                      | 6     |
| Gajdosik (2007)  | 1                    | 0                    | 1                                   | 1                    | 0                   | 0                     | 0                               | 1                                  | 1   | 1  | 1                                      | 6     |
| Brusco (2019)    | 0                    | 0                    | 1                                   | 0                    | 0                   | 0                     | 1                               | 1                                  | 1   | 1  | 1                                      | 5     |
| Marshall (2011)  | 1                    | 0                    | 1                                   | 0                    | 0                   | 0                     | 1                               | 1                                  | 1   | 1  | 1                                      | 6     |
| Oba (2021)       | 1                    | 0                    | 1                                   | 0                    | 0                   | 0                     | 1                               | 1                                  | 1   | 1  | 1                                      | 6     |
| Kudo (2002)      | 1                    | 0                    | 1                                   | 0                    | 0                   | 0                     | 1                               | 1                                  | 1   | 1  | 1                                      | 6     |
| Ryan (2008)      | 1                    | 0                    | 1                                   | 0                    | 0                   | 0                     | 1                               | 1                                  | 1   | 1  | 1                                      | 6     |
| Guissard (2004)  | 0                    | 0                    | 1                                   | 0                    | 0                   | 0                     | 1                               | 1                                  | 1   | 1  | 1                                      | 5     |
| Nakao (2021)     | 0                    | 0                    | 1                                   | 0                    | 0                   | 0                     | 1                               | 1                                  | 1   | 1  | 1                                      | 5     |
| Hatano (2022)    | 1                    | 0                    | 1                                   | 0                    | 0                   | 0                     | 1                               | 1                                  | 1   | 1  | 1                                      | 6     |
| Palmer (2019)    | 1                    | 0                    | 1                                   | 0                    | 0                   | 0                     | 1                               | 1                                  | 1   | 1  | 1                                      | 6     |
| Rodrigues (2017) | ) 0                  | 0                    | 1                                   | 0                    | 0                   | 0                     | 1                               | 1                                  | 1   | 1  | 1                                      | 5     |

PEDro, Physiotherapy evidence database; 1, one point scored; 0, no points scored; \* Criteria of random allocation was not counted for the total score.

## Table 3. Results of subgroup analysis.

| Subgroup  | Subgroup         | Number of<br>studies | Std diff in means<br>(95%CI) | Z-Value | p-Value | Q statistics                      |
|-----------|------------------|----------------------|------------------------------|---------|---------|-----------------------------------|
| Acute     | Hamstrings       | 3                    | -0.440 (-0.878 - 0.001)      | -1.964  | 0.050   |                                   |
|           | Plantar flexors  | 4                    | -1.118 (-2.3110.074)         | -1.838  | 0.066   |                                   |
|           | Overall          | 7                    | -0.520 (-0.9320.109)         | -2.478  | 0.013 * | (Q = 1.096, df(Q) = 1, p = 0.295) |
| Long-term | Hamstrings       | 4                    | -0.466 (-0.8710.061)         | -2.255  |         |                                   |
|           | Plantar flexors  | 6                    | -0.986 (-2.2060.234)         | -1.585  |         |                                   |
|           | Overall          | 10                   | -0.518 (-0.9020.133)         | -2.639  |         | (Q = 0.630, df(Q) = 1, p = 0.427) |
|           | Male             | 5                    | -0.348 (-0.6960.000)         | -1.958  | 0.050   |                                   |
|           | Mixed and female | 5                    | -1.412 (-3.034 - 0.210)      | -1.706  | 0.088   |                                   |
|           | Overall          | 10                   | -0.395 (-0.7360.054)         | -2.273  | 0.000 * | (Q = 1.579, df(Q) = 1, p = 0.209) |

Std diff in means, standardized difference in means; 95% CI. 95% confidence interval; mixed, male and female; \* Significant difference within a group.



Figure 3. Funnel plot analysis for acute (A) and long-term (B) stretching.



Figure 4. Funnel plot analysis for acute (A) and long-term (B) stretching.

## Discussion

To the best of our knowledge, this is the first meta-analysis examining the acute, as well as the long-term, effects of static stretching on MTS. The results of this study showed that acute static stretching significantly decreased MTS with a moderate effect size (effect size = -0.772, p < 0.01). The subgroup analysis indicated no significant differences between the hamstrings and plantar flexors (p = 0.295). In addition, there was a significant association between the

total stretching duration and the magnitude of the effect size for MTS (p = 0.011), indicating that a longer stretch duration can decrease the MTS to a higher extent. Previous studies have shown that static stretching duration is associated with an acute decrease in the MTS of the plantar flexors (Ryan et al., 2008; 2009) and hamstrings (Matsuo et al., 2013; Nakamura et al., 2019; Takeuchi et al., 2021b; 2022a). Ryan et al. (2009) reported that the MTS of the plantar flexors decreased with the onset of acute static stretching and significant decreases occurred with stretching for longer than 90 seconds. In addition, more than 180 seconds of static stretching effectively decreases the MTS of the hamstrings (Matsuo et al., 2013; Nakamura et al., 2019; Takeuchi et al., 2022a). In the present study, the average stretching duration of acute static stretching was 248.6 seconds and this stretching duration was considered sufficient to decrease the MTS of the plantar flexors and hamstrings. Moreover, a previous study showed that the acute effects of static stretching interventions on the MTS of the hamstrings are influenced by the stretching load (Takeuchi et al., 2021b). Stretching load is calculated from the duration and intensity (torque) of stretching (Takeuchi et al., 2021b), and longer-duration or higher-intensity stretching demonstrates a greater stretching load. Thus, the duration of static stretching is associated with acute MTS decrement, which supports the results of the meta-regression of this study. Therefore, it was indicated that static stretching for an average of approximately 240 seconds (4 minutes) decreased the MTS of the plantar flexors and hamstrings.

We were unable to get the data from three papers on acute static stretching (Halbertsma et al., 1996; Herda et al., 2009; 2010). Regarding papers for which data have not been obtained, Herda et al. (1200 seconds of acute stretching) (Herda et al., 2009; 2010) reported a significant decrease in the MTS of the plantar flexors, although Halbertsma et al. (1996) (600 seconds of acute stretching) found a non-significant change in the MTS of the hamstrings (p = 0.372). Thus, 9 out of the 10 papers that met the inclusion criteria of this study showed a decrease in MTS (Ryan et al., 2008; Herda et al., 2009, 2010; Rodrigues et al., 2017; Konrad et al., 2017; 2019; Palmer et al., 2019; Oba et al., 2021b; Hatano et al., 2022), and 1 paper showed no change (Halbertsma et al., 1996). Therefore, the presence of missing data may not significantly affect the results of the main meta-analysis of acute static stretching.

For long-term static stretching, the results of the meta-analysis showed a non-significant moderate effect size (effect size = -0.608, p = 0.078). The average total stretching duration, intervention term, and weekly dose were 147.6 minutes (range between 60 - 450 minutes), 5.6 weeks (range between 2.9 - 12 weeks), and 26.4 minutes/week (range between 10 - 50 minutes/week), respectively. Freitas et al. (2018) conducted a meta-analysis of the effects of long-term stretching including all stretching types (static, dynamic, and proprioceptive neuromuscular facilitation stretching), regardless of the health condition of the participants, on MTS and reported a non-significant trivial effect of stretching for 8 weeks or less (effect size = -0.30, p = 0.345). Randomized controlled trials of long-term dynamic stretching (LaRoche and Connolly, 2006; Konrad and Tilp, 2014b) and proprioceptive neuromuscular facilitation stretching (Konrad et al., 2015) did not show significant changes in the MTS of the plantar flexors. Furthermore, a significant increase has been reported in the MTS of people with osteoarthritis of the knee joint after 6 weeks of static stretching (Reid and McNair, 2011). Therefore, it was suggested that the difference in the magnitude of effect size between the present study (magnitude of moderate) and the previous study (magnitude of trivial) (Freitas et al., 2018) is due to the inclusion criteria that Freitas et al. included all stretching types regardless of the health condition of the participants, while the present study included only static stretching of healthy participants.

There are three potential explanations as to why there was no significant change in MTS after the average of 5.6 weeks of static stretching. Firstly, the intervention term may have been insufficient to change the mechanical properties of the muscle-tendon unit. Three studies examined the change in the mechanical properties of the muscle after more than 12 weeks of static stretching (Andrade et al., 2020; Longo et al., 2021; Moltubakk et al., 2021), and all studies found significant changes in the mechanical properties. Andrade et al. (Andrade et al., 2016) and Longo et al. (Longo et al., 2021) reported a significant decrease in the muscle stiffness and MTS of the plantar flexors after 12 weeks of static stretching. Moltubakk et al. (2021) examined the effects of 24 weeks of static stretching and found a significant decrease in the passive torque at a given angle in the plantar flexors, which reflects the extensibility of the muscle-tendon unit. Consequently, the effect of static stretching for a longer term ( $\geq 12$  weeks) can likely reduce MTS, however, it needs to be further examined. Secondly, MTS is affected by various tissues, such as nerves (Andrade et al., 2016; 2018; 2020), tendons (Kubo et al., 2001; 2002b; Mahieu et al., 2009), and connective tissue (Gajdosik and Williams, 2002; Morse et al., 2008; Sato et al., 2020). A recent meta-analysis examining the effects of long-term static stretching (average of 5.8 weeks, range between 3 - 12 weeks) showed a significant decrease in the muscle stiffness of the plantar flexors and hamstrings with a moderate effect size (effect size = -0.749, p < 0.01) (Takeuchi et al., 2023). Furthermore, previous studies reported that tendon stiffness was not changed after 3-6 weeks of static stretching (Kubo et al., 2002a; Mahieu et al., 2007; Konrad and Tilp, 2014a; Blazevich et al., 2014). Thirdly, the MTS calculation method may be involved. MTS is calculated from the slope of the linear region of the torque-angle curve during passive joint movement (Magnusson et al., 1996a; b). Because deflection of the Achilles tendon, called slack, is related to stiffness at a position close to plantar flexion of the ankle joint (Hug et al., 2013; Hirata et al., 2016), many studies on the ankle joint have calculated MTS from the slope of the torque-angle curve in the ankle dorsiflexed position (Kubo et al., 2002a; Guissard and Duchateau, 2004; Akagi and Takahashi, 2014; Longo et al., 2021). However, the two studies with positive effect sizes in this study (both, effect size = 0.565) calculated MTS in the range of 0-100% ROM of ankle joint (Gajdosik et al., 2007; Konrad and Tilp, 2014a), suggesting that differences in the calculation method of MTS may have influenced the results.

We were unable to obtain the data from three studies on long-term static stretching (Magnusson et al., 1996b; Reid and McNair, 2004; Blazevich et al., 2014). Those studies show different results. Blazevich et al. (2014) (4 weeks, total stretching duration of 5400 seconds) reported a significant decrease in the MTS of the plantar flexors. Reid and McNair (2004) (6 weeks, total stretching duration of 2700 seconds) reported a significant increase in the MTS of the hamstrings. Moreover, Magnusson et al. (1996b) (3 weeks, total stretching duration of 9000 seconds) reported a non-significant change in the MTS of the hamstrings. Thus, even if these studies were added to the meta-analysis of the present study, the result that MTS is not changed by long-term static stretching would not change.

In the present study, the results revealed a significant publication bias (acute, intercept = -9.52, p = 0.018; long-term, intercept = -5.65, p = 0.018) and high heterogeneity (acute,  $I^2 = 79.098$ ; long-term,  $I^2 = 83.061$ ). The effect sizes of Ryan et al. (2008) (effect size = -3.790) and Guissard and Duchateau (2004) (effect size = -10.748) were very large and led to a potential risk of publication bias and heterogeneity. Even when we excluded these studies, the overall results of the meta-analysis were similar but the effect sizes became lower (acute static stretching, effect size = -0.398, Z = -2.656, 95% CI = -0.692 - -0.104, p =0.008; long-term static stretching, effect size = -0.253, Z = -1.595, 95% CI = -0.563 - -0.058104, p =0.008), although there was no significant publication bias (acute, intercept = 0.017, p = 0.991; long-term, intercept = -0.205, p = 0.928) or heterogeneity (acute,  $I^2 = 0.000$ ; long-term,  $I^2 = 24.409$ ).

There were some limitations in the present study. First, only two moderating variables were considered for the subgroup analysis. Therefore, the effects of potential variables for decreasing MTS, such as stretching intensity (Takeuchi and Nakamura, 2020b; Nakamura et al., 2021a; 2021b; Takeuchi et al., 2021a; 2021b; 2021c; 2021d; Fukaya et al., 2022), stretching load (Takeuchi et al., 2021b; 2022a), frequency (Nakamura et al., 2020), rest interval (Nojiri et al., 2021; Oba et al., 2021a), and MTS calculation method (Nordez et al., 2006) were obscured. Second, the papers that met the inclusion criteria for this metaanalysis were hamstrings and plantar flexors. For the shoulder joint, the MTS measurement method was reported (Wight et al., 2018), but the effect of static stretching on the MTS of the shoulder joint was not examined. For other joints, no method of measuring MTS has been shown. However, it has been shown that the muscle stiffness of the rectus femoris (Nakamura et al., 2021a; Takeuchi et al., 2021d), iliopsoas (Nojiri et al., 2021), teres minor (Yamauchi et al., 2016), and infraspinatus (Yamauchi et al., 2016) are decreased by static stretching. When studies are pooled, the different effects of static stretching on different muscles need to be further examined. Third, the meta-analysis was conducted with young participants with an average age in their 20s. Thus, future studies should also investigate younger and older populations. Finally, the results of the subgroup analysis revealed that there was no significant difference between the muscles (plantar flexors vs. hamstrings) or sex (mixed sex and female vs. male). However, no studies have directly examined the effects of sex or muscle on change in the MTS after static stretching interventions.

## Conclusion

The results of the main meta-analysis showed that MTS significantly decreased after acute static stretching with a moderate effect size, but there was no significant change in MTS after long-term static stretching. Subgroup analysis

showed no significant difference between muscle or sex. Furthermore, there was a significant relationship between total stretching duration and MTS in acute static stretching, but not in long-term stretching.

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## **Key points**

- The meta-analysis revealed that acute static stretching training can decrease muscle-tendon unit stiffness in young healthy participants.
- Long-term static stretching did not change muscle-tendon unit stiffness.
- There was a significant relationship between total stretching duration and muscle-tendon unit stiffness in acute static stretching, but not in long-term stretching.

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