LEUPHANA
UNIVERSITÄT LÜNEBURG

Influence of Long-Lasting Static Stretching on Functional and Morphological Parameters

Dissertation
submitted to the Faculty "Education"
of the Leuphana University, Lüneburg (Germany)
in Fulfilment of the Requirements for the Degree

Doctor of Philosophy (Dr. phil.)

by

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Date of Submisson: 02/22/2023

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Day of Disputation:	07/10/2023

The included studies as well as the writing of this paper were performed from April 2020 to February 2023 at the Department of Exercise, Sport and Health at the Leuphana University Lüneburg in cooperation with the University Sports Center of the Carl von Ossietzky University of Oldenburg.
No further than the listed authors and resources contributed to this work neither it was submitted elsewhere.

Lüneburg, 02/15/2023

Konstantin Warneke

List of Abbreviations

ALD	Anterior Latissimus Dorsi
ANOVA	Analysis of Variance
ATPase	Adenosinetriphosphatase
CG	Control Group
CGr	Right Leg of the Control Group
CGl	Left Leg of the Control Group
CMD	Calf Muscle Testing Device
CV	Coefficient of Variability
CI	Confidence Interval
cl	Control Leg
d	Cohens d
df	Degrees of Freedom
DNA	Deoxyribonucleic Acid
DOMS	Delayed Onset Muscle Soreness
EIMD	Exercise-Induced Muscle Damage
ES	Effect Size
η^2	Partial Eta Square
f	Female
FGF	Fibroblast Growth Factor
FCSA	Fiber Cross-Sectional Area
FN	Fiber Number
FL	Fiber Length
FT	Fast Twitch
Fz	Vertical Force
GSK3ß	Glykogensynthase-Kinase 3ß
H1	Hypothesis 1
H2	Hypothesis 2
H3	Hypothesis3
H4	Hypothesis 4
IG	intervention Group
ICC	Intraclass Correlation Coefficient
IGF-1	Insulin-Like Growth Factor-1
IGFR	IGF-1 Specific Receptors
il	Intervened Leg
IRS-1	Insulin Receptor Substrate 1
I^2	Measurement for Level of Heterogeneity
kg	Kilogramm
M	Mean
m	Male

MAE	Mean Absolute Error
MAPE	Mean Absolute Percentage error
MCSA	Muscle Cross-Sectional Area
MCSAL	Muscle Cross-sectional Area in the Lateral Head of the Gastrocnemius
MCSAM	Muscle Cross-sectional Area in the Medial Head of the Gastrocnemius
ME	Mean Error
MGF	Mechano Growth Factor
MHz	Megahertz
mm	Millimeter
MSt	Maximum Strength
MSt90	Maximum Strength in the Flexed Knee Joint
MSt180	Maximum Strength in the Extended Knee Joint
MiSt	Maximum Isometric Strength
MdSt	Maximum Dynamic Strength
MTh	Muscle Thickness
MThL	Muscle Thickness in the Lateral Head of the Gastrocnemius
MThM	Muscle Thickness in the Medial Head of the Gastrocnemius
mTOR	Mechanistic Target of Rapamycin
MVC	Maximum Voluntary Contraction
MVC180	Maximum Voluntary Contraction in the Plantar Flexors with Extended Knee joint
MVC90	Maximum Voluntary Contraction in the Plantar Flexors with Flexed Knee Joint
MGF	Myogenic Growth Factor
MTU	Muscle Tendon Unit
N	Newton
Nm	Newtonmeter
mN	MilliNewton
ORTH	ROM Measurement in the Ankle via the Goniometer of the Orthosis
OA	Old Animals
OCG	Old Control Group
OIG	Old Intervention Group
PAT	Patagialis Muscle
p70s6K	Ribosomal Protein S6 Kinase
PI3K	Phosphoinositid-3-Kinase
PA	Pennation Aangle
PaL	Pennation Angle in the Lateral Head of the Gastrocnemius
PaM	Pennation Angle in the Medial Head of the Gastrocnemius
PKB/AKT	Proteinkinases B
Pretest immo	Pretest with Immobilized Leg
Posttest immo	Posttest with Immobilized Leg
RM	Repetition Maximum
ROM	Range of Motion
RNA	Ribonucleinacid
SAC	Stretch-Activated Channels

SD	Standard Deviation
SMD	Standard Mean Difference
ST	Slow Twitch
3T	3 Tesla
τ^2	Measurement for Level of Heterogeneity
TUT	Time under Tension
TSC1	Hamartin (Protein)
TSC2	Tuberin (Protein)
YA	Young Animals
YCG	Young Control Group
YIG	Young Intervention Group

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1 Introduction

Stretching is primarily used to improve flexibility [208, 210], decrease stiffness of the muscle-tendon unit [110, 361] or reduce risk of injury [29, 40, 44]. However, using prolonged static stretching in warm up routines is discussed and questioned, since the literature provides evidence for detrimental effects on subsequent performance parameters [40, 44, 43, 70]. Hence, some authors recommended implementing static stretching sessions without a relation to following exercises and to avoid performing prolonged static stretching sessions in movement preparation. Thus, authors investigated the effects of stretching interventions in separated sessions over a period of some weeks on flexibility showing significant enhancements in range of motion (ROM) as long-term effects in the stretched muscles [92, 208, 210]. Since previous animal studies in $1970 - 1990$ showed significant hypertrophy effects in skeletal muscle in response to chronic stretching intervention with stretching durations of 30 minutes to 24 hours per day [18, 85, 105, 282], a growing number of authors investigated changes in strength capacity and/or hypertrophy due to long-term stretching training [160, 229, 262, 277, 359]. However, no study was performed using comparatively long stretching durations of more than 30 minutes per day with a daily frequency in humans.

The present cumulative dissertation includes six studies aiming to investigate the effects of long-lasting static stretching training on maximum strength capacity, hypertrophy and flexibility in the skeletal muscle. Before starting own experimental studies, a meta-analysis of available animal research in this topic was conducted to analyze the potential of long-lasting stretching interventions on muscle mass and maximum strength [326]. To induce long-lasting stretching on the plantar flexors and to improve standardization of the stretch training by quantifying the angle in the ankle joint while stretching, a calf muscle stretching orthosis was developed. In the following experimental studies, the orthosis was used to induce daily long-lasting static stretching stimuli with different stretching durations [325, 329, 327, 330, 331] and intensities [332] in the plantar flexors to assess different morphological and functional parameters. For this, a total of 311 participants were included in the studies and, dependent on the investigation, the effects of stretching durations of $10 - 120$ minutes were analyzed. Therefore, to investigate effects in functional parameters maximal isometric and dynamic strength and flexibility of the plantar flexors were investigated with extended and flexed knee joint. The investigation of morphological parameters of the calf muscle was performed by determining the muscle thickness and the pennation angle by using sonographic imaging and the muscle cross-sectional area by using a 3 Tesla magnetic resonance imaging measurement. Results are discussed considering hypotheses stated in animal studies. Mentioned studies are the first investigations examining the effects of long-lasting static stretching of up to two hours on functional and morphological parameters in human skeletal muscle. Consequently, results lead to further research questions, especially to obtain better insights of physiological/biochemical adaptations in response to mechanical tension induced via stretching, which are discussed in

the final section of this work in combination with possible practical applications in the future. Before presenting own results, the theoretical background of improvements in maximal strength, muscle thickness and flexibility will be described in the following.

2 Studies Included in this Work

Warneke, K., Freund, P.A., Schiemann, S. (2022). Long-Lasting Stretching Training Produces Muscle Hypertrophy – A Meta-Analysis of Animal Studies, J Sci Sport Exerc, https://doi.org/10.1007/s42978-022-00191-z

Warneke, K., Wirth, K., Keiner, M., Schieman, S. (2023). Improvements in Flexibility Depend on Stretching Duration, Int J Exerc Sci 16(4): 83-94.

Warneke, K., Brinkmann, A., Hillebrecht, M., Schiemann, S. (2022). Influence of Long-Lasting Static Stretching on Maximal Strength, Muscle Thickness and Flexibility, Front Physiol. https://doi.org/10.3389/fphys.2022.878

Warneke, K., Keiner, M., Hillebrecht, M., Schiemann, S. (2022). Influence of One Hour versus Two Hours of Daily Static-Stretching for six Weeks Using a Calf-Muscle-Stretching Orthosis on Maximal Strength, Int. J. Environ. Res Public Health, 19, 11621, https://doi.org/10.3390/ijerph191811621

Warneke, K., Wirth, K., Keiner M., Lohmann, L.H., Hillebrecht, M., Brinkmann, A., Wohlann, T., Schiemann, S. (2023). Comparison of the Effects of Long-Lasting Static Stretching and Hypertrophy Training on Maximal Strength, Muscle Thickness and Flexibility in the Plantar Flexors. Eur. J. Appl. Physiol, https://doi.org/10.1007/s00421-023-05184-6.

Warneke, K., Keiner, M., Wohlann, T., Lohmann, L.H., Schmitt, T., Hillebrecht, M., Brinkmann, A., Hein, A., Wirth, K., Schiemann, S. (2023). Influence of Long-Lasting Static Stretching Intervention on Functional and Morphological Paramters in the Plantar Flexors: A Randomised Controlled Trial, J Strength Cond Res 00(0)/1-9, 2023.

3 Theoretical Background and Thematic Derivation

The first section of this work serves to provide relevant background information on the current state of research, describing adaptations of known training methods, and to give a brief insight into possible underlying biological adaptation processes. Based on this, the research question underlying this work is derived.

3.1 Definition of Terms

Performing resistance training is associated with improved sports performance [60, 133, 178]. Furthermore, it is well known to induce hypertrophy resulting in enhanced muscle cross-sectional area and muscle thickness [184, 268, 351] which is related to a wide range of advantages regarding health and fitness in daily life [340, 344]. Benefits in muscle function due to resistance training can be attributed mainly to improvements in maximum strength capacity which can be seen as a crucial factor in human movements [292, 344, 353]. Maximum strength is defined as the *"ability to produce a maximal voluntary muscular contraction against an external resistance"* [344, p.2084] and can be measured dynamically via one repetition maximum (1RM) strength testing [59, 125, 344] or under isometric conditions [259, 327]. This means performing a maximal voluntary contraction against an unyielding resistance (isometric contraction) [327]. In literature, Goldspink & Harridge [119] referred to muscle force as a reflection of cross-bridges working in parallel. Consequently, there seems to be a link between fiber cross-sectional area and muscle force *"Fibre cross-sectional area is thus a reasonably accurate way of predicting the force that a muscle fibre can develop"* [119, p.233]. However, since force output also depends on neuromuscular factors, such as simultaneous activation of the available muscle potential via recruitment of related motor neurons with high to maximal frequencies [98, 119], this *"make it impossible to determine specific force generation accurately"* [119, p.233]. Thus, increased maximum strength capacity induced by resistance training can be assumed to be the result of a wide range of specific neuromuscular and structural adaptations in response to a specific training stimulus [45, 46, 119, 339].
Enhancements of muscle thickness and maximum strength can lead to improvements in injury prevention [214, 272], a higher performance level in competitive sports [291, 292] and seems to be of major impact in rehabilitation of orthopedic indications for example in the therapy of muscular atrophy and loss of strength due to immobilization after an injury or a surgery [192, 288, 347]. Resistance training performed over the full range of motion (ROM) is also sufficient to improve flexibility [2, 235] – which can be divided into static and dynamic flexibility. Static flexibility can be defined as *"the range of motion [...] available to a joint or series of joints"* [114, p.289] while dynamic flexibility *"refers to the ease of movement within an obtainable ROM"* [114, p.290f]. In the presented work, the definition of static flexibility is used and measured. In addition to maximum strength, improvements in flexibility are important in many fields of physical exercise, e.g. in injury prevention [29, 114, 356] but also in rehabilitation

[159, 320]. Consequently, improvements in (skeletal muscle) performance in many fields of sports can be attributed to a large extent to enhancements in maximum strength and flexibility, leading to a high importance of investigating corresponding training methods.

3.2 Effects of Resistance Training

If the aim is to induce significant increases in maximum strength, there is a huge amount of literature demonstrating effectiveness of resistance training for a period of many weeks. Some studies are exemplary included in this section to provide general evidence. Wirth et al. [352] showed an increase of up to 6.7% (d = 0.28) using an eight-week strength training program for the lower extremity, while strength training performed for up to two years showed increases of more than 100% (d = 6.13) [258]. Furthermore, Lynch et al. [188] performed a whole-body resistance training protocol for twelve weeks including the bench press, leg press, seated leg curls and leg extensions three days per week. The authors pointed out enhancements in maximum strength of 14.39% (d = 0.45) and 32.32% (d = 1.01) in the leg extension and 11.9% (d = 0.47) as well as 33.72% (d = 1.20) in the leg muscles after six and twelve weeks, respectively. Green & Gabriel [124] demonstrated an increase in maximum strength of up to 27% (d = 0.84) following six weeks of resistance training for the forearm and calf musculature. A meta-analysis by Ralston et al. [246] determined that a high number of weekly sets would lead to higher increases in maximum strength (with pooled effect size of 0.97) compared with a low number of weekly sets (with a pooled effect size of 0.86). Examining the influence of the training frequency on training effects, Grgic and colleagues [126] reviewed the current literature and pointed out that higher increases in strength capacity can be assumed for high compared to low training frequency. Accordingly, Borde et al. [58] demonstrated significant improvements in maximum strength following resistance training of $13 - 90\%$ (with an overall effect size of 1.57 [1.20, 19.4 95% Confidence interval (CI)]) from 25 studies and hypertrophic adaptations of $1 - 21\%$ (with an overall effect size of 0.42 [0.18,0.66 95% CI]) from nine studies in a random-model meta-analysis, using standardized mean differences (SMD). Schoenfeld et al. [268] included 24 studies in their meta-analysis and showed increases in maximum strength due to different resistance training protocols with an overall effect size of 1.50 ± 0.23 and a mean percentage change of $31.6 \pm 4.5\%$ in 1RM with a mean effect size of Δ = -0.37 $\pm$ 0.1 between high and low load resistance training, highlighting the importance of high intensities in resistance training. Furthermore, authors stated a mean hypertrophy effect of $7.6 \pm 1.2\%$ with an effect size (ES) of 0.47 ± 0.08, however, they were not able to determine a significant difference in ES between high load and low load training programs for muscle cross-sectional increases.

Using resistance training programs, increases in maximum strength are often accompanied by hypertrophic effects which are commonly measured via enhancements in muscle thickness and/or muscle cross-sectional area [170, 268, 267]. Vikberg et al. [319] pointed out increases in arm lean mass of 5.35% (d = 0.18) by using bodyweight exercises with ten to twelve repetitions.

Evangelista and colleagues [94] showed significant increases in muscle thickness of 17.78% (d = 0.66) in response to an eight-week traditional resistance training program with four sets of eight to twelve repetitions while Ozaki et al [234] were able to increase muscle thickness significantly with 13.06% (d = 0.59) in a twelve-week training intervention in older adults. Further investigations showed significant increases of muscle cross-sectional area in a six-week [285] to six months [27] training period with $5.8 - 6.1\%$ [27, 285, 303, 337] (no original values available to calculate Cohen's d).

3.3 Impact of Mechanical Tension on Adaptations

The literature shows several factors influencing the effectiveness and the outcome of resistance training routines. To maximize training effects regarding maximum strength gains and hypertrophy, it seems beneficial to use high training frequencies [126, 246] and high intensities [170, 173, 268], whereby intensity in training routines is commonly stated as a percentage of the 1RM [173]. From a physiological point of view, increasing weight in resistance training leads to enhanced mechanical tension which can be seen as one factor to induce microtraumatization and exercise induced muscle damage [100, 265, 266]. Additionally, the literature also demonstrated that high load resistance training provides a sufficient stimulus to induce several anabolic responses due to anabolic signaling pathways, for example the mechanistic target of rapamycin/ribosomal protein S6 kinases/Phosphoinositide 3-kinases (mTOR/p70s6k/PI3K) pathway, which seems to be related to an enhancement in muscle protein synthesis [117, 147, 321, 322]. There are many studies supporting the hypothesis that p70S6k plays a fundamental role in hypertrophy after resistance training [55, 316, 323]. Hartmann et al. [134] described the influence of mechanical tension due to muscle contractions on the gene expression of adults. Therefore, the stimuli from mechanical overload would lead to the release of insulin like growth factor-1 (IGF-1), which can be seen as an important signal to induce muscle growth [187, 261] and myogenin growth factor (MGF), which binds on IGF related protein (IGFR) leading to an activation of anabolic kinases by phosphorylation of insulin receptor substrate 1 (IRS-1). Tidall [309] described the activation of proteinkinasis B (PKB) activating the mTOR pathway with a downstream phosphorylation of p70S6K positively influencing muscle growth [134, 167]. Furthermore, anti-anabolic pathways as glycogen synthase kinase 3 beta (GSK3ß) were mentioned to be inhibited. As it can be assumed that muscle protein synthesis should exceed protein degradation, both reduction of catabolic as well as increase in anabolic processes are of high interest when designing exercise programs in therapy and prevention [186, 187, 256]. Therefore, it could be hypothesized that mechanical tension (induced by using high loads in resistance training) seems to be of crucial importance to induce skeletal muscle hypertrophy as well as maximum strength gains [108, 187, 261, 265]. Mechanical tension can be described as $\text{Tension} = \frac{\text{Force}}{\text{Area}}$. Consequently, to reduce the tension (stressor) on the muscle [146] by using constant forces (weight), the area (muscle cross-section) would increase through hypertrophy. This hypothesis would be in accordance with

the Response-Matrix Model from Toigo & Boutellier [312], describing that a training stimulus (mechanical tension) leads to specific adaptations in the phenotype due to cellular and molecular adaptations by stimulating specific muscle protein synthesis [167, 309, 312]. Therefore, it may be hypothesized that mechanical tension could also be induced by stretching the muscle [105, 282]. The importance of including high degree of stretch to training routines was already propagated by Arnold Schwarzenegger, who is well known as one of the greatest bodybuilders in history. In an interview, he was discussing the chest exercises "flys" performed with dumbbells which *"was an exercise that gave me the full pectoral muscle development because I went all the way out and almost hitting the ground and I was a big believer in expanding the chest as much as possible and giving that stretch because remember with muscles the important thing always is to get the stretch and to get the flex"*. Furthermore, *"those are the kind of exercises to me that were like, you could not replace them with any machine"*. Arnold Schwarzeneggers anecdotal evidence indicates that not only the contraction of the muscle but also movements inducing a high degree of stretch to the muscle may play a major role for inducing hypertrophy in the muscle. Schoenfeld [265, p.2863] pointed out that *"mechanically induced tension produced both by force generation and stretch is considered essential to muscle growth, and the combination of these stimuli appears to have a pronounced additive effect"*. Accordingly, Barbalho and colleagues (2020) demonstrated higher increases in muscle thickness in the glutes (9.4% vs 3.7%) and quadriceps (12.2% vs 2%) following deep barbell back squats – leading to a higher degree of stretch – compared to hip thrusts. From this, it could be hypothesized that stretching plays an important role in muscular adaptations. While stretching the muscle is mostly associated with improvements in flexibility leading to higher degrees in ROM [210], in 1993, Smith and others described delayed onset muscle soreness, which is possibly related to microtraumatization of muscle tissue after muscle stretching. Damage of the muscle due to mechanical stimuli is also known from resistance training [266]. Interestingly, Kremer [167, p.186−188] describes the activation growth factors as FGF, IGF-1, and mTOR leading to stimulation of anabolic pathways as PKB, Tuberous sclerosis 1 and 2 (TSC1, TSC2) due to stretching via so-called stretch activated channels [294, p.54], [261]. Therefore, Coffey & Hawley [76, p.738] stated that *"the process of converting a mechanical signal generated during contraction to a molecular event that promotes adaptation in a muscle cell involves the upregulation of primary and secondary messengers that initiate a cascade of events that result in activation and/or repression of specific signaling pathways regulating exercise-induced gene expression and protein synthesis/degradation"*.

It is hypothesized that there are contractile and metabolic adaptations due to changes in protein synthesis via changes in protein kinases and transcription factors after inducing mechanical tension, which could be attributed to suprathreshold mechanical tension, independent on resistance training or stretching training [304].

3.4 Derivation of the Research Question

Based on this, the question arises about the role of stretching in adaptations of the muscle and if – when performed with adequate intensity and volume – stretching alone could induce significant hypertrophy and maximum strength increases. As seemingly there are many similarities in physiological adaptations and both, resistance- and stretch training, can lead to high mechanical tension, it is hypothesized that stretching could also lead to significant strength and muscle mass gains. Furthermore, as stated in meta-analysis including resistance training studies investigating the effects of varying frequencies, intensities and volumes on performance, it is hypothesized that longer stretching durations, higher training frequencies and higher stretching intensities would lead to higher adaptations in humans.

4 Effects of Stretching Using Animal Model

When aiming to investigate a research question in a new field, there are many possibilities to design the respective studies. It is common to first perform studies in an animal model [140], especially if there is no possibility or high ethical obstacles for studies in humans. To investigate the adaptability of muscle tissue on external factors, to the best knowledge, the first study was performed as early as 1887 by Marey [198]. The investigator changed the position of the distal end of the triceps surae to under the calcaneum showing increased muscle length after a few weeks. In 1958, Alder et al.[5] showed reduced muscle length by immobilizing a muscle in a shortened position, while new contractile tissue in serial and parallel was developed by immobilization of the muscle in a stretched position [72, 300]. However, most studies in animals investigated skeletal muscle adaptations induced by stretching were performed between 1970 and 1996 [20, 69, 105, 153, 282]. Therefore, authors included chicken or quail [9, 18, 36, 105, 139] because of their life span of about two years and were classified as adult when growing was finished after about six weeks after hatching [8]. Before the start of the investigation, the birds were caged under standardized conditions with a 12:12-hours light-dark cycle with free access to water and food. Before the intervention started, all birds were weighed to ensure comparability in starting conditions. In most studies, the muscle of one wing was stretched by using a stretching device [35, 36, 139] or adding weight of $10 - 35\%$ of the own bodyweight to the wing [7, 12, 20, 282]. Most of the studies investigated the influence of different stretching times ranging from 2x15 minutes per day (using an intermittent stretching protocol [36, 105] to chronic 24 hours stretching per day [8, 20, 19, 68, 105, 176] on the morphological parameters muscle mass, muscle cross-sectional area, fiber cross-sectional area, fiber length or fiber number and connective tissue. There are also studies investigating physiological and gene expression changes as well as muscle protein synthesis [35, 85, 155, 301], myosin isoform [9, 155, 203] as well as myosin heavy and light chains [8, 155, 251]. To collect data for the listed parameters, investigators had to remove the intervened and control muscles, consequently, animals had to be dissected. Therefore, there was obviously no possibility to

evaluate an appropriate pre-test value as well as a control group.
In few studies, some animals were dissected at day 0 of the intervention to clarify differences in baseline [10], but mostly, authors did not include a real control group. To include a control condition, the contralateral, non-stretched muscle was determined as an intraindividual control condition [9, 8, 18, 20] and mentioned increases of examined parameters in the studies had to be stated as differences in the posttest between the intervened muscle and the non-stretched control muscle. There are few procedures listed to investigate muscle morphology, however, most studies used the following. Before morphometric assessment, the tissue was stored at -70°C in an ultra-low freezer [12, 63]. Furthermore, the sections were cut at a determined thickness of for example ten micrometer to stain the muscle for myosin adenosintriphosphatase (ATPase). The fiber cross-sectional area was determined by planimetry of a large number of muscle fibers (e.g. 900 in [12]) by using light micrography. To investigate the fiber number, connective tissue was removed after nitric acid digestion and dissection of muscle fibers from the muscle. Guth & Samaha [129] showed that slow twitch (ST) muscle fibers stained with myosin ATPase showed low ATPase activity at pH 9.4 but high activity at pH 4.35 while fast twitch (FT) fibers showed the opposite result [282].
In some studies, the fiber type was determined additionally from the myosin-ATPase after acid preincubation at pH 4.35 and alkaline preincubation at pH 10.45 [12]. Most of the studies investigating the muscles in dissected quails and chicken and examined the anterior latissimus dorsi [20, 67, 203, 350] or the patagialis (PAT) muscle, which is also known as the flight muscle [63, 99, 105]. While the anterior latissimus dorsi can be assumed to consist of a high percentage of ST muscle fibers (up to 95%) the patagialis muscle is stated to consist of a large amount of FT muscle fibers [20, 18]. Many studies found large differences in muscle mass between the intervened muscle and the control muscle following a chronic stretching stimulus of 24 hours per day for a few days [12, 35, 68] up to six weeks [105]. After only two days of stretch, Alway et al. [11] pointed out significant increases in muscle mass and fiber length of 21.3 ± 4.7% (d = 3.37) and 35.7 ± 5.1% (d = 4.56), respectively in the anterior latissimus dorsi of quails. Many authors attributed the increases in muscle mass to mechanical overload via stretch *"It is Stretch that Causes the Hypertrophy of Muscle"* [282, p.93]. After seven days of stretching, authors pointed out increases of 64.0 ± 8.4% (d = 6.5) in muscle mass with an increase of 40.1% (d = 6.18) in fiber length, 29.9 ± 12.3% (d = 2.22) in fiber cross-sectional area as well as 27.3 ± 3.0% (d = 7.16) in fiber number [11]. Bates [36] and Carson et al. [68] confirmed fast increases in muscle mass due to chronic stretching interventions for up to 14 days showing increases of 94.1 ± 7.4% (d = 12.07) after seven days [68] and 96% after ten days of stretch [36]. After 14 days of stretch, increases in muscle mass of 134.7 ± 5.8% (d = 18.59) [68] and 141.6 ± 9.5% (d = 15.26) [67] were found. Furthermore, Bates [36] pointed out enhancements of 116% after 20 days of stretch. Overall, most authors investigated intervention periods of 28 − 42 days of stretch with increases in muscle mass of up to 318 ± 31.5% in the anterior latissimus dorsi [20] and 121% (d = 7.48) in the patagialis muscle [105]. Hereby, stretch was performed using chronic stretching (24 hours per day and seven days per week) [8, 69] or with intermittent

stretching protocols [18]. Results from intermittent stretching protocols used by Bates [36] and Frankeny et al. [105] led to the authors' assumption of a dose-response relationship for daily stretching durations because enhancing the time under tension (TUT) showed higher increases in muscle mass over a intervention period of five and six weeks, respectively. Furthermore, authors showed significant increases in muscle cross-sectional area of 141.6 ± 32.5% (d = 5.83) or in fiber cross-sectional area of 96.5 ± 3.2% (d = 13.62) [105]. Those adaptations were accompanied by an increase in fiber length of 80.4 ± 11.8% (d = 16.28) and hyperplasia effects of 82.2 ± 17.1% [20]. Adaptations of the patagialis muscle seemed to be lower than those in the anterior latissimus dorsi which could possibly be attributed to differences in the fiber distribution [12]. Authors found that increases in muscle mass and muscle cross-sectional area were accompanied by enhanced level of deoxyribonucleinacid (DNA), ribonucleic acid (RNA) [23, 35, 63, 117, 286] as well as enhanced muscle protein synthesis [117]. Furthermore, Alway [9] showed significant maximum strength increases in vitro of 95% (d = 11.13) due to chronic stretching interventions of 30 days. More detailed information about the main outcome and interpretation of the authors about the examined parameters for each study are provided in brief summaries in the Supplemental Material. Previous to own experimental investigations and to get a quantitative value of the conducted effects in animals, the first study included to this work was a meta-analysis.

4.1 Long-Lasting Stretching Produces Muscle Hypertrophy – A Meta-Analysis of Animal Studies

In *Journal of Science in Sport and Exercise*,
https://doi.org/10.1007/s42978-022-00191-z

To investigate long-term effects of stretching interventions on muscle mass, muscle cross-sectional area, fiber cross-sectional area, fiber number, and fiber length, a meta-analysis was performed by using RevMan version 5.4.1 [78] to quantify the effects of the current literature. For this, the following search terms were defined to search the databases of PubMed, Web of Science, and Scopus:

((“hypertrophy” OR “hyperplasia”) AND (“stretch-induced growth” OR “stretch-induced hypertrophy” OR “fiber number” OR “fiber length” OR “sarcomere length” OR “sarcomere number”) AND “skeletal muscle”) NOT (“exercise induced” OR “endocrine” OR “nervous system” OR “electrical stimulation” OR “cardiomyocytes”).

The search strategy was limited to English language sources only. A total of 89 publications were found from this combination of terms. The references of those publications were also examined for further relevant studies. However, this did not yield in any additional studies. After reviewing the titles, 47 studies remained, which were then screened to exclude studies that only indirectly investigated structural adaptations and those studies that focused on hormonal adaptations, muscle fiber distribution, or signal transduction pathways without collecting the

target parameters of muscle mass, muscle cross-sectional area, fiber cross-sectional area, fiber length, or fiber number. Afterwards, 23 studies remained, which were then subjected to full-text analysis using inclusion and exclusion criteria (see original paper in the supplemental material) established in advance of the meta-analysis for the final selection. Studies including objective measurement of listed parameters with a stretching intervention of at least one week and stretching times of at least 15 minutes per day were included in the meta-analysis. Furthermore, mean (M) and standard deviation (SD) had to be given. The results of included studies answering the research question are stated in Table 1.

Table 1: Description of included studies, providing details regarding the intervention and an overview of results

Source	**Subjects**	**Muscle group**	**Intervention**	**Measured parameters**
Alway et al.[11]	N=63	ALD	seven day stretching intervention, nine animals examined every day	MM:+64±8.4% MCSA:+29.9±12.3% FL:+40.2±2.2% FN:+27.3±3%
Alway [7]	N=36; 22 in intervention group	ALD	30-day stretching with 12% of bodyweight	MM:+161.5±7.9% FL:+25.4±4.6%
Antonio et al.[20]	N=26	ALD	intermittent stretching protocol with progressive weight increase, followed by continuous stretching at 35% of own body weight. Intervention period 37 days	Maximal values MM:+318±31.5% FN:+82.2±17.1% MCSA:+141.6±32.5%
Antonio & Gonyea [18]	N=7	ALD	stretching with 10% of bodyweight; intermittent stretching protocol	MM:+53.1±9% FCSA:+27.8±6% FL:+26.1±7.3%
Antonio & Gonyea [19]	N=18	ALD	28-day stretching intervention with 29% of bodyweight, animals examined after 16 days and 28 days	MM: day16:+188.1±15.6%; day28:+294.3±39.1% FL: day16:+80.4±11.8%; day28:+74.6±9.7% FN: day16:-6.7% ±4.6%; day28:+29.7±6.8%
Barnett et al. [35]	N=63	PAT, biceps brachii	unilateral stretching for up to 10 days, animals examined after 1, 2, 3, 7 and 10 days	MM: PAT: CG: 0.1474±0.0142g IG: 0.2461±0.0239g Biceps brachii: CG: 0.5914±0.0607 IG: 0.7644±0.0646

Brown et al. [63]	N=40	PAT	16-day stretching intervention, animals were examined after 6 days and 16 days	Muscle mass increased for 61% in 6-week-old chicken and 34% in 10-month-old chicken. 28-month-old animals had an 18% loss of muscle mass during passive stretch.
Carson et al. [68]	N=94, young (YA) and old (OA) animals	ALD	30-day stretching intervention with 10% of bodyweight, animals were examined after 7, 14 and 30 days in both ages	MM: YA: 7d: 94.1±7.4%; 14d:134.7±5.8% OA: 7d: 82.1±4.9%; 14d:102.4±6.5% FL: YA: 7d: 37.7±2.0%; 14d:28.9±4.0% OA: 7d:39.8±4.1%; 14d:21.3±5.3% FN: YA:14d:31.6±2.1%; OA:14d:19.2±2.2% FCSA: YA:14d:51.6±7%; OA:14d:39.6±8.5%
Carson et al. [69]	N=32, (young (YA) n=16 vs old (OA) n=16 animals)	ALD	unilateral stretching with 10% of bodyweight, contralateral muscle was control muscle	MM:YA:+178.7±7.1% OA:+142.8±7.9% FN:YA:IG:22.5±0.4 vs. CG: 18.5±0.4 OA: IG: 22.8±1.2 vs. CG:18.4±0.9 MCSA: YA: +63.8±7.8%; OA: +49.1±5.4% FN:YA:+59.6±8%; OA: +47.2±8.1%
Carson & Alway [67]	N=30, young (YA) (n=15), old (OA) (n=15).	ALD	unilateral stretching for 7 and 14 days	MM: YA;7d:+98.7±12% YA;14d: +141.4±9.5% OA;7d:+83.9±6.6% OA;14d:+106.9±11%
Czerwinski et al. [85]	N=57, chicken	PAT	11-day intervention, stretched muscle versus control muscle, banded stretch for one wing	MM: CG: 1.3±0.07g vs. IG: 1.88±0.09g

Frankeny et al. [105]	N=54	PAT	Six week stretching intervention with several stretching protocols, 8,4,2+2,2,1,0.5 und 0.25+0.25 hours of intermittent stretching and 24h of permanent stretching	MM: 24h: +121% MCSA: up to +111% FCSA: up to +110%
Matthews et al. [203]	N=10	PAT	33-day stretching intervention with 10% of bodyweight	MM: +247±91% FCSA: IG: 985±291µm² CG: 520±96 µm²
Roman & Alway [251]	N=28	ALD	21 days stretching intervention, animals examined after 7, 14 and 21 days	MM: 7days: CG:37.2±1.8mg IG: 54.6±2.9mg 14days: CG:43.5±2.7mg IG:67.8±4.3mg 21days: CG:42.6±3.2mg IG:71.2±3.7mg
Sparrow [286]	N=60	ALD	30-day stretching intervention, 30 animals examined after 3, 7, 13 and 29 days, remaining animals examined after 5, 13, 25 and 35 days after stretching without intervention to investigate regression	MM: CG: 0.928±0.026g IG: 1.850±0.07g

Abbreviations: ALD = anterior latissimus dorsi muscle; PAT = patagialis muscle; MM = muscle mass; MCSA = muscle cross-sectional area; FCSA = fiber cross sectional area; FL = fiber length; FN = fiber number; YA = young animals; OA = old animals;IG = intervention group; CG = control group.

A random effects model was used to take into account any heterogeneity resulting from the use of different species in the studies and all other potential between-study differences. Figures 1 and 2 report the empirical mean (M), standard deviation (SD), and number of test subjects (N) for the parameters muscle mass and fiber cross-sectional area. For all analyses, the SMD (with inverse variance weighting) and its 95% confidence interval were computed as the effect size of interest in RevMan.

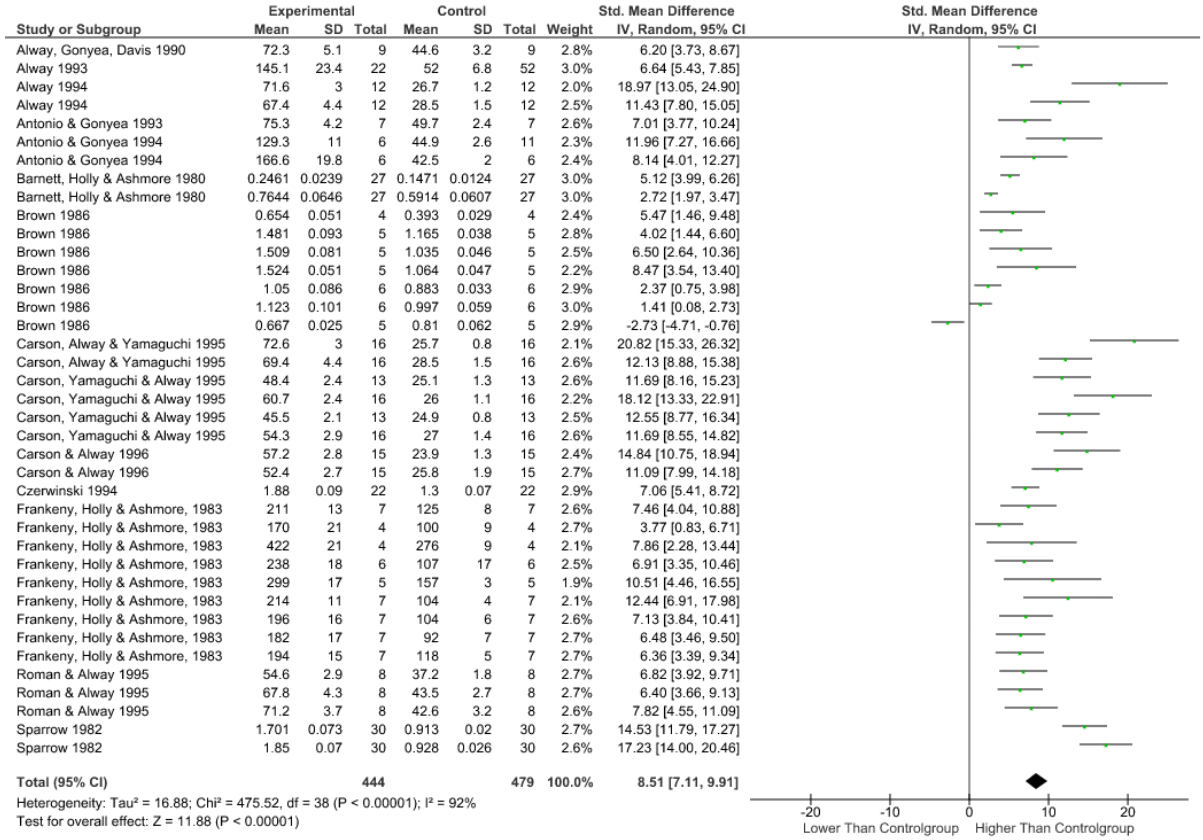

Figure 1: Forest plot from included studies investigating the parameter muscle mass using mean, standard deviation and sample size in a random model

Figure 1 shows a consistent very large magnitude overall effect size for the parameter muscle mass with d = 8.51, p < 0.001, 95% CI 7.11 − 9.91 with a peak increase of 318 ± 39.1% and d = 7.01, 95% CI 3.77 − 10.24 due to a combination of chronic and intermittent stretching protocols in a 37- day stretching period. There were also consistent very large magnitude effects for muscle cross sectional area (d = 7.91, p < 0.001, 95% CI 5.75 − 10.08), fiber cross-sectional area (d = 5.81, p < 0.001, 95% CI 4.32 − 7.31), fiber number (d = 4.62, p < 0.001, 2.54 − 6.71) as well as fiber length (d = 7.86, p < 0.001, 95% CI 4.00 − 11.72). Remaining forest plots are provided in the original paper attached to this work (see supplemental material).
Considering the results, it can be assumed that stretching leads to sufficient mechanical tension to produce increases in muscle mass due to hypertrophy (and hyperplasia) in animal model, if stretching is induced by sufficient volume: *"We conclude that daily stretching for as little as 30 minutes per day is a powerful inducer of growth in normal [. . .] muscle"* [105, p.276]. Results demonstrated a dose-response relationship for daily stretching time [36, 105], intervention period [68] and intensity [18] for enhancing muscle mass following stretching in animals. Thus, two papers requested studies on the transferability of results to humans [36, 105], stating *"Thirty minutes of stretching per day is certainly within normal physiological limits, and as a result may be applied to human muscle with hopes that similar adaptations would occur"* [105, p.275f].

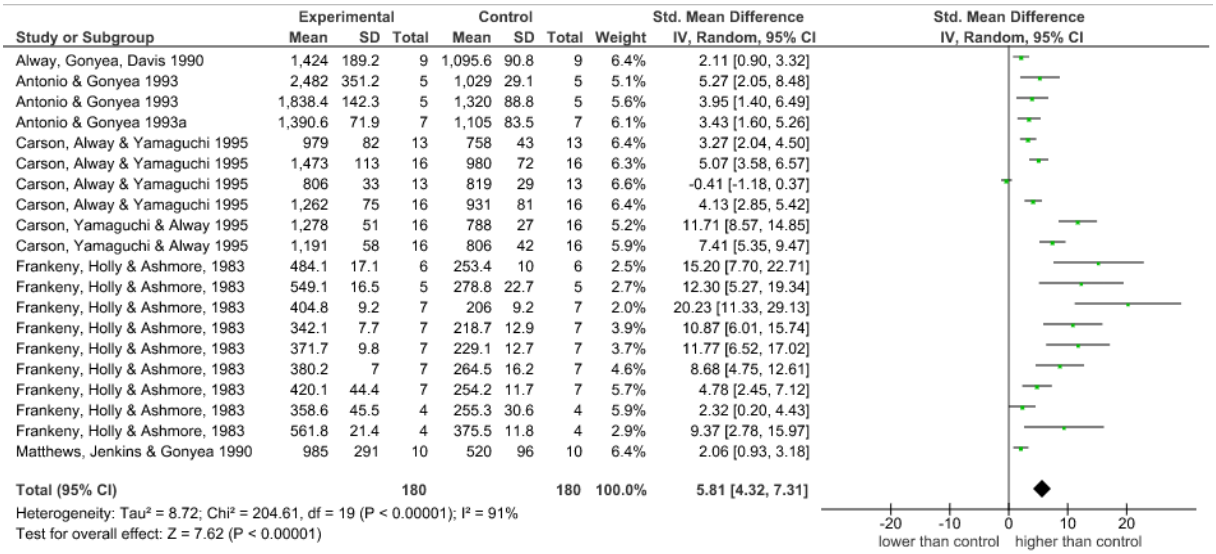

Figure 2: Forest plot from included studies investigating the parameter fiber cross-sectional area using mean, standard deviation and sample size in a random model

It can be hypothesized that the magnitude of adaptations depends on rate of muscle protein synthesis. Assuming that stretching increases morphological parameters and maximal strength in an animal model, a very different rate of muscle protein synthesis must be considered regarding animals and humans [112, 306]. Early experiments by Williams & Goldspink from the 1970s indicated two to three days for length adaptation of muscle in mice, but two to three weeks in cats and humans [343]. For the species primarily studied in this meta-analysis (chickens/quail), Sayegh & Lajtha [263] indicated a lower protein synthesis rate compared to mice. Furthermore, more factors influencing the protein synthesis rate, as it seems to depend on sex hormones (e.g., testosterone) [311], age, and muscle fiber distribution or the expression of distribution of myosin heavy chains [222, 273]. Those factors could also be responsible for the very different magnitude of increases in muscle mass considering the anterior latissimus dorsi and the patagialis, which were discribes to have very different fiber distributions (and therefore differences in myosin isoforms [7, 9, 12, 155, 251]. Additionally, most studies investigated intervention periods of up to six weeks with consistent hypertrophy [105]. *"If stretch can be maintained, there appears to be little limit to extent and duration of the hypertrophy."* [282, p.95], however, this statement was not fully substantiated. It is obvious that several factors influence the responses of skeletal muscle when exposed to long-lasting stretching interventions, consequently, there is a need to conduct studies investigating the transferability of results to humans. Thus, the following hypotheses were developed.

5 Resulting Hypotheses for Human Research

H1: Long-lasting static stretching of at least 60 minutes per day provide sufficient stimulus to increase in maximum strength
H2: Long-lasting static stretching of at least 60 minutes per day provide sufficient stimulus to induce hypertrophy
H3: There is a dose response relationship of static stretching regarding flexibility adaptations
H4: There is a dose response relationship of static stretching regarding maximum strength adaptations

6 Long-Term Effects of Long-Lasting Static Stretching in Humans

Before presenting own experimental studies, results from available literature investigating the effects of long-term stretching interventions on human skeletal muscle will be reviewed. For a more detailed description of the available literature, see the section "Supplemental Material".

6.1 Human Studies from Literature

There are some limitations and differences to be considered if transferability is to be investigated. First, animals were dissected for examination which is on the one hand problematical from an ethical point of view, and on the other hand, there would be strong limitations in the acquisition of test subjects willing to participate in the study. Furthermore, it seems difficult to include participants performing long-lasting static stretching training while they still need to take care of their daily life. To this point, no studies could be found investigating comparatively long stretching interventions with a daily frequency as performed in animal studies. Consequently, no final statement can be given regarding the possibility to increase muscle thickness, muscle cross-sectional area or maximum strength due to long-lasting stretching interventions. In the following, *"long-lasting"* will be defined as more than 30 minutes per session, since this was the minimal stretching duration in listed animal studies showing significant increase in muscle mass [36, 105] and the highest duration in human studies, performed by Yahata [359] using 6x5 minutes of stretch training. Most commonly, stretching duration used in human studies was less than five minutes per session, consequently, this was defined as *"short-lasting"* stretching.
However, there are numerous studies examining the effects of long-term (intervention period lasting for weeks) but short-lasting static stretching training on maximum strength and/or hypertrophy. Kokkonen and colleagues [160] investigated the effects of a ten-week stretch training routine including 15 stretching exercises for the lower extremity which were performed for 3x15 seconds on three days per week with a total stretching time of 40 minutes per session.

The authors determined a moderate magnitude effect size increase of 32.4% (d = 0.72) in maximum strength of the knee extensors, small magnitude 15.3% (d = 0.44) of the knee flexors, trivial magnitude improvements in jumping performance of 2.3 − 6.7% (d = 0.11 − 0.14) as well as sprinting performance of 1.3% (d = 0.1). Chen et al. [73] showed enhanced maximum strength of 3.04 − 8.67% (d = 0.47 − 2.03) with a 30 x 30 sec stretching training on three days per week in the leg muscles. Nelson and colleagues [229] demonstrated significant large magnitude improvements in maximum strength in the plantar flexors of the stretched leg of 29% (d = 1.24) while the non-stretched intraindividual control leg showed a small magnitude increase of 11% (d = 0.46). Furthermore, Abdel-aziem & Mohammad [1] pointed out changes in maximum strength with -0.15 − 12.51% and d = -0.01 − 1.09 by performing stretching five times for 30 sec in the plantar flexors twice a day on five days per week for six weeks. Furthermore, a number of studies (Nakao et al. [226], Yahata et al. [359], Ikeda & Ryushi [145], LaRoche and colleagues [171], Caldwell et al. [66] and Mizuno [212]) confirmed enhanced maximum strength due to stretching interventions with 0.9 − 23.79% (d = 0.087 − 0.47) for stretching durations between three times 30 seconds and six times five minutes per session with two to 14 sessions per week. However, several studies did not find significant improvements in maximum strength in response to stretch training for four to twelve weeks with stretching durations of three times for 60 seconds to once for three minutes per session [4, 37, 185, 225, 277]. Barbosa et al. [34] even determined significant large magnitude decreases of 18.26% (d = -0.92) in eccentric peak torque following stretching three times 30 seconds three times per week for 3 weeks.

While in animal studies, most authors investigated the effects on muscle growth [20, 36, 69, 85, 105, 153] there are only few studies investigating the effects of stretching on hypertrophy in humans. Longo et al. [185] showed trivial magnitude effects with 2.04% (d = 0.17) after stretching the triceps surae five times 45 seconds five times per week for 12 weeks and Simpson et al. [277] stated an increase in muscle thickness of 5.6% in response to five stretching sessions per week with a stretching duration of three minutes. Other authors were not able to find any hypertrophic response due to stretching in humans [4, 216, 359]. In their review, Nunes et al. [232] confirmed the results by reviewing current literature showing no significant effects of stretching times of up to two minutes per session. In 2021, Panidi and colleagues [236] determined increases in muscle thickness of up to 24% due to stretching times of 15 minutes per session with five sessions per week over twelve weeks.

The conflicting results of the current literature can possibly be attributed to high heterogeneity of measurement procedures (measuring maximal isometric, concentric or eccentric maximum strength or peak torque) or differences in stretching procedures (using own bodyweight) [37, 229], stretching devices [225, 359] or strength machines [277]. Furthermore, vastly different stretching durations from four times 30 seconds per session [212, 229] to six times five minutes per session [359] in combination with different number of stretching sessions ranging from one session [262] to 14 sessions per week [66] were used. In addition, authors used very different intervention periods ranging from two [66] to 12 weeks [236]. Apostolopoulos et

al. [21] and Thomas et al. [308] highlighted the crucial importance of intensity and training frequency regarding physiological responses to stretch training. The authors stated that only high intensities would lead to structural adaptations in the muscle tissue compared to low- or moderate stretching intensities and only high intensities could be responsible for inflammation, myofiber degeneration or dysfunction. However, in current literature an objective quantification of intensity is rarely given. Consequently, the aim of the self-conducted studies in this work was to investigate long-term effects on maximum strength and flexibility increase as well as hypertrophy in response to daily long-lasting stretching of up to two hours per day performed over a period of six weeks. From this, a comparison with listed animal experiments seems to be feasible.

6.2 Designing Experimental Studies

When evaluating the current literature, no long-lasting, high frequency (daily) and high volume (> one hour per week) stretching interventions in humans could be found [359]. Consequently, a comparison to animal study results seems not possible. To examine a potential transferability of results from animals to humans, investigations of daily training with long-lasting stretching durations on maximum strength as well as muscle thickness or muscle cross-sectional area is required. Since daily stretching with a stretching device of 30 minutes to one hour led to enhancements in muscle mass of up to 59% in animal studies [36, 105], the studies included in this work are designed to investigate the effects of daily training performed with long-lasting stretching stimulus on different functional and morphological parameters in the calf muscle in humans with comparable stretching durations and by using a stretching device as well. A calf muscle stretching orthosis was developed, designed, and built as a feasible solution to induce an adequate stretching stimulus with constant stretching intensity for up to two hours per day.

6.3 Developing the Orthosis to Induce Long-Lasting Static Stretching Tension

The triceps surae comprises of the medial and lateral gastrocnemii as well as the soleus. Its main function is the plantar flexion of the foot. The medial head of the gastrocnemius originates from the medial condyle while the lateral head originates from the lateral condyle. The two heads merge in caudal direction. Together with the soleus they merge into the Achilles tendon which in turn inserts onto the tuber of the calcaneus.
Stretching the triceps surae is achieved by positioning the ankle joint in dorsi flexion as this opposes the triceps surae's main function which is plantar flexion. However, because the gastrocnemius is also involved in knee flexion, knee extension is obligatory when aiming to stretch all muscles of the triceps surae. To implement an extensive triceps surae stretch training into a person's everyday life and additionally be able to achieve stretching times that

Figure 3: First self-conducted calf muscle stretching orthosis

are comparable to those in animal experiments[1], an ankle orthosis was designed, developed and built.
The very first prototype was a self-built model made of wooden boards and leather (see Figure 3). This orthosis already comprised two main elements: a "lower leg piece" and a "foot piece". The foot was placed on the "foot piece" with the plantar side facing the wooden material. The heel was positioned in a way that it was as close to the center of rotation (in this model the intersection of the two wooden pieces) as possible. The lower leg was thus enclosed by the leather with the dorsal side facing the leather's closed side.

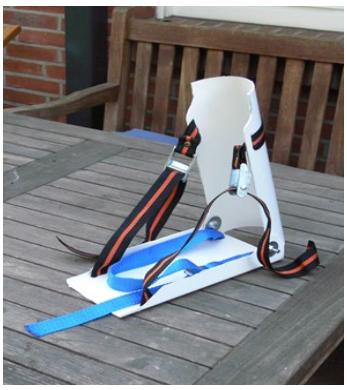

Figure 4: Second self-conducted orthosis to check different types of material and the possibility of mass production

The angle between the foot and lower leg can be adjusted by shortening the cords or latching the ropes to the second pair of hooks on the "lower leg piece". When the front of the "foot piece" is pulled towards the "lower leg piece" the angle between the two elements decreases which results in a dorsiflexed position that leads to a stretch in the triceps surae. To prevent the heel from lifting off the "foot piece" the ankle was fixed into position with a tensioning strap. In order to test the same concept with lighter materials, a new model was built that replaced the wooden boards and leather with plastic shell elements (see Figure 4).

[1]Note that these studies also used stretching devices to induce the stretch stimulus (see [23, 8, 18, 36])

Figure 5: Designing a professional stretching orthosis using Geomagic Free Form

Because the stretch-inducing mechanism worked when testing several people from my social and work environment (including junior basketball players in the highest German division), I aimed at building a "professional" prototype. Through a network contact I got to know RAS Team GmbH – a company that works in the field of orthopaedic technology and specializes in custom orthoses. In cooperation with their orthopaedic technican Paul Hagedorn, a new prototype was designed that could be used for the intervention studies (see Figure 5).
The design and production process started with a plaster cast from a right foot that was scanned with a handheld 3D scanner (Artec Eva) and edited with Artec Studio. The orthosis was designed onto the lower leg scan via the CAD software Geomagic Free Freeform (see Figure 5) and Autodesk Fusion 360. In total, 40 units of the finished design were 3D printed in HP 3D High Reusability PA12 nylon on a HP Multi Jet Fusion 5200 (see Figure 6).

Figure 6: Used orthoses to perform long-lasting stretch training in the calf muscle

7 Material and Methods

The studies presented in the following sections include a variety of tests. Participants performed maximum strength tests for the plantar flexors with extended and flexed knee joint and flexibility tests with extended and flexed knee joint. Furthermore, muscle thickness and the pennation angle were determined using ultrasound sonography and muscle cross-sectional area was examined by MRI-imaging. Mechanical stretching tension was induced with different intensities and durations by using the calf stretching orthosis described above. Since the effects of different modalities were investigated in different studies included in this work not all parameters were examined in every study. The mentioned test conditions will each be described in detail in the following.

7.1 Participants

A total of 316 participants (m = 189, w = 127, age = 25.4 ± 3.2 height = 176.6 ± 3.1, weight = 76.2 ± 4.4) were recruited from the university sports program and local sports clubs and included in the studies. Untrained participants as well as athletes with injury or risk for thromboses were excluded, consequently the subject pool comprised exclusively recreationally trained participants to well-trained participants. They were classified at least as "trained athletes" if they performed two or more training sessions per week in a gym or a team sport continuously for the previous six months. Participants were divided into intervention groups (IG) and a control group (CG) and were informed about the experimental risks and provided written informed consent to participate in the present studies. Furthermore, approval for this study was obtained from the institutional review board (Carl von Ossietzky Universität Oldenburg, No. 2019-01 and No.121-2021). The study was performed in accordance with the Helsinki Declaration.

7.2 Maximum Strength Measurement

While Arampatzis et al. [22] and Signorile et al. [275] found differences in muscle innervation for high-level force production in the plantar flexors depending on the knee joint angle, Warneke et al. [327] stated that there are high correlations between isometric and dynamic (r = 0.7 – 0.77) maximum strength testing with flexed and extended knee joint (r= 0.63 – 0.76). However, evaluating the concordance of listed parameter (extended and flexed knee joint as well as isometric and dynamic strength testing), it could be demonstrated that there is only moderate concordance with concordance correlation coefficients of 0.62 – 0.77, thus, it cannot be assumed that one test could be replaced by another. Consequently, maximum strength was assessed using extended and flexed knee joint under isometric and dynamic strength testing conditions.

7.2.1 Maximum Strength Measurement with Extended Knee Joint

A 45° leg press was used to measure maximum strength in the extended knee joint. A force plate was attached to the footpad to record the maximal strength in the calf muscles. The used force plate was 50 × 60 cm in size and used a measuring range of ± 5000 N and a 13-bit analog-to-digital converter. The subject was instructed to place the foot on the attached force plate so that the metatarsophalangeal joints of the foot were placed on the edge flush (Figure 7). The starting position was chosen to achieve a 90° ankle joint angle. For isometric strength testing, the leg press sled was fixed via industrial grade tensioning straps to provide an impassable resistance. The subject was instructed to perform a maximal voluntary contraction (MVC) in plantarflexion in response to an acoustic signal. Participants had to hold the maximal contraction for at least three seconds after reaching the perceived maximal strength. The force-time curve was recorded for five seconds. After each trial, the subjects rested for one minute to avoid fatigue. Measurements were conducted until no improvement in maximum strength was recorded but for a minimum of three attempts. Reliability was determined between best trial and second-best trial, for which a high reliability can be assumed with intraclass correlation coefficient (ICC) = 0.995. In the following, after taking a recovery break of five minutes, the maximum dynamic strength of the calf muscles was tested with extended knee joint. The subject was instructed to get in the starting position (90° ankle joint angle) and press the loaded leg press sled into a maximally plantarflexed position. Hereby, the distance covered was recorded with a motion sensor (company "MicroEpsilon") with an accuracy of 0.1 mm. Based on the isometric data of the previous test, a weight corresponding to 60% of the isometric maximum strength was added. After each trial, further weight (first 10 kg, then 5 kg or 2.5 kg) was attached to the leg press until the participant was no longer able to perform the 1RM over the full ROM. Once the distance covered fell below 90% of the first attempt, the test was stopped. The best trial with full ROM was used for further analysis.

7.2.2 Maximum Strength Measurement with Flexed Knee Joint

A calf muscle testing device was used to assess maximum strength with flexed knee joint. The maximum strength was determined using a 10 x 10cm force plate with force sensors "Kistler Element 9251" with a resolution of 1.25 N, a pull-in frequency of 1000 Hertz, and a measurement range of ±5000 N. The vertical forces (Fz) were recorded via a charge amplifier "Typ5009 Charge Amplifier" and a 13-bit analog-to-digital converter NI6009. The participants were positioned in a seated position with a joint angle of 90° in the knee joint and in the ankle joint and instructed to perform a plantar flexion for three seconds with maximal possible force in response to an acoustic signal. Testing was performed until the subject could not improve the achieved maximum strength values with a minimum of three trials. High reliability with ICC = 0.994 can be assumed [327].

Figure 7: Maximum strength testing for the plantar flexors with extended knee joint (a) and with flexed knee joint (b)

7.3 Investigation of Hypertrophy

Hypertrophy of the muscle was measured using common procedure of medical imaging.

7.3.1 Muscle Thickness in the Lateral and Medial Head of the Gastrocnemius

The first measurement of skeletal muscle architecture was the assessment of muscle thickness which was conducted using a two-dimensional B-mode ultrasound (Mindray Diagnostic Ultrasound System)(see Figure 8).

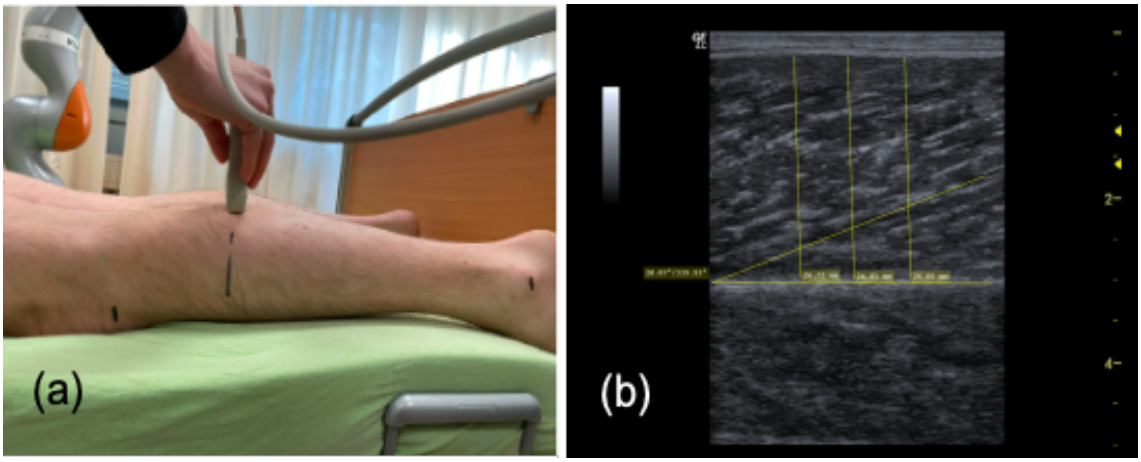

Figure 8: Performing ultrasound sonography (a) to determine the muscle thickness in the gastrocnemius medialis and lateralis (b)

The muscle thickness represents the most employed measure of muscle dimension [260] due to its correlation to muscle cross-sectional area, which is proportional to the number of parallel sarcomeres, thereby possibly influencing maximal force [182, 204, 227]. The pennation angle was evaluated by Warneke et al. [328]. In examinations, ultrasound images from the lateral

and medial gastrocnemius were recorded using a linear transducer with a standardized frequency of 12–13 MHz. Each participant was placed prone on a table with the feet hanging off the edge of the table to ensure no contraction in the calf muscles. Then, the sonographer identified the proximal and distal landmark of the lateral gastrocnemius for each participant and measurement [238]. The transducer was placed at 30% of the distance from the most lateral point of the articular cleft of the knee to the most lateral top of the lateral malleolus (see Figure 8) [238]. Measuring the muscle thickness, the transducer was positioned at the midpoint of the muscle belly perpendicular to the long axis of the leg [260]. The muscle belly was determined as the center of the muscle between its medial and lateral borders. This is the point where the muscle's anatomical cross-sectional area can be assumed to be maximal [111]. In addition, the image plane is best aligned with the muscle's fascicles, including minimal fascicle curvature [48, 204, 245]. Before starting the measurement, transmission gel was applied to improve acoustic coupling and to reduce the transducer pressure on the skin. Then, the sonographer ensured that the superficial and deep aponeuroses were as parallel as possible by holding and rotating the transducer around the sagittal-transverse axis to the determined point on the skin without compressing the muscle. Hence, the visibility of the fascicles as continuous striations from one aponeurosis to the other was optimized. Muscle thickness is defined as the linear, perpendicular distance between the two linear borders of the skeletal muscle and was obtained by averaging three measurements across the proximal, central, and distal portions of the acquired ultrasound images [103, 260]. Two investigators independently produced the images for each participant's muscle thickness. The objectivity of the investigators was found to be between 0.85 (control leg) and 0.94 (intervention leg). Determining muscle thickness via ultrasound can be stated as a reliable procedure as there are high values stated for within-day reliability with ICC [84, 221] and for between day reliability of up to 0.88 [163, 244]. Two investigators also evaluated the ultrasound images independently from each other using the image processing software MicroDicom, Version 2022.1, 64 bit.
It is common to investigate hypertrophy with sonography, which is stated as a cost efficient and reliable method [252, 355]. However, Hebert et al.[135] and English et al.[93] stated limitations of using sonography especially because of serious problems in determination of reliability in previous studies and restricted objectivity due to applied pressure of the transducer on the muscle, which can heavily influence the results. Therefore, investigating interrater reliability, ICCs are also shown to be high, however, calculating the mean error (ME), mean absolute error (MAE) and the mean absolute percentage error (MAPE), differences of 4.63 – 8.57% could be determined, which seems to be comparatively high [331], considering assumed hypertrophy effects of 5-10% due to commonly used strength training in humans for six to ten weeks [94, 234, 285, 303, 337]. To approve results from sonography imaging in included studies and counteract listed limitations in current literature, MRI imaging was additionally used, as it is viewed as the gold standard for hypertrophy investigations with high accuracy, objectivity and reliability [47].

7.3.2 Muscle Cross-Sectional Area in the Medial and Lateral Head of the Gastrocnemius

To determine the muscle cross-sectional area, MRI was performed at the Neuroimaging Unit of the Carl von Ossietzky University of Oldenburg with a 3T Siemens Magnetom Prisma MRI using a T1-weighted turbo-spin-echo sequence (40 slices, slice thickness = 7 mm, TR = 1600 ms, TE = 14 ms, voxel size = 0.4 x 0.4 x 7 mm^3, FOV = 150 x 150, distance factor = 20%, flip angle = 150°, TA = 8:16 min) with a combination of the standard body and spine coil. Each participant was placed on the back with feet first position and the measurement was performed on the left leg first, immediately followed by the right leg. The evaluation of MRI images and therefore examination of the muscle cross-sectional area was performed anonymized for participant and group with MicroDicom (Sofia, Bulgaria) (see Fig. 9(a)), by bordering the fascia layers of the lateral and the medial head of the gastrocnemius. Images were examined by two investigators independently, blinded for the group of the participant. Measurements were taken from the first image distal of the knee joint where a clear bordering of the muscle could be seen to the transition from the muscle to the tendon. The mean of the three highest muscle cross-section values in the lateral and the medial head of the gastrocnemius were considered for evaluation to minimize potential error of location [166, 307]. Reliability of MRI measurements can be assumed to be very high with r=0.99 [324, 351].

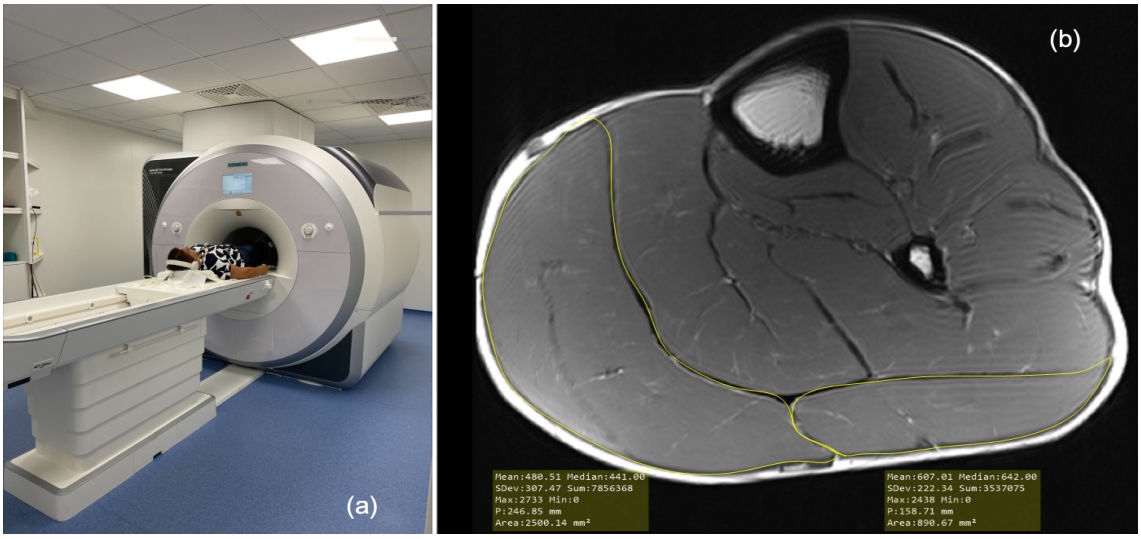

Figure 9: Examples for testing procedure (a) and evaluation of the muscle cross-sectional area of the medial and the lateral head of the gastrocnemius (b)

7.4 Flexibility Testing

As maximum strength was tested with extended and flexed knee joint, assessment in flexibility also included both, flexibility testing with extended and flexed knee joint.

7.4.1 Range of Motion Measurement in Flexed Knee joint

ROM in the upper ankle joint with a flexed knee was recorded in IG and CG via the functional "knee to wall" test (KtW). A sliding device was used for this purpose. Each participant was instructed to place the foot on the attached marker. The contralateral leg did not have ground contact and the subject was allowed to hold onto both sides of a doorframe. To record the ROM, the subject pushed the board of the sliding device forward until the heel of the standing leg lifted off. Meanwhile, the investigator pulled on a sheet of paper placed under the subject's heel. The measurement was finished as soon as this paper could be removed. The mobility was read off in cm from the attached measuring tape (Figure 10a). Depending on ankle ROM, this measurement can be seen as screening flexibility in flexed knee. Three valid trials were performed per leg, and the maximal value was used for evaluation. Reliability can be stated as high with ICC = 0.98 [334].

7.4.2 Range of Motion with Extended Knee Joint

The goniometer on the orthosis was used to examine ROM in dorsiflexion with extended knee joint. While sitting on a chair, the participant was instructed to place the foot on another chair of the same height so that the leg was parallel to the ground. The participant had to sit with the back flat against the backrest. In this position, one investigator pushed the foot into a maximally dorsiflexed position. The ROM value was read off from the goniometer of the orthosis with the starting position being a 90° angle between the foot and lower leg and classified as neutral 0°: each big indentation of the goniometer corresponds to an increase of 5° and each little indentation corresponds to an increase of 2.5°. The same procedure was performed in a previous study with high reliability ICC = 0.98 [334].

7.5 Intervention

In all studies, participants received the described calf-muscle stretching orthosis for the six-week intervention period. The intervention groups (IGs) were instructed to perform daily stretching training between 10 minutes and two hours, dependent on the study design. In most studies included in this work, stretching was performed by constant angle stretching. In this scenario the participants were instructed to adjust the orthosis to the maximal tolerable stretching intensity of an individual stretching pain of $8 - 9$ on a scale to 10. Thus, the stretching intensity in the plantar flexors was regulated by the set angle of the orthosis which was determined by the goniometer included to the orthosis. The participants were instructed to read off the angle while performing the intervention and to document the daily reached

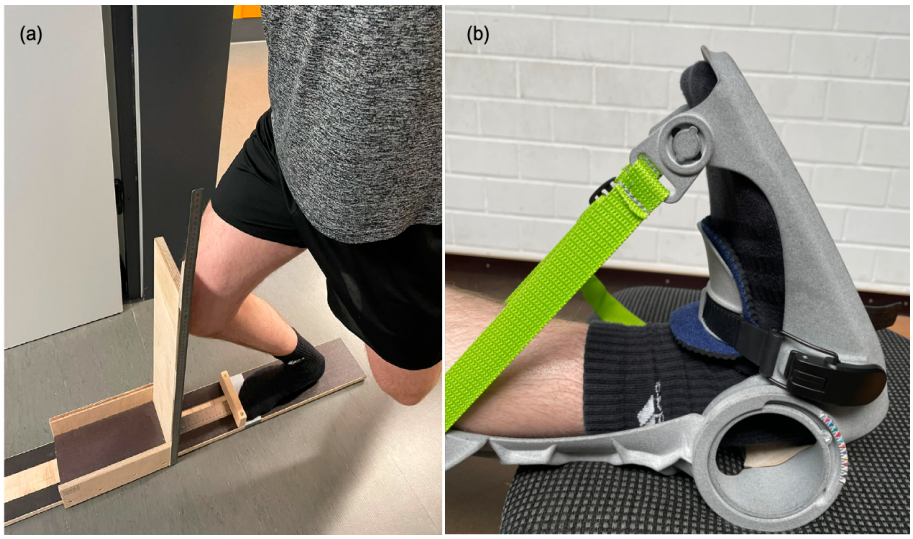

Figure 10: Using the KtW test for assessing ROM in upper ankle with flexed knee joint (a) and the goniometer of the orthosis for ROM measurement in the dorsiflexion with extended knee joint (b)

angle in a stretching diary. This should help to reach consistent intensity throughout the intervention period and quantify stretching intensity. In the last one of the included studies, well-trained participants were recruited to ensure significant effects of the training intervention are not exclusively prevalent in moderate-trained participants. To enhance stretching intensity, the set angle of the orthosis was to be re-adjusted every ten minutes using a 6 x 10 minutes stretching protocol, to counteract hysteresis effects [168]. Stretching was performed with the dominant leg which was determined by asking participants which leg they use when performing single leg jumps. Participants were instructed to wear the orthosis with extended knee joint. During the stretching training participants had to sit with their back as straight as possible against a backrest and to place their foot on an object of the same height for instance a chair (see Figure 11). The control groups (CGs) did not perform any intervention related to the corresponding study.

7.6 Data Analysis

Data analyses were performed with SPSS 28. Data of all parameters are provided using M ± SD. Normal distribution was checked via Shapiro Wilk test. Reliability was determined and is provided in ICC and coefficient of variability (CV) for listed assessments (see Table 2). Reliability for tests was determined between best and second-best value providing the "intraday day" reliability. Levene-test was performed to ensure homogeneity of variance. A one way ANOVA was used to rule out significant differences in pre-test values between groups. If there were only two groups, a t-test for independent values was used to rule out significant differences in the pre-test values, e.g. in MRI measurement [332] or sonography by Warneke et al. [325]. A two-way ANOVA with repeated measures was performed for the collected data. Scheffé test was used as a post-hoc test for mean differences. Tests were performed between

Figure 11: Exemplary procedure for calf muscle stretching using the developed stretching orthosis

the intervened leg, the non-intervened control leg as well as between both legs of the control group separately. Effect sizes are presented as Eta squares (η^2) and categorized as: small effect $\eta^2 < 0.06$, moderate effect $\eta^2 = 0.06 - 0.14$, large effect $\eta^2 > 0.14$ [77]. Additionally, effect sizes are reported with Cohen's d [77] and categorized as: small effects d < 0.5, moderate effect d $= 0.5 - 0.8$, large effect d >0.8. Sample size was calculated via G-Power in Warneke et al. (2022). In further research, power $(1-\beta)$ was calculated post-hoc via G-Power (Version 3.1, Düsseldorf, Germany). The level of significance was set at p < 0.05.

Table 2: Showing calculated ICC and CV of included parameters

Parameter	ICC	CV in %
MVC180	0.984 – 0.996	1.72 – 1.83
MVC90	0.983 – 0.993	1.80 – 1.97
KtW	0.991 – 0.997	0.94 – 0.98
ORT	0.989 – 0.992	0.64 – 1.10
MThL	0.876 – 0.881	4.19 – 5.21
MThM	0.917 – 0.948	2.86 – 3.50

MVC = maximal strength; KtW = knee to wall test; ORT = ROM measurement with orthosis; SONO = measurement of muscle thickness via sonography; Pa = Pennation angle; 180 = MVC measured with extended knee joint; 90 = MVC measured with flexed knee joint; L = lateral head of the gastrocnemius; M = medial head of the gastrocnemius.

8 Experimental Studies

In this chapter results of own studies investigating the effects of long-lasting stretching intervention will be presented.

8.1 Improvements in Flexibility Depend on Stretching Duration

In *International Journal of Exercise Science (2023).*
https://digitalcommons.wku.edu/ijes/vol16/iss4/4

Stretching is commonly performed to increase flexibility in humans [208, 210] to improve performance and to reduce the risk of injury [354]. If the aim is to improve flexibility, Thomas et al. [308] referred to a dose-response relationship regarding stretching duration and weekly volume, while Apostolopoulos et al. [21] pointed out differences in structural adaptations of the muscle by using high stretching intensity. Evaluating current literature in the topic of stretch training, there is high heterogeneity in study designs. While there are studies investigating the influence of one stretching session per week [262], most studies examined the influence of three times stretching per week [160, 225, 229]. Overall, stretching durations per session differed from four times 30 sec to 30 minutes per day [359]. Furthermore, there were large differences in the way stretching was induced. While some authors used a stretching board [236, 359], other participants were instructed to perform stretching by using a leg press machine [277] or to perform stretching by using stretching devices [314]. To clarify the influence of stretching duration on ROM, stretching should be performed using standardized conditions, consequently, the orthosis was used to perform stretching. Therefore, 80 subjects (m=45, f=35, age: 26.4±4.6 years, height: 176.3±8.1 cm and weight: 74.3±5.5 kg) were included in this investigation performing a six-week stretching intervention for the calf muscle for 10 minutes (IG10), 30 minutes (IG30) and 60 minutes (IG60) per day. This study can be seen as verification of the effectiveness of the previously described stretching procedure in general. Flexibility in the calf muscle was measured with flexed and extended knee joint in pre- and post-test by using the knee to wall test and the goniometer of the orthosis. Stretching was performed as mentioned above in section 7.5. Results are stated in Table 3 and illustrated in Figure 12.

Table 3: Descriptive statistics and results of two-way ANOVA for both flexibility tests

Parameter	Pretest (M±SD) in N	Posttest (M±SD) in N	Pre-Post Differences in %	Time effect	Time X group
IG10KtW	11.71±3.33	12.88±3.44	+10.02	p<0.001	p<0.001
IG30KtW	12.39±3.8	13.61±4.0	+9.89	$F_{77,3}$=195.58	$F_{77,3}$=22.5
IG60KtW	11.96±2.37	13.69±2.19	+14.46	$\eta^2 = 0.72$	$\eta^2 = 0.47$
CGKtW	12.29±1.81	12.36±1.9	+0.57		
IG10ORTH	8.65±2.02	9.18±1.9	+6.07	p<0.001	p<0.001
IG30ORTH	8.23±1.75	8.93±1.48	+8.51	$F_{77,3}$=98.7	$F_{77,3}$=16.55
IG60ORTH	9.00±1.5	10.48±1.33	+16.39	$\eta^2 = 0.557$	$\eta^2 = 0.392$
CGORTH	8.74±1.55	8.83±1.69	+1.12		

IG10=intervention group 10 with a daily stretching duration of 10 minutes, IG30= intervention group 30 with a daily stretching duration of 30 minutes, IG60=intervention group 60 with a daily stretching duration of 60 minutes, CG=control group, KtW=range of motion measurement via knee to wall test, ORTH=range of motion measurement via the goniometer of the orthosis.

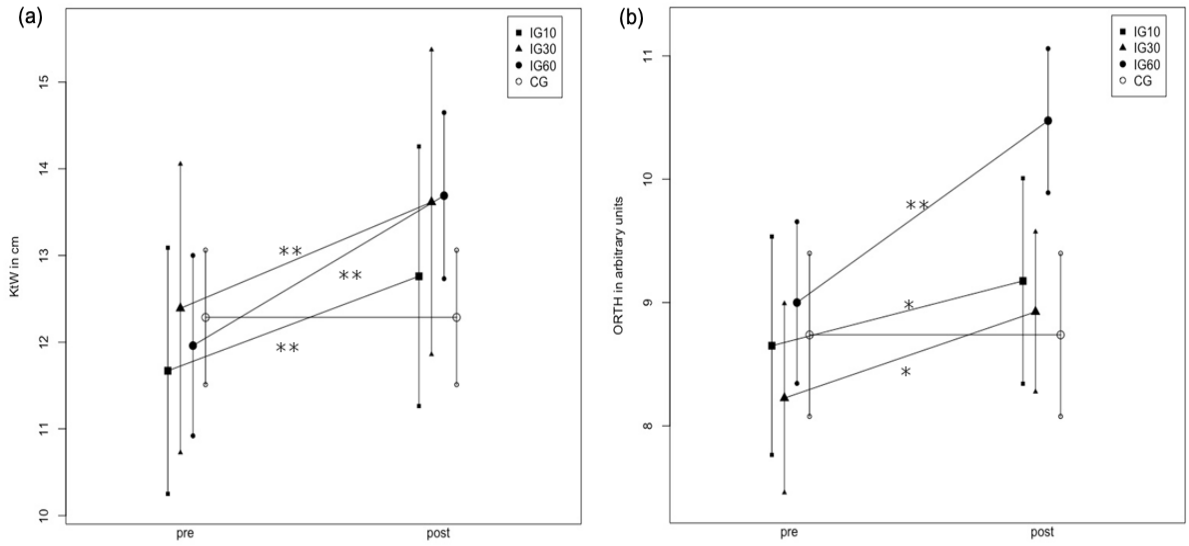

Figure 12: Comparison of progressions measured via the KtW stretch (a) and ORTH (b) between all groups; IG10=intervention group 10 stretching with a daily stretching duration of 10 minutes; IG30= intervention group 30 stretching with a daily stretching duration of 30 minutes; IG60=intervention group 60 with a daily stretching duration of 60 minutes; CG=control group. **=p<0.001, *=p<0.05 for difference to control group

The Scheffé test determined no significant difference for the mean differences in the knee to wall test between pre- and post-test values between IG10 and IG30 as well as between IG10 and IG60 (p = 0.996, p = 0.09) and between IG30 and IG60 (p = 0.14). There were significant differences between CG and IG10 with d = 0.97, p < 0.001, CG and IG30 with d = 1.03, p < 0.001 as well as IG60 and CG d=1.49, p<0.001, showing significant increases in ROM. For the ROM testing using the goniometer of the orthosis, the Scheffé test determined no significant

difference for IG10 compared to IG30 (p = 0.86, d = 0.16) as well as in IG10 compared to CG (p = 0.21, d = 0.38). However, there were significant higher increases in IG60 compared to IG10 (p < 0.001, d = 0.88), IG60 and IG30 (p = 0.004, d = 0.71) as well as IG60 and CG (p < 0.001, d = 1.27) and for IG30 compared to CG (p = 0.03, d = 0.55). Post-hoc analysis of G-Power calculated $1 - \beta$=100% with $\eta^2 = 0.33$ for the within effects and $1 - \beta$=93.1% with $\eta^2 = 0.46$ for the interaction for $\alpha = 0.05$ for four groups and two measuring time points. While increases in ROM measured via KtW showed no significant differences dependent on stretching time, improvements in flexibility measured via the goniometer of the orthosis showed time-dependent increases in ROM. There were no significant differences between stretching time of 10 and 30 minutes, but significant differences to other groups were found when stretching was performed one hour per day. Higher increases in ROM measured via the goniometer of the orthosis may be attributed to the identical execution of the goniometer of the orthosis measurement and the stretch training of the intervention. Since an influence of the knee angle of muscles used in the lower extremity can be assumed [22, 275] and the KtW examines the dorsiflexion with flexed knee joint, results showed that there is high specificity in effects of stretching training on ROM.

Summary of results
Results of the first experimental study compared different stretching durations from ten minutes to one hour per day showing significant high magnitude improvements in flexibility in all three intervention groups with d=0.97 − 1.49, p < 0.001 via the knee to wall test and d=0.38 − 1.27, p < 0.001 via the goniometer of the orthosis compared to the control group. However, results showed no significant differences between the intervention groups (p=0.09 − 0.99) in the knee to wall test, but significant differences when ROM was measured via goniometer of the orthosis (p=0.001 − 0.03, d=0.16 − 1.27) with a dose response relationship.

8.2 Influence of Long-Lasting Static Stretching on Maximal Strength, Muscle Thickness and Flexibility

In *Frontiers in Physiology (2022)*,
https://doi.org/10.3389/fphys.2022.878

Since the first study showed highest increases in flexibility in response to longest examined stretching time and animal studies demonstrated higher effects of long-lasting stretching durations the second experimental study included in this work investigates the effects of a long-lasting stretching intervention using the orthosis. Therefore, a one hour daily stretch with constant angle was performed. Effects on maximal isometric and maximal dynamic strength in the plantar flexors with extended and flexed knee joint, the muscle thickness in the gastrocnemius as well as the ROM in the knee to wall stretch and with extended knee joint using the goniometer of the orthosis were investigated. Fifty-two (52) trained participants (male: 31, female: 21, age: 27 ± 3.1 years, height: 175.9 ± 5.2cm, weight: 80.5 ± 7.3kg) were included to this work. There were significant moderate magnitude increases in maximum isometric

strength of 16.8% (d = 0.79, p < 0.001) while there were no significant changes in the control leg as well as in the control group. Evaluating maximum dynamic strength using 1RM, there was a significant moderate magnitude increase of 25.1% (d = 0.69, p < 0.001) as well as a small magnitude increase in the contralateral leg of 11.4% (d = 0.32, p < 0.001), while there was no significant change in maximum dynamic strength in the control group, see Figure 13.

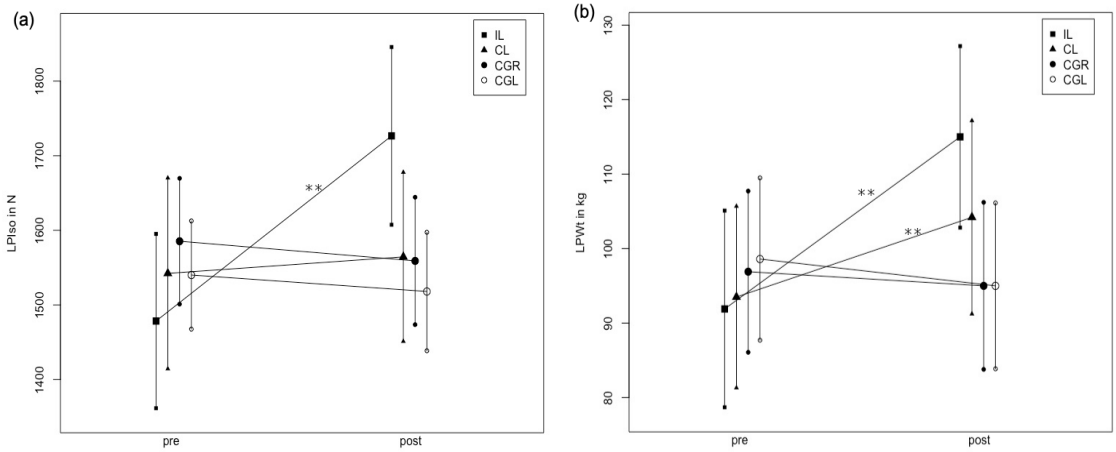

Figure 13: Comparison of progressions in maximum isometric strength (LP) and maximum dynamic strength using the 1RM (LPwt) in the intervened leg (IL), the control leg (CL) and both legs of the control group (CGR, CGL). **=p<0.001, *=p<0.05 for difference to control group

Furthermore, a significant large magnitude increase in muscle thickness of 15.3% (d = 0.84, p < 0.001) was found without significant changes in control conditions. Moderate and large magnitude increases were found in KtW flexibility (13.2%, d = 0.57, p < 0.001) and ROM with extended leg measured via the goniometer of the orthosis (27.3%, d = 0.87, p < 0.001) respectively (Figure 14).

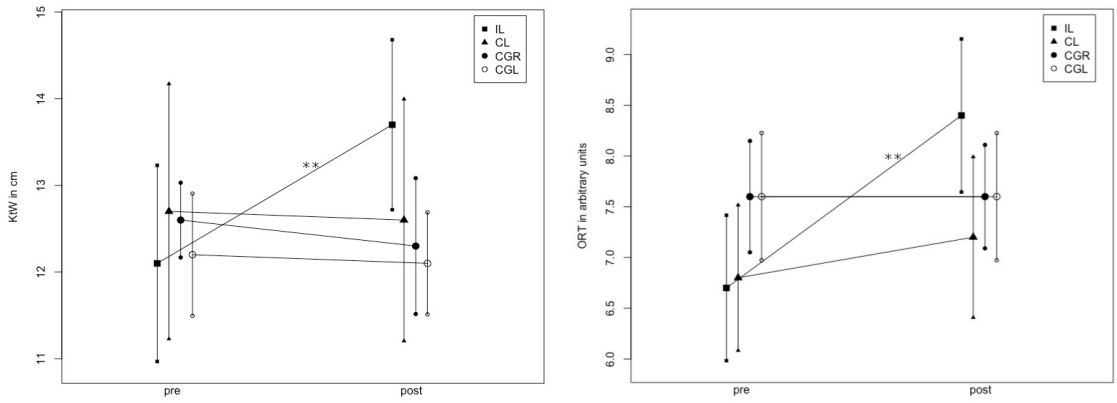

Figure 14: Comparison of progressions in knee to wall test and angle measurement using the goniometer of the orthosis in the intervened leg (IL), the control leg (CL) and both legs of the control group (CGR, CGL). **=p<0.001, *=p<0.05 for difference to control group

Summary of the results

In this work, a significant improvement in maximum strength in the calf muscles was achieved by daily one-hour stretching training. There was a significant improvement in maxiuml isometric strength with an extended knee joint in the stretched leg by approximately 16.8%. In comparison, an average maximum isometric strength increase of 1.4% was determined in the non-stretched control leg while there was no significant increase between legs of the control group. Furthermore, the maximum dynamic strength with an extended knee joint test via 1RM testing enhanced by 25.1% and 11.4% in the stretched and non-stretched control leg, respectively. In both legs in the control group no significant change in 1RM could be determined. For all maximum strength measurements, large effect sizes were found for interaction effects in the ANOVA ($\eta^2 > 0.14$ and $d > 0.8$). Additionally, significant hypertrophy effects in the lateral head of the gastrocnemius of 15.2% in the intervention leg versus 2.1% in the control leg were found. In the intervened leg, muscle thickness increased by 15.3% from 14.31 ± 2.42 mm to 16.5 ± 2.78 mm. In the control leg, there was no significant increase by 2.1% from 14.54 ± 2.32 mm from pre-test to 14.85 ± 2.08 mm in post-test. Flexibility improved with 13.2% and 27.3% ($p < 0.001$) significantly due to the stretching intervention.

8.3 Influence of One Hour versus Two Hours of Daily Static-Stretching for six Weeks Using a Calf-Muscle-Stretching Orthosis on Maximal Strength

In *International Journal of Environmental Research and Public Health, 19 (2022)*,
https://doi.org/10.3390/ijerph191811621

As previously demonstrated, stretching performed with adequate volume and intensity seems to be sufficient to induce hypertrophy and improvements in maximum strength in animals and humans as well. Improving muscle mass in animals, a dose-response relationship can be assumed [36, 105]. Therefore, the question arises about a transferability of this phenomenon to humans regarding maximum strength increases. Furthermore, in the previous study [325], cross educational effects were measured for dynamic maximal strength with an increase of 11.4% ($p < 0.001$, $d = 0.32$). Cross-education effects are known from unilateral strength training [16, 17, 193], but were also obtained from unilateral stretch training in Caldwell et al. [66] and Nelson et al. [229]. Nelson and colleagues demonstrated a high magnitude increase in maximum strength of up to 29% ($d = 1.24$) in the stretched leg which was accompanied by a moderate magnitude contralateral force transfer of 11% to the contralateral leg ($d = 0.46$) by inducing 4x30 sec of stretching, three days per week for ten weeks. Furthermore, Caldwell et al. [66] showed that stretching the quadriceps twice daily for two weeks resulted in significant, high magnitude increases in maximum strength of 7.1% ($d = 0.8$) in the stretched and of 6.6% ($d = 0.45$) in the contralateral leg while stretching once per day did not lead to a contralateral force transfer. To investigate the time-dependent increase in maximum strength due to static

stretching as well as the contralateral force transfer, 70 participants were included in this study and divided into two intervention groups and a control group (see Table 4). Stretching was performed by using the stretching orthosis for one hour (IG1) and two hours (IG2). Descriptive statistic is provided in Table 5 and illustrated in Figure 15.

Table 4: Characteristics of included participants

Group	N	Age (in years)	Height (in cm)	weight (in kg)
Total	70 (f=24; m=46)	24.1±3.5	178.3±8.9	74.1±10.5
IG1	25 (f=7; m=18)	23.4±4.7	180.3±4.5	76.4±9.2
IG2	15 (f=3; m=12)	27.2±5.3	181.3±8.2	78.9±12.7
CG	30 (f=14; m=16)	24.6±3.8	176.2±6.4	71.5±10.2

IG1 = intervention group 1 stretched one hour per day; IG2 = intervention group 2 stretched two hours per day; CG = control group.

Table 5: Descriptive statistics of the maximum strength values in intervention groups as well as in control group in pre- and post-testing for the stretched leg.

Group	Pretest (M±SD) in N	Posttest (M±SD)in N
IG1il	1195.3±321.1	1364.5±355.4
IG1cl	1210.6±371.8	1277.2±343.2
IG2il	1144.2±244.7	1397.9±366.5
IG2cl	1151.7±306.5	1277.2±380.8
CGl	1076.3±364.5	1056.0±332.7
CGr	1100.9±346.1	1088.9±364.8

IG1 = intervention group 1 stretched one hour per day; IG2 = intervention group 2 stretched two hours per day; CG = control group; il = intervened leg; cl = non-stretched intraindividual control leg; l = left leg of the control group; r = right leg of the control group.

In the overall statistics, the two-way ANOVA revealed large effects for the time effect ($F_{1,69}$ = 48.48; η^2 = 0.275) as well as the interaction effect group*time ($F_{2,68}$ = 10.06; η^2 = 0.28, p < 0.001) showing significant increases in maximum strength. The mean value in IG1 increased moderately by 14.2% (p < 0.001, d = 0.51) from pre-test to post-test and with a large magnitude 22.3% (p < 0.001, d = 0.91) in IG2; CG did not change significantly by 1.9% (p = 0.45).

The group differences determined by the Scheffé test showed significant differences between IG1il and CG (p = 0.003 – 0.004) as well as between IG2il and CG (p < 0.001). No significant differences could be determined between IG1il and IG1cl (p = 0.392) or between IG2il and IG2cl (p = 0.41). Furthermore, the Scheffé test showed no significant differences for IG1cl and control groups (p = 0.56 - 0.60) and between IG2cl and control groups (p = 0.14 – 0.16).

Analysis of maximum strength tests of the intervened leg

There were large magnitude effects for time effect ($F_{1,69}$ = 54.245; η^2 = 0.430, d = 1.74) as well as the interaction effect group*time ($F_{2,68}$ = 18.494; η^2 = 0.325, d = 1.39) with p <

0.001. The mean value in IG1 moderately increased by 14.2% ($p < 0.001$, $d = 0.51$) from pre-test to post-test and a large magnitude 22.3% ($p < 0.001$, $d = 0.91$) in IG2; CG did not change significantly by 1.9% ($p = 0.45$). The group differences determined by the Scheffé test exhibited significant differences between the mean of IG1 and CG ($p < 0.001$), IG2 and CG ($p < 0.001$). No significant differences were found between IG1il and IG2il ($p = 0.23$).

Analysis of maximum strength tests of the non-intervened leg
Statistics showed moderate effects for the time effect ($F_{1,69} = 10.761$; $p = 0.002$; $\eta^2 = 0.130$, $d = 0.77$) and the interaction effect group*time ($F_{2,68} = 5.063$; $p = 0.009$; $\eta^2 = 0.123$, $d = 0.749$) pointing out significant increases in maximum strength. The mean value in IG1cl increased trivially by 5.5% ($p = 0.024$, $d = 0.18$) from pre-test to post-test and by 10.9% ($p = 0.011$, $d = 0.36$) in IG2; the control group did not change significantly by 1.1% ($p = 0.45$). The Scheffé test showed a significant difference only for IGcl2 vs. CG ($p = 0.014$).
Figure 15 shows the mean value progression of the maximum strength values in intervention groups as well as in the control group in pre- and post-testing for the stretched leg. Values in IG1 and IG2 represent the stretched leg. Values in CG represent the left leg of the control group.

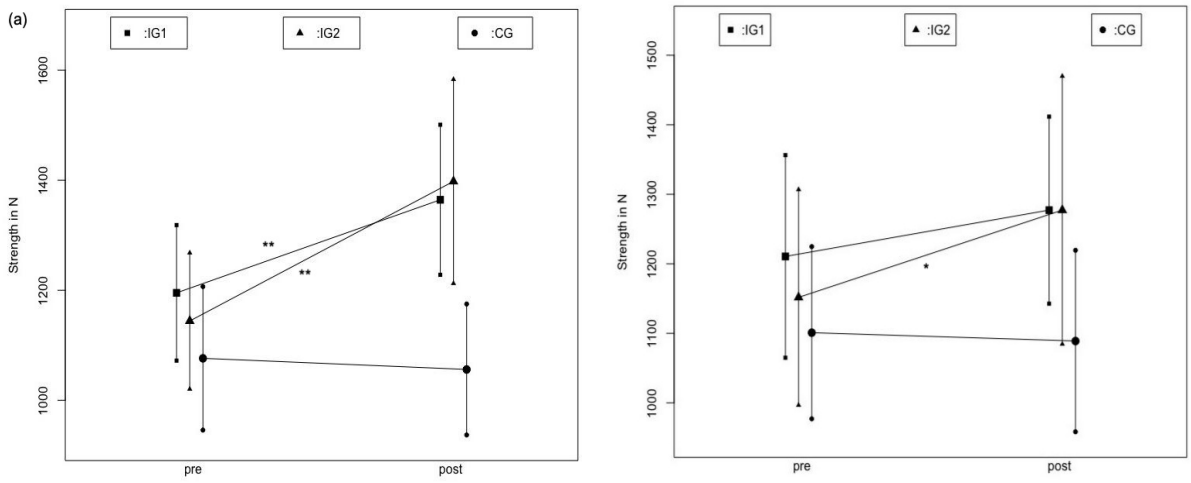

Figure 15: Comparison of maximum strength in pre- to post-test between IG1il, IG2il and CG (a) as well as between IG1cl, IG2cl and CGr (b). **=p<0.001, *=p<0.05 for difference to control group.

Analysis of the stretched leg versus the non-stretched leg within one group to examine the contralateral force transfer.
For IG1, results pointed out a significant moderate time effect ($F_{1,116}$=17.78; $p < 0.001$; η^2=0.13) and a significant high interaction effect group*time ($F_{3,116}$=12.84; $p < 0.001$; $\eta^2 = 0.25$) showing significant increases in maximum strength. The Scheffé test showed significant differences between the intervened leg of IG1 and both legs of the control group ($p < 0.001$) but no significant difference between the intervened and control leg of IG1 ($p = 0.062$). No

significant differences between the control leg of IG1 and both legs of the CG could be determined (p = 0.96 – 0.156). For IG2, the two-way ANOVA revealed a significant moderate time effect with $F_{1,98} = 28.95$; $p < 0.001$; $\eta^2 = 0.23$ and a significant high interaction effect group*time ($F_{3,78} = 15.48$; $p < 0.001$; $\eta^2 = 0.32$) showing significant increases in maximum strength as well. The Scheffé test showed significant higher increases in the intervened leg of the intervention group compared to both legs of the control group ($p < 0.001$) but no significant difference between the intervened and the control leg ($p = 0.14$). A significant higher increase in the control leg compared to both legs of the control group could also be determined ($p = 0.02 - 0.033$). The results are graphically illustrated in Figure 16 presenting changes in isometric maximum strength from pre- to post-test for the intervened and control leg of IG1il and IG2 respectively and both legs of the control group.

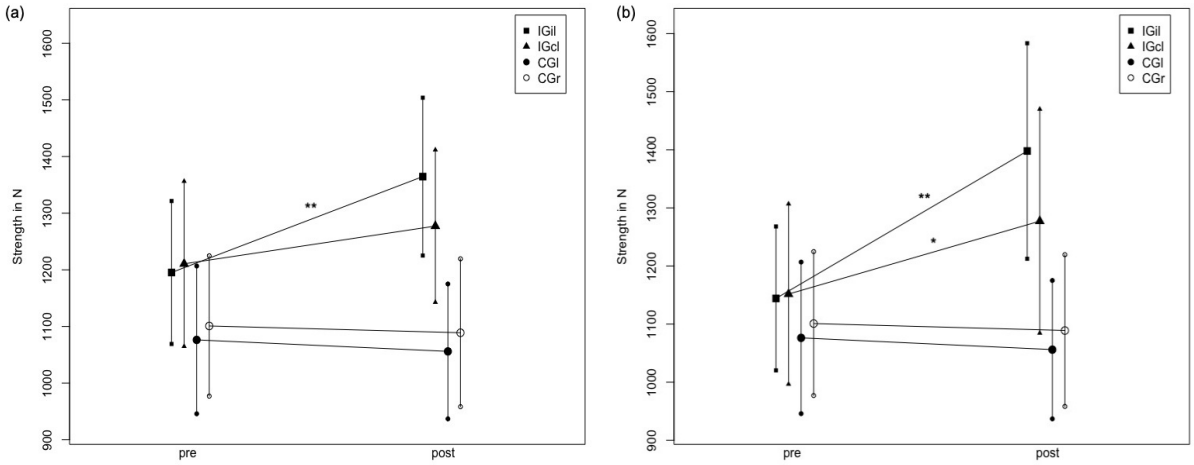

Figure 16: Illustrating the mean value curve of the maximum strength values in IG1il, IG1cl and both groups of CG (CGl and CGr) (a) as well as the mean value curve of the maximum strength values in IG2il, IG2cl and both groups of CG (b). **=p<0.001, *=p<0.05 for difference to control group

Post-hoc analysis for F-tests of G-Power calculated $1 - \beta = 42.00\%$ for the lowest effect size with $\eta^2 = 0.123$ and $1 - \beta = 99.99\%$ for the highest effect size with $\eta^2 = 0.430$ with $\alpha = 0.05$ for three groups and two time points for the interaction.

Summary of the results
Stretching training of one and two hours, respectively, resulted in significant increases in maximum isometric strength ($p < 0.001$) while no significant increases could be measured in CG ($p = 0.45$). Two hours of daily stretch training resulted in an average maximum strength increase of 22.3% in the intervened leg and a contralateral force transfer to the non-intervened leg of 10.9%, $p = 0.011$ in the control leg. Stretching the calf muscle for one hour daily resulted in a 14.2% ($p < 0.001$) increase in maximum strength in the calf muscles of the intervened leg and of 5.5% ($p = 0.024$) in the control leg. Furthermore, results showed a significant contralateral force transfer in IG2 (10.9%, $p = 0.011$), but not in IG1.

8.4 Comparison of the Effects of Long-Lasting Static Stretching and Hypertrophy Training on Maximal Strength, Muscle Thickness and Flexibility in the Plantar Flexors

In *European Journal of Applied Physiology (2023),*
https://doi.org/10.1007/s00421-023-05184-6

Since previous studies showed significant improvements in maximum strength, flexibility, as well as muscle thickness of the stretched muscles and the Scheffé test did not show significant differences between improvements of one versus two hours of static stretching, the following study included in this work aimed to investigate the possibility to replace a commonly used strength training for the plantar flexors by long-lasting static stretching routine using daily stretching of one hour. Based on the results reported above, it could be hypothesized that both interventions, a daily performed one-hour stretching training as well as a commonly used strength training for the calf muscles, would lead to comparable results regarding maximum strength and muscle thickness. Sixty-nine (69) trained participants ($f = 30$, m =3 9, age: 27.4 $\pm$ 4.4 years, height: 175.8 $\pm$ 2.1 cm, weight: 79.45 $\pm$ 5.9 kg) were recruited. Participants were classified as trained if they performed two or more training sessions per week in a gym or a team sport continuously for the previous six months. Included subjects were randomly divided into three groups (IG1, IG2 and CG) each with $n = 23$. IG1 performed stretching as previously described (see section 7.5), while IG2 performed unilateral strength training for the plantar flexors with five sets of 12 repetitions with extended knee joint in a leg press on three non-consecutive days per week. Effects on maximal strength with extended (MVC180), and flexed (MVC90) knee joint, ROM using the knee to wall test and the goniometer of the orthosis as well as hypertrophy in the lateral and medial head of the gastrocnemius including the pennation angle were examined. Table 6 provides descriptive statistics for listed parameters and each group and results of the two-way ANOVA.

Table 6: Descriptive statistics and results of two-way ANOVA of included parameters

Parameter	Pretest (M±SD) in N	Post-test (M±SD) in N	Pre-Post-Diff. in %	Time effect	Time x group
IG1MVC180	1522.61±310.25	1796.8±368.08	+18.00	p<0.001	p<0.001
IG2MVC180	1594.00±321.78	1807.80±361.11	+13.36	F=88.26	F=15.49
CG	1557.05±284.46	1585.57±292.04	+1.80	$\eta^2 = 0.57$	$\eta^2 = 0.32$
IG1MVC90	1314.7±305.79	1440.61±332.67	+9.58	p<0.001	p=0.006
IG2MVC90	1371.8±289.45	1508.44±258.70	+9.96	F=25.908	F=5.51
CG	1334.8±235.36	1340.33±205.81	+0.42	$\eta^2 = 0.28$	$\eta^2 = 0.14$
IG1KtW	11.72±2.52	12.98±2.55	+10.75	p<0.001	p=0.046
IG2KtW	12.26±2.10	13.36±2.31	+8.97	F=48.96	F=3.24
CG	11.71±12.17	12.17±2.0	+3.93	$\eta^2 = 0.43$	$\eta^2 = 0.09$
IG1ORT	8.35±2.08	9.39±1.41	+12.46	p<0.001	p<0.001
IG2ORT	7.92±1.637	8.64±1.31	+9.09	F=39.37	F=8.85
CG	8.17±1.25	8.21±1.03	+0.49	$\eta^2 = 0.37$	$\eta^2 = 0.21$
IG1MThL	14.53±2.43	15.21±2.11	+4.68	p<0.001	p=0.021
IG2MThL	14.83±2.91	16.09±3.35	+8.5	F=15.51	F=4.08
CG	14.33±2.48	14.40±2.32	+0.49	$\eta^2 = 0.19$	$\eta^2 = 0.11$
IG1MThM	19.55±2.59	21.06±2.88	+7.72	p<0.001	p=0.006
IG2MThM	19.25±3.47	20.87±3.09	+8.42	F=19.46	F=5.58
CG	18.49±3.13	18.41±2.87	-0.43	$\eta^2 = 0.23$	$\eta^2 = 0.14$
IG1PaL	13.39±2.33	13.49±2.73	+0.75	p=0.549	p=0.625
IG2PaL	14.14±2.91	14.59±2.28	+3.18	F=0.36	F=0.47
CG	12.67±2.86	12.55±2,76	-0.95	$\eta^2 = 0.01$	$\eta^2 = 0.02$
IG1PaM	17.32±4.07	19.46±3.24	+12,3	p<0.001	p=0.077
IG2PaM	16.92±3.18	19.07±3.04	+12,71	F=12.81	F=2.66
CG	16.51±3.92	16.62±3.67	+0.67	$\eta^2 = 0.16$	$\eta^2 = 0.08$

IG1 = stretching group; IG2 = strength training group; CG = control group MVC = maximal strength; KtW = ROM measurement via Knee to Wall test; ORT = ROM measurement via goniometer of the orthosis; 180 = maximal strength testing in extended knee joint; 90 = maximal strength testing in flexed knee joint; MThL = muscle thickness in the lateral head of gastrocnemius; MThM = muscle thickness in the medial head of gastrocnemius; PaL = pennation angle in the lateral head of the gastrocnemius; PaM = pennation angle in the medial head of the gastrocnemius

Results of the Scheffé test for maximum strength, ROM and muscle thickness are provided in Table 7. Results are graphically illustrated in Figures 17 and 18.

Table 7: Overview for results of the Scheffé test for MVC180, MVC90, KtW, ORTH, MThL, MThM

	MVC180	**MVC90**	**KtW**	**ORTH**	**MThL**	**MThM**
IG1 - CG	√ p<0.001 d=1.15	√ p=0.029 d=0.06	√ p=0.062 d=0.53	√ p<0.001 d=0.9	× p=0.36	√ p<0.027 d=0.6
IG2 - CG	√ p<0.001 d=0.9	√ p=0.013 d=0.65	× p=0.152 d=0.42	√ p=0.022 d=0.61	√ p<0.021 d=0.61	√ p<0.014 d=0.65
IG1 - IG2	× p=0.387	× p=0.986	× p=0.882	× p=0.378	× p=0.37	× p=0.979

IG1 = stretching group; IG2 = strength training group; CG = control group; MVC180 = maximum strength in the plantar flexors with extended knee joint; MVC90 = maximum strength in the plantar flexors with flexed knee joint; KtW = ROM measurement using the knee to wall test; ORTH = ROM measurement using the goniometer of the orthosis; MThL = muscle thickness in the lateral head of the gastrocnemius; MThM = muscle thickness in the medial head of the gastrocnemius.

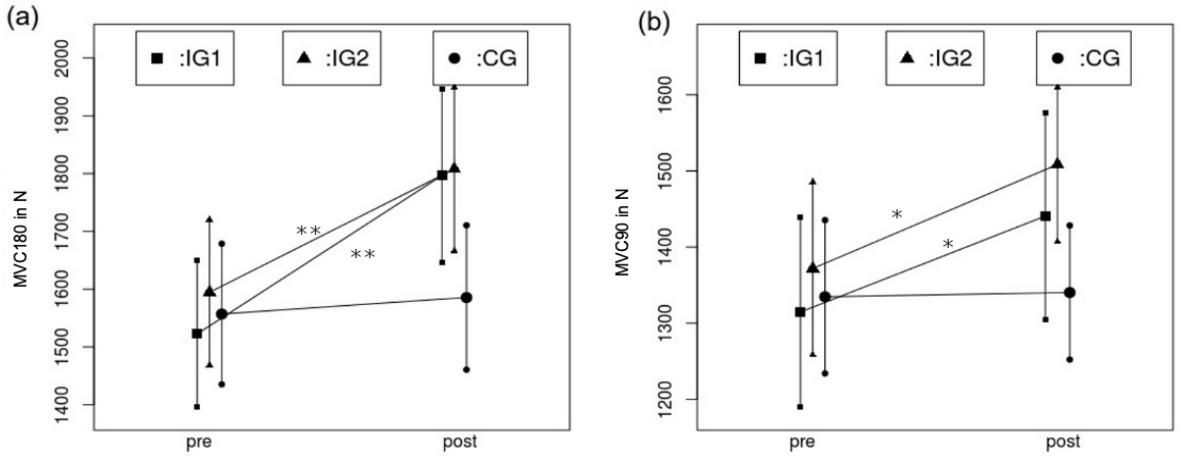

Figure 17: Comparison of progression in maximum strength from pre- to post- test considering group with extended knee joint (a) and flexed knee joint (b), IG1 = stretch training group, IG2 = strength training group, CG = control group. **=p<0.001, *=p<0.05 for difference to control group

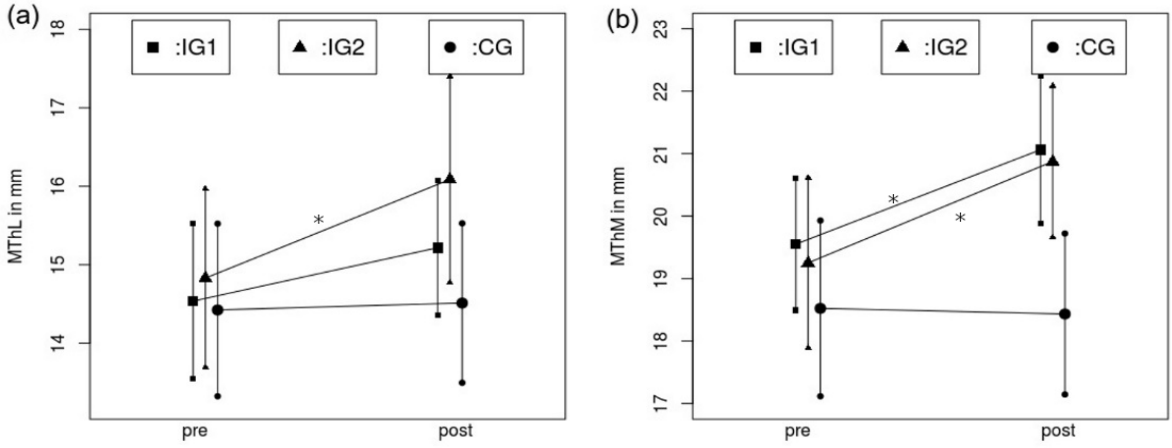

Figure 18: Comparison of progression in muscle thickness from pre- to post- test considering group in the lateral head of the gastrocnemius (a) and the medial head of the gastrocnemius (b); IG1 = stretch training group; IG2 = strength training group; CG = control group. **=p<0.001, *=p<0.05 for difference to control group

Post-hoc analysis for F-tests of G-Power calculated $1 - \beta = 23.4\%$ for the lowest effect size with $\eta^2 = 0.089$ and $1 - \beta = 99.79\%$ for the highest effect size with $\eta^2 = 0.321$ with $\alpha = 0.05$ for three groups and two time points for the interaction and $1 - \beta = 65.5\%$ for the lowest effect size with $\eta^2 = 0.282$ and $1 - \beta = 99.9\%$ for the highest effect size with $\eta^2 = 0.572$ with $\alpha = 0.05$ for three groups and two time points for the time effect.

Summary of the results

The study compared the effects of a daily long-lasting stretching intervention of the plantar flexors with a commonly used strength training routine to achieve hypertrophy. Participants of IG1 and IG2 achieved an increase in MVC force in the plantar flexors, enhancements in muscle thickness in the gastrocnemius, and improved ROM in the upper ankle. CG showed no significant changes in measured values. Since there were no significant differences between the effects in IG1 and IG2, it can be assumed that using long-lasting stretching training for one hour of continuous stretching per day leads to similar improvements as commonly used strength training to induce hypertrophy training.

8.5 Influence of Long-Lasting Static Stretching Intervention on Functional and Morphological Parameters in the Plantar Flexors: A Randomised Controlled Trial

In *Journal of Strength and Conditioning Research 00(0)/1-9, 2023*

Previous studies showed that stretch training performed with adequate duration and frequency seems to be sufficient to induce significant increases in maximum strength, muscle thickness,

and flexibility comparable to adaptations induced by a commonly used strength training routine. However, Apostolopolous et al. [21] and Antonio et al. [20] indicated that stretching intensity seems to be of high importance for structural adaptations. It was hypothesized that using constant angle stretching would lead to decrements in stretch intensity based on relaxation effects, leading to a significant decrease in stretch intensity over time. Therefore, participants joining the last experiment were instructed to stretch 6x10 minutes and to re-adjust the stretch intensity every ten minutes. Furthermore, to improve the quality of the morphological assessment, magnetic resonance imaging was used in addition to sonography measurements. MRI is stated as the gold-standard procedure to investigate morphological changes in muscular tissue. Forty-five (45) strength-trained participants (f: 17, age: 26.6 $\pm$ 3.2, height: 168.7 $\pm$ 3.4, weight: 63.7 $\pm$ 2.4, m: 28, age: 28.3 $\pm$ 3.0 years, height: 184.5 $\pm$ 4.3 cm, weight: 86.4 $\pm$ 4.2 kg) from sports study programs and local sports clubs were randomly divided into an intervention group (IG) and a control group (CG). Participants were classified as strength trained if they performed two or more training sessions per week in a gym continuously for the previous six months and included training of the plantar flexors in their training routines. Two participants were excluded from the data analysis due to problems with the Achilles tendon or being unable to train for a period of two weeks due to illness. Table 8 provides descriptive statistics and results of the two-way ANOVA for included parameters

Table 8: Descriptive statistics and results of two-way ANOVA for functional and morphological parameters

Parameter	Pretest (M±SD)	Post-test (M±SD)	Pre-Post-Diff. in %	Time effect	Time X Group
IGMVC180IL	1697.67±389.746	1856.81±431.28	9.44±7.67	p<0.001	p<0.001
IGMVC180CL	1643.76±334.69	1678.1±349.75	2.15±6.16	$F_{80,1}$=30.83	$F_{80,3}$=7.67
CMVC180Gr	1623.86±251.52	1645.00±275.20	1.28±5.52	η^2=0.278	η^2=0.223
CMVC180Gl	1559.57±245.79	1601.33±267.44	2.80±6.53		
IGMVC90IL	1507.19±333.16	1580.29±364.56	4.84±7.42	p=0.019	p=0.003
IGMVC90CL	1470.86±341.48	1514.10±340.45	3.3±6.35	$F_{80,1}$=5.74	$F_{80,3}$=4.99
CGMVC90r	1413.10±273.79	1415.29±266.23	0.44±5.01	η^2=0.067	η^2=0.158
CGMVC90l	1378.81±266.05	1353.57±235.10	-1.31±4.70		
IGKtWIL	11.38±3.47	13.3±3.43	18.79±8.35	p<0.001	p<0.001
IGKtWCL	12.45±3.74	12.54±3.72	0.86±3.00	$F_{80,1}$=33.45	$F_{80,3}$=13.63
CGKtWr	11.79±3.16	12.17±2.70	5.97±22.23	$\eta^2 = 0.295$	$\eta^2 = 0.338$
CGKtWl	11.93±2.90	12.19±3.03	2.40±7.86		
IGORTHIL	7.5±2.07	9.12±1.92	24.56±17.02	p<0.001	p<0.001
IGORTHCL	8.02±1.83	8.19±1.87	2.09±4.47	$F_{80,1}$=42.67	$F_{80,3}$=21.46
CGORTHr	8.214±1.45	8.38±1.22	3.02±11.13	$\eta^2 = 0.348$	$\eta^2 = 0.446$
CGORTHl	8.24±1.53	8.36±1.40	2.51±12.7		
MCSALIL	1015.33±269.78	1095.87±275.74	8.82±5.70	p<0.001	p=0.014
MCSALCL	1002.06±216.72	1022.06±236.02	1.80±5.79	$F_{40,1}$=18.38	$F_{40,1}$=6.66
				$\eta^2 = 0.315$	$\eta^2 = 0.143$
MCSAMIL	1715.54±529.18	1803.00±535.64	5.68±4.87	p<0.001	p=0.003
MCSAMCL	1617.41±428.08	1630.35±417.95	1.26±5.92	$F_{40,1}$=9.83	$F_{40,1}$=9.83
				$\eta^2 = 0.308$	$\eta^2 = 0.197$
MThLIL	14.58±3.17	15.54±2.77	7.90±11.42	p=0.40	p=0.013
MThLCL	14.58±2.36	14.45±2.68	-0.74±11.10	$F_{80,1}$=4.36	$F_{80,3}$=3.81
MThLCGr	14.25±2.52	14.36±2.53	0.91±4.63	$\eta^2 = 0.05$	$\eta^2 = 0.125$
MThLCGl	14.54±2.20	14.63±2.20	0.75±4.34		
MThMIL	18.43±3.31	19.66±3.15	7.29±7.81	p<0.001	p=0.002
MThMCL	18.24±2.86	18.37±2.71	1.00±6.66	$F_{80,1}$=12.95	$F_{80,3}$=5,54
MThMCGr	17.64±3.29	17,90±3.28	1.65±4.48	$\eta^2 = 0.139$	$\eta^2 = 0.172$
MThMCGl	18.25±2.44	18.30±2.67	0.23±4.40		

MVC180 = isometric MVC in plantar flexors with extended knee joint; MVC90 = isometric MVC in plantar flexors with flexed knee joint; MThM = MTh in the medial head of the gastrocnemius via sonography; MThL = MTh in the lateral head of the gastrocnemius via sonography; KtW = ROM measurement with flexed knee joint via knee to wall test; ORTH = ROM measurement with extended knee joint via the goniometer of the orthosis; MCSAL = MCSA of the lateral head of the gastrocnemius (with MRI); MCSAM = MCSA of the medial head of the gastrocnemius (with MRI); MThL = MTh of the lateral head of the gastrocnemius (with sonography); MThM = MTh of the medial head of the gastrocnemius (with sonography); IL = intervened leg of IG; CL = control leg of IG, CGr = right leg of CG; CGl = left leg of CG

Figure 19 illustrates changes in maximum strength with extended knee joint (MVC180) and with flexed knee joint (MVC90) of the intervened leg (IL) and the control leg (CL) of the

intervention group as well as both control legs. Tables 9 and 10 provide information regarding group differences from the Scheffé tests.

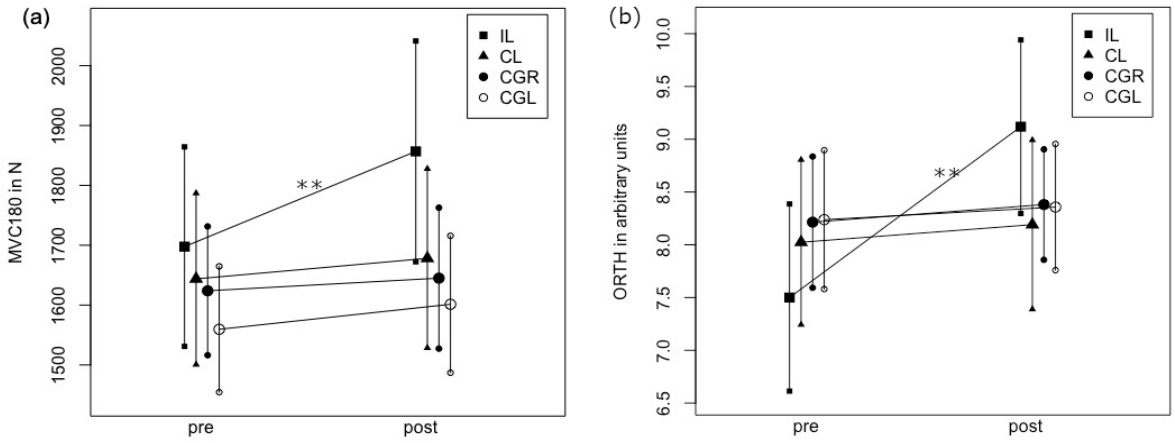

Figure 19: Illustrates progressions from pre- to post for MVC180 (a) and for ORTH (b). IL corresponds to the courses of the intervened leg of IG; CL corresponds to the control leg of IG; CGr to the right leg of CG and CGl to the left leg of CG. **=p<0.001, *=p<0.05 for difference to control group

Table 9: Overview for results of the Scheffé test for MVC180, MVC90, KtW and ORTH

	MVC180	**MVC90**	**KtW**	**ORTH**
Il-CL	√ $p = 0.005$, $d = 0.69$	× $p = 0.759$, $d = 0.2$	√ $p < 0.001$, $d = 1.01$	√ $p < 0.001$, $d = 1.16$
IL-CGr	√ $p < 0.001$, $d = 0.76$	× $p = 0.093$, $d = 0.46$	√ $p < 0.001$, $d = 0.85$	√ $p < 0.001$, $d = 1.16$
IL-CGl	√ $p = 0.007$, $d = 0.64$	√ $p = 0.008$, $d = 4.09$	√ $p < 0.001$, $d = 0.92$	√ $p < 0.001$, $d = 1.2$
CL-CGr	× $p = 0.976$, $d = 0.07$	× $p = 0.531$, $d = 0.27$	× $p = 0.849$, $d = 0.16$	× $p = 1.00$, $d = 0$
CL-CGl	× $p = 0.999$, $d = 0.05$	× $p = 0.112$, $d = 0.45$	× $p = 0.964$, $d = 0.1$	× $p = 0.997$, $d = 0.037$
CGr-CGl	× $p = 0.938$, $d = 0.11$	× $p = 0.803$, $d = 0.18$	× $p = 0.987$, $d = 0.06$	× $p = 0.997$, $d = 0.037$

IL = intervened leg of IG; CL = control leg of IG; CGr = right leg of CG; CGl = left leg of CG; MVC180 = isometric MVC measurement with extended knee joint; MVC90 = isometric MVC measurement with flexed knee joint; KtW = ROM measurement via KtW test; ORTH = ROM measurement via goniometer of the orthosis

Table 10: Overview for results of the Scheffé test for muscle thickness in the lateral and medial head of the gastrocnemius

	MThL	**MThM**
IL-CL	√	√
	p = 0.025, d = 0.56	p = 0.015, d = 0.6
IL-CGl	×	√
	p = 0.123, d = 0.44	p = 0.04, d = 0.53
IL-CGl	×	√
	p = 0.114, d = 0.44	p = 0.008, d = 0.64
CL-CGr	×	×
	p = 0.922, d = 0.13	p = 0.985, d = 0.07
CL-CGl	×	×
	p = 0.933, d = 0.12	p = 0.996, d = 0.05
CGr-CGl	×	×
	p = 1.00, d = 0.02	p = 0.942, d = 0.11

IL = intervened leg of IG; CL = control leg of IG; CGr = right leg of CG; CGl = left leg of CG; MThL = MTh in the lateral head of the gastrocnemius; MThM = MTh in the medial head of the gastrocnemius.

Post-hoc analysis for F-tests of G-Power calculated $1-\beta = 23.8\%$ for the lowest effect size with $\eta^2 = 0.125$ and $1-\beta = 99.99\%$ for the highest effect size with $\eta^2 = 0.446$ with $\alpha = 0.05$ for four groups (parameters: intervened leg of IG, control leg of IG, right leg of CG, left leg of CG) and two time points for the interaction. For the time effect there was o $1-\beta = 7.5\%$ for the lowest effect size with $\eta^2 = 0.005$, while there was a $1-\beta = 97.4\%$ for the highest effect size with $\eta^2 = 0.348$ with $\alpha = 0.05$ for four groups and two time points.

Summary of results

Results of the present study show significant increases in maximum isometric strength in the plantar flexors measured with extended and flexed knee joint of 9.44% and 4.84% (d = 0.2 − 0.76) as well as in both flexibility tests 18.79% and 24.56% (d = 0.85 − 1.16). Furthermore, significant hypertrophic effects were determined by sonography imaging for increases in muscle thickness of 7.29 − 7.9% (d = 0.53 − 0.6) as well as via MRI for muscle cross-sectional area of 5.68% and 8.82% (d = 0.16 − 0.3). There were no significant changes in CG values from pre- to post-test in any measured parameter. There were moderate correlation coefficients between increases in muscle cross-sectional area measured via MRI and muscle thickness measured via sonography (r = 0.36 − 0.43, p = 0.005 − 0.021).

9 Discussion

The aim of this work was to investigate the influence of long-lasting static stretching training of up to two hours per day, seven days per week on functional (maximum strength and flexibility) and morphological parameters (muscle thickness, muscle cross-sectional area, and the pennation angle).

9.1 Overview of Results

In the meta-analysis included in this work, it could be evaluated that long-lasting stretching for up to 24 hours per day for seven days per week for up to six weeks can be seen as a sufficient stimulus to induce very large magnitude increases in muscle mass ($d = 8.51$, $p < 0.001$), muscle cross-sectional area ($d = 7.91$, $p < 0.001$), fiber cross-sectional area ($d = 5.81$, $p < 0.001$), fiber length ($d = 7.86$, $p < 0.001$) and fiber number ($d = 4.62$, $p < 0.001$) in animals. Furthermore, Alway [9] demonstrated significant increases in maximum strength of 95% ($d = 11.13$) in the stretched muscle using in vitro studies. The transferability of results from animal studies to humans was requested as early as 1983 by Frankeny and colleagues [105]. Since there were only studies investigating the influence of four times 30 seconds of stretching with three to seven days per week [66, 229] to six times five minutes twice per week [359], no studies with comparable stretching durations and/or stretching frequencies could be obtained in humans. To be able to conduct studies answering this request, a stretching orthosis was built and used to induce long-lasting static stretching training of up to two hours per day for seven days per week in humans.
Experimental studies from our laboratory showed a range of trivial to large magnitude increases in maximum strength of 4.84% to 22.9% with $d = 0.2 - 1.17$ and ROM of 6.07 − 27.3% with $d = 0.16 - 0.87$ dependent on stretching time, training level and testing procedure. Furthermore, significant moderate to large magnitude hypertrophy effects of 7.29 − 15.3% with $d = 0.53 - 0.84$ in muscle thickness and trivial to small increases of 5.68% and 8.82% ($d = 0.16 - 0.3$) in muscle cross-sectional area could be demonstrated.

9.2 Discussion of Results from Animal Studies

9.2.1 Hypertrophy

In the literature, there are some main hypotheses stated explaining the high increases in muscle mass in the animal studies. It was suggested that stretch-induced mechanical tension on sarcomeres may play a major role in stimulating hypertrophic processes in the overloaded muscle. Devol et al. [89] described a large increase in muscle mass after the first days, however, the curve of increases plateaus by prolonging the intervention time without re-adjusting the stretch intensity. The authors suggested that the mechanical tension per sarcomere could be responsible for muscle hypertrophy, which can be assumed to decrease if stretching leads

to a serial accumulation of sarcomeres over time. From this, to induce further increases in hypertrophy (and induce further mechanical tension on serial sarcomeres) the stretch-stimulus should be re-adjusted (increasing stretching stimulus due to more weight or using higher degree of abduction, if an adductor is stretched).
Therefore, it seems that intensity, measured by tension, could be of high importance for stretch-mediated hypertrophy. Accordingly, the results from Antonio & Gonyea [18] showed the highest hypertrophy effect of 318.6 ± 31.5% by using a progressively increasing intensity by adding weight to the wing of quails (starting at 10% of the own body weight and ending at 35%). Furthermore, Sola et al. [282] also showed that higher muscle mass gains due to higher stretch intensities. This indicates that *"It is Stretch that Causes the Hypertrophy of Muscle"* [282, p.93] and adaptations are related to the magnitude of intensity which is in accordance with Apostolopoulos and colleagues [21]. Summers et al. [293] pointed out a 50% muscle mass increase after five days of stretching which was accompanied by three times higher creatine kinase activity. Increases in creatine kinase can be seen as a predictor of microtraumatization and therefore for damaged muscles fibers that was found after few days of chronic stretch [25]. The same authors also stated a significant increase of growth-promoting factors [293] which were confirmed by Sasai et al. [261] describing the stimulation of growth-promoting pathways due to mechanical stretching of the muscle. This is stated as one important factor for an increased rate of protein synthesis [117, 119, 261]. Furthermore, some authors suggested the involvement of satellite cell activation to hypertrophy of damaged muscle fibers due to microtraumatization after mechanical overload [53, 301, 350]. Satellite cells are described as resident myogenic stem cells staying in the basal lamina surrounding the muscle as well as in the sarcolemma of adult muscle fibers in a quiescent state [301]. It was further hypothesized that increased level of hepatocyte growth factor, which is known as a heterodimer related to regeneration processes in the liver, could be involved in the activation of satellite cells [50]. Furthermore, authors listed other known growth factors such as IGF-1 and fibroblast growth factor, which seem to play a minor role in activation of satellite cell activation [301]. While HGF associates to damaged muscle cells to induce repairing process, another factor called NO-radicals seems to respond with increased level to mechanical stress and is involved to muscle growth and muscle regeneration [14].
As described by Goldspink [117] there are many mechanisms to translate mechanical changes in muscle tissue into chemical signals that can activate satellite cells [301]. Further, the activation of satellite cells could additionally play a role in muscle hyperplasia [11], which was also found in many animal studies contributing to stretch-mediated muscle mass increase [11, 18, 282]: *"From studies of many previous workers and the results of this investigation, we have come to the opinion that hyperplasia follows adequate stretch"* [282, p.97]. There are two prominent hypotheses for explaining muscle hyperplasia due to stretching.

9.2.2 Hyperplasia

Firstly, the quiescent satellite cells are activated by long-lasting stretching and form new fibers to minimize the damage of existing fibers. Authors described no hyperplasia due to ablation or tenotomy of synergists but exclusively after stretching training [7, 11, 68, 67, 282, 350]. Proponents of this theory referred to the presence of an embryonic type of myosin isoform after chronic stretching interventions. Further indications could be seen in constant relation of FT and ST fibers in the stretched muscle with an increasing fiber number [7]. Based on this, considering the common shift of fibers from embryonic fibers to FT fibers to ST fibers [203], FT fibers could shift to ST fibers, however, Sola et al. [282] demonstrated that newly formed fibers were as big as adult fibers after two to three weeks. This could implicate the shift from embryonic fibers to FT fibers to maintain the equal ratio of ST and FT fibers with an increase in the total fiber number. Authors suggested the explanation that the shift in fiber distribution is due to *"a more favorable energetic state to help overcome chronic muscle overload (Baldwin et al., 1982)"* [203, p.260].

Apart from the theory of hyperplasia due to the activation of satellite cells, proponents of the second theory referred to fiber splitting of the muscle after reaching a critical size. If a muscle is metabolically inefficient, it was hypothesized that the muscle would split into two or more daughter fibers which can also lead to increases in muscle mass due to mechanical overload [18, 19, 25]. Accordingly, some authors stating strongly damaged muscle fibers and the progress of fiber splitting [19, 282]. However, since literature provides information for both theories, no final statement can be given, neverthless, there were significant increases in fiber number in response to chronic stretching interventions. Antonio & Gonyea [18] and Antonio et al. [20] performed first intermittent stretching with progressively increasing stretching intensity starting with 10% and increasing up to 35% of the quails' own body weight. Afterwards, authors induced stretching with a continuous stretching protocol (24 hours seven days per week) with 35% of the own body weight. While the continuously performed stretching led to high hyperplasia effects with 5.25% fibers showing a split fiber profile but minor hypertrophy, the first phase with an intermittent stretching protocol resulted in minor hyperplasia with only 0.3% fibers showing a split fiber profile but large hypertrophy effects. The authors suggested that only chronic stretching would lead to sufficient muscle damage needed to induce hyperplasia while resting intervals in the first phase of the intervention gave the opportunity to repair damaged muscle fibers.

Alway [9, 8] investigated the contractile properties of stretched muscle reporting a reduced contraction time of stretched muscle with a shift to slower isomysoin (slow myosin 1 to slow myosin 2) accompanied with an increase in maximum strength of up to 95%. Additionally, authors attributed increases in maximum strength in vitro to hypertrophy and hyperplasia. *"If hypertrophy could account solely for increased force, we would have expected an increase in Po of 60%, rather than 95%, in stretched muscle. Because the remaining 35% of the P0 could not be explained by fiber hypertrophy, this additional force must have resulted from new fibers*

that contributed to the increased physiological CSA. Because new fibers were functional, control and stretched muscles had both, similar muscle force per unit muscle corrected for nonmuscular tissue (3.0 vs. 2.6N/g, respectively) and specific tension (11.1 vs. 9.2, respectively).” [9, p.140]. The hypothesis is based on the assumption that fibers are cylindrical and have similar length throughout the entire muscle's length which would lead to an overestimation of the FCSA [9]. Apart from parallel hypertrophy increases, augmentation of muscle mass could also be attributed to increased fiber length [7, 20, 19, 68] due to an accumulation of sarcomeres in series. This elongation of the muscle could be hypothesized to be the physiological response to a stretch of sarcomeres leading to a reduced overlap of contractile filaments, leading to higher tensile stress on the sarcomere. To reduce mechanical tension due to stretch per sarcomere, it can be hypothesized that an increased number of sarcomeres in serial would lead to reduced tension per sarcomere [89].

9.3 Discussion of Results from Own Studies in Humans

This section of the chapter serves to place the results obtained from own (experimental) studies in the context of the current literature and to provide explanatory approaches with regard to the underlying background.

9.3.1 Interpretation of the Results for Maximum Strength and Hypertrophy

While there are numerous studies investigating long stretching durations of 30 min – 24 h per day on seven days per week in animals, our studies included in this work are, to the best of my knowledge, the first investigations in humans examining long-lasting static stretching interventions on morphological and functional parameters. As stated above, to increase maximum strength as well as muscle thickness and muscle cross-sectional area, strength training is commonly used. However, long-lasting stretching, performed in the above mentioned studies showed increases in maximum strength of $4.8 - 22.9\%$ with $d = 0.2 - 1.17$ and hypertrophy of $7.29 - 15.3\%$ with $d = 0.53 - 0.84$. There are some hypotheses stated in literature to explain adaptations on the human muscle. First, mechanical tension can be seen as an initiating stimulus to induce various cellular processes or signal transduction and induce changes in muscle morphology [57, 215, 248, 301]. This so-called mechanotransduction can cause tension-induced muscle hypertrophy [3]. Smith et al. [279] and Jacobs & Sciascia [147] previously showed that stretching tension of sufficient intensity can lead to delayed onset muscle soreness and associated inflammation. After this microtraumatization of muscle tissue, the following repairing processes are related to the stimulation of the protein synthesis rate [62, 119]. Increases in muscle thickness and muscle cross-sectional area are known to be a factor influencing maximum strength since it can be assumed that there is more contractile tissue in parallel which could lead to higher strength capacity. Therefore, mechanical tension-induced hypertrophy could be an important factor for the increase in maximum strength and is requested in both, resistance training [170, 269] and static stretching [20, 282]. The included self-conducted stud-

ies used six-week intervention periods to examine increases in maximum strength. However, it can be assumed that strength increases in the first weeks of an unknown training stimulus can be attributed to neuronal adaptations of the muscle to a large extent [38, 88, 158]. Thus, no investigations examining neuronal adaptations to long-lasting static stretching interventions could be found in humans. Animal studies have to be reviewed.
Since blood flow restriction (BFR) training has the potential to increase MCSA [142, 148, 358] and many subjects described numbness in the foot of the stretched leg in the self-conducted studies, it cannot be ruled out that a reduced blood flow to the muscle is present while stretching and thus comparative adaptations might occur in long-lasting static stretching. Hotta et al. [141] also described decreased blood flow by using 30 minutes of stretching in the rat as an acute effect but measured increased blood flow to the working muscle as a long-term effect after a training period of four weeks on five days per week stretching training of 30 minutes which could possibly influence the strength performance in the target muscle. Though, it can be questioned whether and to which extent an increased blood flow to the working muscle can lead to improved maximal strength capacity. Furthermore, evidence is limited because most studies demonstrated hypertrophy effects in untrained participants [148] and by using intervention periods of eight weeks, consequently, the induced stimulus via blood flow restriction must be seen as an unknown stimulus with limited informative value for long-term effects. Jessee et al. [148] showed hypertrophy effects due to BFR training without increases in maximum strength, consequently, effects may play a minor role in stretch induced adaptations. Lastly, hypertrophic effects of BFR were (exclusively) examined by using sonography, which can be assumed to be limited by subjective influence of the investigator [93, 135] and must be questioned without proving results by using more objective morphology measurements as MRI [330]. No studies could be found that investigated long-term effects of long-lasting stretching interventions on blood flow and its impact on maximum strength performance in humans. Since hypoxic conditions seem to play a role in muscle growth and muscle loss [71], further studies could investigate the influence of oxygen saturation while stretching and its impact on structural and functional adaptations.
Based on the results from the own lab and current literature, pointing out a final statement about the origin of the maximum strength increases as well as of hypertrophy is hypothetical. But it can be assumed that stretching with sufficient volume and intensity via a calf muscle stretching orthosis can induce a stimulus leading to adaptations comparable to those of strength training.
Considering results of previously performed studies, one could question the need of daily one hour stretching, as Kokkonen et al. [160] performed a stretching routine including three stretching sessions per week with 15 exercises each performed three times 45 seconds that led to a significant increase in maximum strength in the knee extension of 32.4% ($d = 0.72$) and of 16.2% in the knee flexion ($d = 0.44$). Furthermore, Nelson et al. [229] pointed out significant maximum strength increases of about 29% by stretching the calf muscle four times 30 seconds on three days per week. Panidi et al. [236] found increases in muscle thickness

of 23% in the stretched leg and 13% in the control leg performing stretching training on five days per week for 12 weeks with stretching durations up to 15 minutes per session in volleyball players. Those substantial increases seem to be surprising considering hypertrophy effects in response to commonly used resistance training. Results can be questioned, since authors refer to stabilization of the bodyweight with the contralateral leg [229]. Thus, if stabilizing the bodyweight with the contralateral leg led to significant increases in maximum strength, the training status of participants had to be extremely low. Accordingly, Li et al. [180] were able to report significant moderate magnitude increases in maximum strength of 11% with d = 0.66 only in participants with low baseline strength values (females) by using different flexibility training routines. The male participants with higher baseline strength values showed no significant increase in maximum strength. Furthermore, if those results could be generalizable, the need of resistance training could be questioned in general, as comparative, or even higher results could be reached by performing 4x30 sec stretching on a stair. For this, there are reasonable doubts. Yahata et al. [359] performed stretching with longer durations of 30 minutes twice per week, however, lower increases in maximum strength with 6.6% and no hypertrophy could be observed. Considering our own studies as well as expected increases using resistance training as the most common method with assumed large effectivity results seem to be more realistic.

There is only one literature review examining stretch mediated hypertrophy, performed by Nunes et al. [232] who showed that short-lasting static stretching interventions of up to two minutes per session were not able to induce significant hypertrophy. In general, there is limited literature showing hypertrophy due to static stretching. Simpson et al. [277] pointed out significant increases in muscle thickness due to three minutes of static stretching performed on five days per week. However, Nunes et al. [231] commented on this article, questioning the significance of increases in muscle thickness and suggested a correction of the results. Only Longo et al. [185] were able to show improvements in muscle thickness in the gastrocnemius lateralis of 5.8% (d = 0.37) by using stretching durations lower than one hour without any effect in the soleus and the gastrocnemius medialis. The studies reviewed in the present work showed significant moderate to large magnitude increases in muscle thickness of $7.3 - 15.3\%$ with $d = 0.53 - 0.84$ measured via ultrasound sonography with confirmed increases in muscle cross-sectional area using MRI.

Increases in muscle thickness and muscle cross-sectional area were comparable with those measured in resistance training studies with $5.35 - 17.78\%$ ($d = 0.18 - 0.66$) [94, 319]. Based on this, there is evidence that static stretching performed with adequate duration and/or intensity can be used as an alternative to commonly used resistance training programs if the aim is to improve maximum strength and induce hypertrophy which was also investigated in the fifth study **"Comparison of the Effects of Long-Lasting Static Stretching and Hypertrophy Training on Maximal Strength, Muscle Thickness and Flexibility in the Plantar Flexors"** included in this work. However, regarding training economy, a resistance training program should be preferred, as the required training volume to induce

comparable adaptations.

9.3.2 Classification and Interpretation of Results for ROM

It is well accepted that stretching induces increases in ROM [91, 177, 208, 210, 360]. However, there is high heterogeneity in study design and the techniques to induce stretching. While some investigators induced stretching by using a stretching board [225, 236, 359], others used stretching devices or orthoses – as was employed in the present studies in this dissertation –, strength machines [277] or performed stretching without any external resistance [37]. The intensity of stretch applied was mainly controlled via stretching pain scales but no objective intensity value was measured. High heterogeneity in study design without controlling the intensity leads to difficulties in comparing results. To counteract this limitation and to investigate the dose-response relationship stated by Thomas et al. [308], stretching in the first experimental study included in this work compared different daily stretching times of 10, 30, 60 minutes over a six-week period with extended knee joint. Stretching intensity was quantified and standardized by using the goniometer attached to the orthosis. The results indicated a dose-response relationship for the ROM testing via the goniometer of the orthosis with 16.39% and d = 0.88. In all the included studies, there were higher effects in ROM testing with extended knee joint compared to flexed knee joint showing a specificity in adaptation as stretching was performed with extended knee joint. There are some explanatory approaches for improvements in flexibility due to stretching interventions in humans. Freitas et al. [106] described increased ROM because of changes in pain tolerance following the stretching stimuli and the need of longer stretching durations to induce structural changes in the muscle. However, some authors explained increased ROM via changes in the muscle-tendon unit or via increased stretch tolerance [106, 297]. It is known from animal studies that long-lasting static stretching led to a serial accumulation of sarcomeres [20, 19], which was neither investigated in previous studies nor in the included studies of this work. Hypothesizing an increase in the number of serial sarcomeres [248, 363] could possibly be seen as the explanation for changes in pain tolerance and increasing the time which is needed to reach pain threshold due to stretching as it can be assumed that the maximally stretched position of each sarcomere of the muscle would be reached at higher muscle length. Accordingly, it could be hypothesized that a serial accumulation of sarcomeres lead to changes in pain threshold if stretching pain is related to high length in sarcomeres of the muscle fiber.

9.3.3 Practical Applications

Results indicate a transferability of the results from animal experiments to humans regarding adaptations in maximum strength and muscle thickness and muscle cross-sectional area. The meta-analysis confirmed the dose-response relationship which was previously found in animal studies [36, 105]. As stated by Nunes et al. [232], short-lasting static stretching was not able to induce significant hypertrophy in humans, however, the presented studies showed muscle

hypertrophy increases comparable to increases in muscle thickness and muscle cross-sectional area known from resistance training [94, 234]. Therefore, a daily stretch training can be seen as an alternative to resistance training with widespread application. The main goal of many training routines in fitness [340, 344], competitive sports [291, 292], injury prevention [214] and rehabilitation is to induce hypertrophy and/or maximum strength increases. However, on the one hand, the practicability of daily long-lasting static stretching to induce hypertrophy and maximum strength gains may be questioned because of the comparatively high volume and time requirement of highly frequent used long-lasting stretching routines of one hour per day. On the other hand, the increase of maximum strength by using the developed stretching orthosis can be integrated in daily life (working in a sitting position etc.), consequently, no additional time has to be spent for training a specific muscle. However it could be argued that more muscle groups can be trained with resistance training in a particular period than could be simultaneaously placed under continuous stretch. But, since no active contraction is required with stretching, benefits especially in rehabilitation can be assumed. This is possible if common resistance training would be contraindicated, for example, after surgery of the anterior crucial ligament or hip prothesis. Additionally, Schoenfeld et al. [270] pointed out some applicability of using inter-set stretching in general to enhance muscle hypertrophy in response to resistance training. Neverthless, it might be questionable to include those training procedures in advanced athletes, as it might disturb the needed rest to recover from previous training set, which might lower the intensity of the following one.
Furthermore, improvements in ROM in the upper ankle joint are associated with an improvement in dorsiflexion which can be seen as beneficial in many sport-specific movements such as the (deep) squat [109] and jumping [237]. In addition, there are limited possibilities to perform a resistance training with external weights in space flight. As the orthosis seems to provide sufficient stimuli to induce hypertrophy and strength gains without the need of external weights or gravity, an application of this training methods in space flight could be promising, as there are limitations in designing resistance training programs [152, 239]. Considering results from animal studies a transferability of results in many other indications have to be investigated (see outlook). As Sola et al. [282] determined comparative effects in hypertrophy even in denervated muscles, transferability to humans should be tested because of a potential application in (for example) people using wheelchairs to reduce atrophic effects.

9.4 Limitations

Most strength increases in the present studies were measured via maximum isometric strength testing procedures. However, some limitations of maximum isometric strength tests regarding the transferability to sport-specific movements can be assumed [220, 345] and literature illustrates that differences between results of isometric and dynamic strength measurements in the plantar flexors exist [327]. However, to counteract this issue, dynamic and isometric testing was performed in the study **“Influence of Long-lasting Static Stretching on Maximal**

Strength, Muscle Thickness and Flexibility". Furthermore, there is limited reliability stated for sonography to examine muscle morphology. English et al. [93] and Hebert et al. [135] stated limitations in standardization of sonography which can be attributed to challenges in localizing the same spot in pre- and post-testing and applying the same transducer pressure of the transducer. Based on this, there seems to be limited objectivity in sonography [330], however, it is the most common method to investigate changes in muscle thickness following to training interventions [31, 80, 79]. To rule out listed disadvantages, the mean of three measurements per picture as well as the mean from two sonography images per muscle group were evaluated. Furthermore, to standardize the procedure, important information addressing location of the measurement and usage of the transducer were stated detailled. Additionally, MRI-imaging which is stated as the gold standard method to investigate muscle cross-sectional area was included to the testing routine confirming hypertrophy effects following six weeks of stretching. Measuring the flexibility of the upper ankle while maintaining an extended knee joint was performed via the goniometer of the orthosis. Therefore, maximal ROM was tested by pushing the foot into the maximal dorsiflexed position using the orthosis. This measurement was performed by the investigator, consequently, it cannot be ensured that in post-test the same pressure was used to reach the dorsiflexed position in pre- and post-test. Furthermore, to standardize the knee to wall test, a self-built knee to wall measuring device was used with a measuring scale to read off the flexibility value. The measurement was finished when the investigator was able to pull a sheet of paper from underneath the heal of the participant. As stated for the measurement via the goniometer of the orthosis, it could not be ensured, that the investigator pulled the sheet of paper with a standardized force. To balance this limitation, three measurements were performed and ICC as well as CV were measured and confirmed as high.

As mentioned above, there is a general limitation in stretching literature regarding the quantification and standardization of intensity. Without quantification, it cannot be ensured that "static stretching" used similar mechanical tension stimuli between subjects or even between training sessions. Since it can be assumed that pain feeling is subjective, a quantification of intensity by using pain scales seem to be a sham standardization leading to inconsistency in procedures. Accordingly, Lim & Park [183] pointed out that stretching pain seems not to be well correlated with the passive resistance of the stretched muscle, which could be assumed to be of high interest inducing mechanical tension. Thus, this would lead to inability of comparing results and procedure because of differences in physiological responses of the muscle due to usage of different intensities, as it can be observed in resistance training. It must be assumed that performing three repetitions with 95% of the 1RM would lead to different adaptations compared to 20 repetitions with 40% of the 1RM. Using five repetitions with 40% of the 1RM can be assumed to be ineffective in any way. Transferred to the present topic of stretching: Using 4x30 sec stretching without measuring the mechanical tension (e.g. via passive torque, passive resistance) cannot be assumed to induce a suprathreshold mechanical stimulus per se, leading to increases in measured parameters in one study [1, 229] without significant improve-

ments in another [34, 37, 262, 277]. However, since stretching duration seems to be of high importance for adaptations of stretching training [106, 308], results from studies highlighting the relevance of high intensities [21, 110, 298] should be interpreted carefully because of a lack of quantification and an objectivation of intensity. Since no long-term investigations were found in literature nor included to this work, side effects of the long-term use cannot be excluded. Furthermore, assuming a sustained increase in flexibility, at some point the maximum range of motion will be reached. Latest at this point, no further mechanical tension could be induced by the orthosis.

9.5 Conclusion

It can be concluded that long-lasting static stretching between one and two hours per day provides a sufficient stimulus to induce significant improvements in maximum strength as well as stimulate hypertrophy. Different daily stretching times in long-lasting static stretching increased ROM in the upper ankle joint with a dose-response relationship. However, based on current literature, no final statement about underlying physiological mechanisms in humans can be given. There are few hypotheses listed previously including suprathreshold mechanical stimuli triggering anabolic signaling pathways leading to hypertrophy. Furthermore, neuronal adaptations due to stretching or modified blood flow to the working muscles were also discussed in previous literature. In conclusion, the described training method might be a potential alternative to commonly used resistance training programs if one is not willing to or able to perform a training routine in the weight room e.g. after injury and surgery, in space flight or in pandemic quarantine.

9.6 Outlook

The self-conducted studies provided much information about the influence of long-lasting stretching interventions in the human gastrocnemius. However, numerous further research questions arise from current literature. The loss of skeletal muscle due to aging, also known as sarcopenia, is a widespread problem because of the demographic change in western populations. It is recommended to reach higher levels of physical activity and induce mechanical tension to reduce the velocity of muscle and strength loss. In rehabilitation, high intensities in resistance training are also of high relevance. Husby et al. [144] showed benefits of using resistance training to improve physical capacity in rehabilitation compared with commonly used methods such as physiotherapy. However, the presented work showed adaptations due to long-lasting static stretching in young and adult individuals, consequently the usage in rehabilitation setting is still hypothetical. Therefore, the use of stretching interventions should be addressed in further studies, as it is recommended to implement safe and effective physical activity in numerous settings to counteract muscle loss [97, 127, 333].
In animal research, Alway and Lee & Alway [8, 176] demonstrated that aging seems to influence the magnitude of adaptations, although there were still significant increases in muscle mass. It

can be suggested to investigate the effects of this training method in the elderly as a preventive training program or as a training intervention for sarcopenia in humans. Furthermore, there are many orthopedic indications requiring surgery with following phases of immobilization and corresponding muscle and strength loss [192, 288, 347]. Topp et al. [313] showed the efficacy of prehabilitation programs to enhance efficacy of rehabilitation after knee surgery. For this, using long-lasting stretching could possibly be implemented in prehabilitation and post-surgery programs, as no active muscle contraction takes place while also minimizing injury risk compared to resistance training can be hypothesized: *"The therapeutic application of stretch should therefore be kept in mind when designing regimes for rehabilitation"* [119, p.237].

Furthermore, it is known from animal studies that the highest hypertrophy occurred after the first week of stretching while after prolonged stretching durations with a constant stretching stimulus, the increases in muscle mass flatten [89]. Additionally, Bates [36] and Frankeny et al. [105] demonstrated increases in muscle mass with a stretching duration dependency. 50% of the muscle mass increases of a continuous 24h daily stretching intervention were observed for daily stretching times of 30 minutes [105]. Bates [36] showed increases in muscle mass in the anterior latissimus dorsi in a five-week training period of 57% with a stretching time of 30 minutes per day, 59% with a stretching time of one hour per day, 67% with two hours of stretching per day and 72% with four hours of daily stretching. Thus, a doubling of the stretching duration led to comparatively small increases in muscle mass. Based on this, it seems not to be worthwhile to stretch the muscle for one or two hours in animals when trying to optimize the effort-to-result ratio. Further studies are requested to investigate the effects of different stretching interventions to point out the optimal stretching time and intervention period in humans.

In animal studies, authors stated that long-lasting stretching seems to be responsible for new fiber formation [18, 20, 282]. In humans, no study was able to point out a significant increase in fiber number, as it seems difficult to determine the fiber number in humans.

It is possible that hyperplasia effects were not investigated previously because of the missing triggering stimulus: Authors described that there may be more evidence for hypertrophy but this *"does not mean that there may not be a gradual replacement of fibers in normal life, nor that there may not be hyperplasia if the stimulus is adequate"* [282, p.78]. A very interesting hypothesis regarding hyperplasia effects in humans is discussed by MacDougall [190] in Komi [162]. It is stated that postnatal muscle growth which consists of increased fiber area as well as fiber length with a proportional increase in number of myoneclei is present until bone growth is complete – assuming that this would lead to a constant stretch of the muscle. However, in humans there are controversial results in regard to new fiber formation due to exercise. MacDougall et al. [191] as well as Tesch & Larsson 1982 [305] refer to the calculation of fiber number based on fiber size while Larsson & Tesch 1986 [172] estimated the fiber number based on motor units suggesting that some bodybuilders could have more fibers than untrained participants, however, those results are of indirect evidence, consequently attention should be

paid when interpreting these results. Accordingly, it is stated that in trained bodybuilders biceps fiber count did not differ compared to untrained subjects [189]. However, finally it is stated that *"it thus appears that net increases in fibre numbers do not occur in healthy adult human muscle in response to resistance exercise; or, if they do, that they are of little numerical significance. How then does one reconcile the clear evidence for abundant fibre hyperplasia in certain animal models, with its lack in the human model? One possible explanation is that hyperplasia occurs only in response to a significant stretch overload that also causes muscle lengthening, and that conventional resistance training does not impose such a stimulus. Let us examine the rationale for this explanation."* [190] in Komi [162, p.258].

Furthermore, studies indicated that a serial accumulation of sarcomeres is induced or enhanced if a contraction is performed in addition to stretching [136, 317]. Williams et al. [342] showed that there was a faster decrease in serial sarcomeres due to immobilization of a muscle in a shortened position when an electrical stimulation was added to the immobilization in a shortened position assuming that the muscle aims for optimal length to contract. Mizuno [212] investigated the influence of stretching four times 30 seconds in combination with electrical stimulation showing non-significant higher effects compared to stretching without electrical stimulation. Consequently, further studies could include electrical stimulation in combination with long-lasting static stretching to investigate additional effects.

The experiments from this dissertation are limited to the plantar flexors. Transferability to the upper body muscles or bigger muscle groups in the lower extremity should be tested in further investigations. As maximum strength is stated as a basic ability in sport performance, further investigations should investigate whether long-lasting stretching induced increases in maximum strength lead to significant improvements in sport-specific movements such as jumping and sprinting based on the fact that Alway [8, 9] demonstrated reduced contraction velocities after long-lasting stretching in quails which could possibly be attributed to a shift in isomyosin to a slow type of myosin. Consequently, further investigations should include the effects on contraction velocity and therefore sport-specific abilities such as the stretch-shortening cycle and jumping and sprinting performance.

10 References

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11 Contributions to and Status of the Included Studies

The initial research idea was figured out by Martin Hillebrecht and me. The software and measurement devices were provided by Martin Hillebrecht. The study conduction was supervised by Stephan Schiemann. Furthermore, Stephan Schiemann provided the financial resources to perform MRI measurements. The orthoses were developed by me with the support of my father, Martin Hillebrecht and optimized and produced in collaboration with RAS GmbH and the Novecor GmbH.

Study 1
Warneke, K., Freund, P.A., Schiemann, S. (2022). Long-Lasting Stretching Training Produces Muscle Hypertrophy – A Meta-Analysis of Animal Studies
published in **J Sci Sport Exerc**

Before starting the experimental investigations I summarized the available literature in animal and human studies and developed the idea for performing a literature research article. The meta-analytic procedure was instructed and supervised by Alexander Freund, who also contributed in writing of the meta-analysis specific aspects. After writing the first draft of the manuscript, I discussed the content and structure with the co-authors. As the corresponding author, I submitted the study to the journal and revised it based on the reviewer's comments.

Study 2
Warneke, K., Wirth, K., Keiner, M., Schieman, S. (2023). Improvements in Flexibility Depend on Stretching Duration
published in **Int J Exerc Sci 16(4): 83-94**

In the study, in sixty participants (CG, IG10 and IG60) the data collection was performed alone, while for IG30 I got support in data collection by a master's student. Furthermore, I performed the data analysis and wrote the first draft of the manuscript. Afterwards, I discussed the results and the manuscript with my co-authors and included their feedback to the study. As the corresponding author, I submitted the manuscript to the Journal and revised the manuscript based on the reviewer's comments.

Study 3
Warneke, K., Brinkmann, A., Hillebrecht, M., Schiemann, S. (2022). Influence of Long-Lasting Static Stretching on Maximal Strength, Muscle Thickness and Flexibility.
published in **Front Physiol**

In the study I performed the data collection as well as the data analysis. To perform ultrasound measurements I received an instruction by Anna Brinkmann from the Carl von Ossietzky University Oldenburg. Furthermore, I wrote the first draft of the manuscript. Afterwards, I discussed the results and the manuscript with my co-authors and included their feedback to the study. As the corresponding author, I submitted the manuscript to the Journal and revised the manuscript based on the reviewer's comments.

Study 4
Warneke, K., Keiner, M., Hillebrecht, M., Schiemann, S. (2022). Influence of One Hour versus Two Hours of Daily Static-Stretching for six Weeks Using a Calf-Muscle-Stretching Orthosis on Maximal Strength.
published in **Int J Environ Res Public Health, 19, 11621**

In the study I performed the data collection as well as the data analysis. I wrote the first draft of the manuscript. Afterwards, I discussed the results and the manuscript with my co-authors and included their feedback to the study. As the corresponding author, I submitted the manuscript to the Journal and revised the manuscript based on the reviewer's comments.

Study 5
Warneke, K., Wirth, K., Keiner, M., Brinkmann, A., Wohlann, T., Lohmann, L.H., Hillebrecht, M., Schiemann, S. (2023). Comparison of the Effects of Long-Lasting Static Stretching and Hypertrophy Training on Maximal Strength, Muscle Thickness and Flexibility in the Plantar Flexors.
published in **Eur J Appl Physiol 2023**

The research idea was conducted by Klaus Wirth and me. In the study I performed the data collection with the assistance of two of my Co-Authors (Lars Lohmann, Tim Wohlann). The data analysis was performed exclusively by me. Afterwards, I discussed the results and the manuscript with my co-authors and included their feedback to the study. As the corresponding author, I submitted the manuscript to the Journal and revised the manuscript based on the reviewer's comments.

Study 6
Warneke, K., Keiner, M., Wohlann, T., Lohmann, L.H., Schmitt, T., Hillebrecht, M., Brinkmann, A., Hein, A., Wirth, K., Schiemann, S. (2023). Influence of Long-Lasting Static Stretching Intervention on Functional and Morphological Paramters in the Plantar Flexors: A Randomised Controlled Trial.
published in **J Strength Cond Res 2023**

In the study I performed the data collection with the assistance of two of my Co-Authors (Lars Lohmann, Tim Wohlann) to ensure a time efficient data collection, as the data collection was performed in collaboration with the Imaging Unit of the Carl von Ossietzky University Oldenburg. Anna Brinkmann was involved in the investigation of ultrasound imaging, while Andreas Hein collaborated with the MRI measurement. The MRI measurements were supervised by Tina Schmitt and mainly performed by Gülsen Yanc. The data analysis was performed exclusively by me. Afterwards, I discussed the results and the manuscript with my co-authors and included their feedback to the study. As the corresponding author, I submitted the manuscript to the Journal and revised the manuscript based on the reviewer's comments.

Table 11: Status of the Included Studies

Nr	Title	Status
1	Long-Lasting Stretching Training Produces Muscle Hypertrophy – A Meta-Analysis of Animal Studies	published
2	Improvements in Flexibility Depend on Stretching Duration	published
3	Influence of Long-Lasting Static Stretching on Maximal Strength, Muscle Thickness and Flexibility	published
4	Influence of One Hour versus Two Hours of Daily Static-Stretching for six Weeks Using a Calf-Muscle-Stretching Orthosis on Maximal Strength	published
5	Comparison of the Effects of Long-Lasting Static Stretching and Hypertrophy Training on Maximal Strength, Muscle Thickness and Flexibility in the Plantar Flexors	published
6	Influence of Long-Lasting Static Stretching Intervention on Functional and Morphological Paramters in the Plantar Flexors: A Randomised Controlled Trial	published

12 Acknowledgement

At this point, I want to thank Prof. Dr. Stephan Schiemann for the supervision of my PhD project. He gave significant input in all regards to my work and helped with all available resources on 7 days per week to every time of the day, even on holidays, which enabled me to conduct many studies and write the papers. Secondly, I want to thank Prof. Dr. Astrid Zech and Prof. Dr. Andreas Konrad for supporting my research, discussing further research possibilities during the last year and acting as reviewers for this PhD thesis paper. Thirdly, I am grateful to PD Dr. Martin Hillebrecht, who also put in much work in figuring out the topic of the project, provided the measurement devices and corresponding software and also spent many hours discussing further research questions in his free time, and furthermore, Prof. Dr. Klaus Wirth, who awaked my enthusiasm for research in exercise science and sports medicine with the focus on strength training as early as in the Bachelor studies, discussed the research questions and provided (critical) feedback to the manuscript drafts of my work.

Prof. Dr. Michael Keiner acted as my mentor in the mentoring program of the "German Association of Sport Science". He also gave his input to included studies as well as to research we performed in addition to the PhD project and therefore, he was a big support in discussing research and statistics and expanding my list of research.

In the final stage of this project, I had (with significant help of Prof. Dr. Stephan Schiemann and PD Dr. Martin Hillebrecht) the great privilege to get another scholarship to stay abroad and work under the supervision of Prof Dr. David G. Behm at the Memorial University of Newfoundland. Therefore, at this place, I want to thank Prof. Behm for this possibility and the great experiences I was able to enjoy in academic settings, but also for the activities he let me be part of in the free time, leading to an unforgettable stay in St. John's. Furthermore, many thanks for reviewing this thesis paper as a native speaker and expert in the topic.

Apart from those, I experienced much support from different other people. Consequently, I want to thank my great parents for their tireless support and in all cases, independent on developing the orthosis, helping with graphical design or even with organizational stuff. Furthermore, I got a huge amount of support by my good friend Lars H. Lohmann, who assisted me in study conduction, in the development of the orthosis as well as in proofreading of my studies.

Furthermore, I want to thank Bert Lange, Paul Hagedorn and Friedrich Jahns from RAS GmbH for their knowledge and help in the development of the orthosis from the prototype to the current state. Without the production and providing of the used orthosis (for free, only guided by their interest) in collaboration with the Novecor GmbH, it would not have been possible to conduct this work with high quality products.

For measuring the muscle cross-sectional area, many MRI measurements were performed in the University of Oldenburg. Therefore, I want to thank Gülsen Yanc for her work, even in the evening to enable this amount of measurements, and Dr. Tina Schmitt for collaborating in this study.

Last, I want to thank all other friends and colleagues who accompanied me on my way, such as my girlfriend Maja Hinrichs who needed a huge amount of tolerance for my moods while helping me with my work, but also Prof. Dr. Dr. Tobias Schmidt, Dr. Masatoshi Nakamura, Dr. Jonas Warneke for proofreading this work.

Again, I thank all listed persons for their motivation and help.

13 Original Studies Included in this Work

Journal of Science in Sport and Exercise
https://doi.org/10.1007/s42978-022-00191-z

REVIEW ARTICLE

Long-Lasting Stretching Induces Muscle Hypertrophy: A Meta-Analysis of Animal Studies

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Received: 17 February 2022 / Accepted: 7 August 2022

Abstract
Muscular hypertrophy depends on metabolic exhaustion as well as mechanical load on the muscle. Mechanical tension seems to be the crucial factor to stimulate protein synthesis. The present meta-analysis was conducted to determine whether stretching can generate adequate mechanical tension to induce muscle hypertrophy. We used PubMed, Web of Science, and Scopus to search for literature examining the effects of long-term stretching on muscle mass, muscle cross-sectional area, fiber cross-sectional area, and fiber number. Since there was no sufficient number of studies investigating long-lasting stretching in humans, we only included original animal studies in the current meta-analysis. Precisely, we identified 16 studies meeting the inclusion criteria (e. g. stretching of at least 15 min per day). The 16 studies yielded 39 data points for muscle mass, 11 data points for muscle cross-sectional area, 20 data points for fiber cross-sectional area, and 10 data points for fiber number. Across all designs and categories, statistically significant increases were found for muscle mass ($d = 8.51$; 95% CI 7.11–9.91), muscle cross-sectional area ($d = 7.91$; 95% CI 5.75–10.08), fiber cross-sectional area ($d = 5.81$; 95% CI 4.32–7.31), and fiber number ($d = 4.62$; 95% CI 2.54–6.71). The findings show an (almost) continuous positive effect of long-term stretching on the listed parameters, so that it can be assumed that stretch training with adequate intensity and duration leads to hypertrophy and hyperplasia, at least in animal studies. A general transferability to humans—certainly with limited effectiveness—can be hypothesized but requires further research and training studies.

Keywords Muscle mass · Static stretching · Hyperplasia · Chronic stretching

Introduction

To achieve muscular hypertrophy, strength training needs—in addition to metabolic exhaustion—a high mechanical load on the muscle, which leads to micro-traumatization of the muscle fibers [63]. In this regard, the crucial factor is high mechanical tension on the muscle. Resulting hypertrophy effects depend on an increased (myofibrillar) protein synthesis rate, which is stimulated via corresponding signaling pathways. In particular, activation of the Akt/mTOR/p70S6K signaling pathway appears to be of high importance for the stimulation of muscular protein synthesis and is primarily induced by mechanical loading [1, 17, 46]. A corresponding mechanical stimulus can be initiated not only by high loads in strength training, but also through stretching with appropriate intensity. Smith et al. [55] demonstrated that mechanical stress generated by stretching can be sufficient to induce delayed onset muscle soreness (DOMS) [55]. Accordingly, it can be assumed that stretching stimuli can cause adequate micro-traumatization. The resulting repair processes can trigger hypertrophy-stimulating signaling pathways to increase protein synthesis rates [29]. The resulting activation of stretch-activated channels alters the cytoplasmic membrane and initiates signal transduction processes via mTOR [59, 61].

Against this background, the following hypothesis can be derived: stretch training performed with sufficient intensity leads to high mechanical load that can trigger muscular hypertrophy as a long-term training effect. This hypothesis has already been discussed previously: “It is well known that application of chronic stretch is a very potent model for inducing muscle enlargement” [36]. However, to date,

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Published online: 21 October 2022

studies examining adaptations of stretch training have generally focused either on increasing range of motion (ROM), or on other parameters describing flexibility [38, 40]. Moreover, acute effects of stretching interventions on muscular performance mostly show negative effects regarding maximum strength and explosive power [13, 71].

Initial human studies show that long-term stretching interventions for several weeks can induce hypertrophic effects and/or increase maximum strength. For example, Simpson et al. [53] were able to achieve an average increase of 5.6% in muscle cross-sectional area through a stretching intervention with a duration of three minutes, three days per week, for 6 weeks. Panidi et al. [44] found an increase in muscle cross sectional area (MCSA) of 23% ± 14% after a 12-week stretching intervention with stretching durations up to 15 min per training session. Nelson et al. [43] demonstrated a 29% increase in maximal strength after stretching the calf muscles for 4 × 30 s, 3 days a week for 10 weeks. In addition, Kokkonen et al. [34] achieved significant improvements in various performance tests, such as 1 RM knee extension and knee flexion, standing long jump, and high jump, with static stretching for 40 min per session, 3 days per week for 10 weeks.

Longitudinal studies using animal experiments have been available for some time and have demonstrated significant hypertrophy effects after continuous stretching from 30 min to 24 h per day over an intervention period of several weeks, reflected by an increase in muscle mass (MM), MCSA, fiber cross sectional area (FCSA) and/or hyperplasia effects with an increased fiber number (FN) [8, 10, 15, 23, 25]. Data of muscle weight were collected by removing the connective tissue and weighing the wet muscle weight. MCSA and FN were investigated by placing the muscle in a solution in which the different muscle fibers were stained in different colors (fast twitch fiber stained lightly, slow twitch fibers stained darkly). Subsequently, the muscle cross-section and fiber cross-sectional area were determined from a given number of fibers (for example 500 slow twitch and 200 fast twitch fibers in Antonio et al. [10] using light micrography images and an image analysis computer program). In addition, in vitro condition a significant increase in maximum strength was demonstrated by continuous stretching, so that these hypertrophy effects are functional in animals [3, 4]. The muscle fiber type was determined by ATPase-activity using an ATPase staining method and fiber number was investigated by counting fibers running from origin to insertion [10]

Since animal studies play a vital role in research to investigate human health, and systematic reviews or meta-analyses provide a suitable basis for drawing evidence-based conclusions concerning a research topic, we decided to create a transparent overview of the available information on effects of long-lasting stretching intervention on muscle tissue, especially to check if the applicability of the training method appears worthwhile for human studies [31]. There is one meta-analysis available from Kelley [33] that has addressed this issue before. In Kelley's meta-analysis, however, the muscular overload was not generated exclusively by stretching but also by other methods (weight training, ablation), so that no conclusion could be drawn about the specific effects of long-term stretching. Moreover, comprehensive analysis on distinct outcomes such as MM, MCSA, FCSA and FN are not available in the study by Kelley [33]. Consequently, a distinct base of empirical evidence needs to be researched to investigate the questions of the present meta-analysis. In particular, the present meta-analysis of animal studies aims to provide a comprehensive and differentiated overview of the effects of (continuous) stretching interventions on MM, MCSA and FCSA, and on hyperplasia effects (FN). Subsequently, the relevance of these results with regard to the potential use of stretching training with the goal of muscle and strength building in athletic and therapeutic training will be discussed.

Methods

The following search terms were defined to search PubMed, Web of Science, and Scopus databases: [("hypertrophy" OR "hyperplasia") AND ("stretch-induced growth" OR "stretch-induced hypertrophy" OR "fiber number" OR "fiber length" OR "sarcomere length" OR "sarcomere number") AND "skeletal muscle") NOT ("exercise induced" OR "endocrine" OR "nervous system" OR "electrical stimulation" OR "cardiomyocytes"]. The search strategy was limited to English language sources only.

A total of 89 publications were found from this combination of terms. The references found in these publications were examined for further relevant studies. However, this did not yield any additional studies. After reviewing the titles, 47 studies remained, which were then screened to exclude studies that only indirectly investigated structural adaptations and those studies that focused more on hormonal adaptations, muscle fiber distribution, or signal transduction pathways without collecting the target parameters of muscle mass, muscle cross-sectional area, fiber cross-sectional area, fiber length, or fiber number. After this step, 23 studies remained, which were then subjected to full-text analysis using inclusion and exclusion criteria established in advance of the meta-analysis for the final selection.

The following parameters were defined as inclusion criteria:

- Objective measurement of muscle mass and/or muscle cross-sectional area and/or fiber count and/or fiber

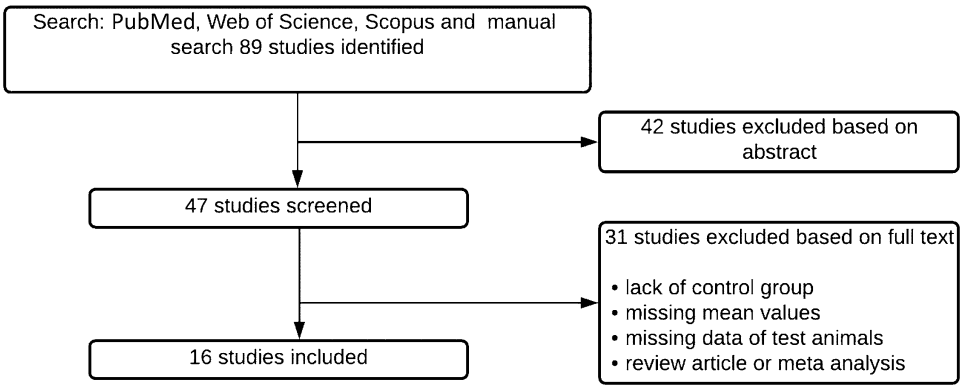


Fig. 1 Searching method via PRISMA method

cross-sectional area and/or fiber length and/or number of muscle fibers.

- Stretching interventions of at least one week.
- Stretching times of at least 15 min per day.
- Specification of mean values and standard deviations.
- Studies on animals.

Accordingly, the following were considered exclusion criteria:

- No measurement of muscle mass and/or muscle cross-section and/or fiber number and/or fiber cross-section and/or fiber length and/or number of muscle fibers.
- Missing or insufficient information on the duration of the intervention and on the stretching times.
- Missing data concerning mean values and standard deviations, absence of absolute values.
- Missing data of number of test animals.
- Missing control group/control condition.

The final sample in the meta-analysis included 16 studies, whereby some studies with multiple effect sizes were included in the analysis because they either included different variables (e.g. muscle mass, fiber cross-section and/or hyperplasia effects) or because they described the effects of different intervention periods (a few days to several months). Figure 1 illustrates the procedure for study selection and Table 1 details the included studies.

Quality Assessment

The quality assessment was based on the Delphi list [62]. The Delphi method was chosen as a reliable and valid tool for the assessment of the quality of the included studies [54]. The assessment items for the current meta-analyses can be found in Table 2. The evaluation was performed by two independent raters. If question 2 received an affirmative answer, it was assumed that the age of the test animals, the species or breed of the animal as well as the initial weight were given. In all studies listed, mean values and standard deviations were given (see inclusion criteria) and the objective of the study was clearly stated. In none of the studies was information provided on blinding of the "care provider" and "outcome provider." Only Czerwinski et al.[23] provided information on randomization.

Meta-analytic Procedure

Using the meta-analysis software RevMan, version 5.4.1 [22], 5 separate analyses were performed for the following parameters: muscle mass, muscle cross-sectional area, muscle fiber cross-sectional area, muscle fiber length, and number of muscle fibers. The following parameters from each of the studies were included in the analysis: number of experimental animals, and the respective mean values and standard deviations of the experimental and control conditions. Since several studies involved different durations, the studies were listed in alphabetical order with a lowercase letter to allow assignment of the elongation period to the respective representation in the forest plot. We used a random effects model to take into account any heterogeneity resulting from the use of different species in the studies and all other potential between-study differences (study characteristics are summarized in Table 1).[1]

Tables 3–7 report the empirical M, SD and N for the parameters muscle mass, muscle cross sectional area, fiber cross sectional area, muscle fiber number, and fiber length. For all analyses, the standardized mean difference (with inverse variance weighting) and its 95% confidence interval were computed as the effect size of interest in RevMan.[2] Since for the evaluation of MM, MCSA, FCSA, FN and FL in laboratory studies, animals had to be dissected and flight muscles (ALD, PAT) had to be removed, no pre-post comparison of the same subjects could be performed. Therefore,

[1] In addition, we provide funnel plots for each outcome parameter as supplemental material to illustrate potential publication bias.

[2] Using the formulae. $SMD_i = \frac{m_{1i}-m_{2i}}{s_i}\left(1-\frac{3}{4N_i-9}\right)$ and $SE\{SMD_i\} = \sqrt{\frac{N_i}{n_{1i}n_{2i}}+\frac{SMD_i^2}{2(N_i-3.94)}}$.

Table 1 Description of included studies

Source	Subjects	Muscle Group	Intervention	Measured Parameters
Alway et al. [7]	$N=63$	ALD	7-day stretching intervention, nine animals examined every day	MM: $+64\% \pm 8.4\%$ MCSA: $+29.9\% \pm 12.3\%$ FL: $+40.2\% \pm 2.2\%$ FN: $+27.3\% \pm 3\%$
Alway [2]	$N=36$, 22 in intervention group	ALD	30-day stretching with 12% of bodyweight	MM: $+161.5\% \pm 7.9\%$ FL: $+25.4\% \pm 4.6\%$
Alway [3]	$N=24$, 12 young (Y) and 12 old (O) quails	ALD	30 days of unilateral stretching with 12% bodyweight	MM: YCG:26.7 ± 1.2 mg; YIG:71.6 ± 3.0 mg OCG:28.5 ± 1.5 mg; OIG: 67.4 ± 4.4 mg maximal strength: YCG:58.3 ± 2.8 mN; YIG 115.4 ± 5.9 mN OCG:57.4 ± 3.1 mN; OIG:112.1 ± 6.1 mN
Alway [4]	$N=18$, 12 in intervention group, 6 in control group	ALD	Unilateral stretching with 12% of body weight	MM: $+162.5\% \pm 3.4\%$ FN: $+48.4\% \pm 3.2\%$ relative maximal strength 23.6 ± 0.9 mN vs. 18.9 ± 0.6 mN absolute maximal strength 95% increase vs. control muscle
Antonio et al. [10]	$N=26$	ALD	Intermittent stretching protocol with progressive weight increase, followed by continuous stretching at 35% of own body weight Intervention period 37 days	Maximal values MM: $+318\% \pm 31.5\%$ FN: $+82.2\% \pm 17.1\%$ MCSA: $+141.6\% \pm 32.5\%$
Antonio and Gonyea [8]	$N=7$	ALD	Stretching with 10% of bodyweight; intermittent stretching protocol	MM: $+53.1\% \pm 9\%$ FCSA: $+27.8\% \pm 6\%$ FL: $+26.1\% \pm 7.3\%$
Antonio and Gonyea [9]	$N=18$	ALD	28-day stretching intervention with 29% of bodyweight, animals examined after 16 days and 28 days	MM: day16: $+188.1\% \pm 15.6\%$; day28: $+294.3\% \pm 39.1\%$ FL: day16: $+80.4\% \pm 11.8\%$. day28: $+74.6\% \pm 9.7\%$ FN: day16: $-6.7\% \pm 4.6\%$; day28: $+29.7\% \pm 6.8\%$
Barnett et al. [14]	$N=63$	PAT, biceps brachii	Unilateral stretching for up to 10 days, animals examined after 1, 2, 3, 7 and 10 days	MM: PAT: CG: 0.1474 ± 0.0142 g IG: 0.2461 ± 0.0239 g Biceps brachii: CG: 0.5914 ± 0.0607 IG:0.7644 ± 0.0646
Brown et al. [18]	$N=40$	PAT	16-day stretching intervention, animals were examined after 6 days and 16 days	Muscle mass increased for 61% in 6-week-old chicken and 34% in l0-month-old chicken. 28-month-old animals'had an 18% loss of muscle mass during passive stretch

Table 1 (continued)

Source	Subjects	Muscle Group	Intervention	Measured Parameters
Carson et al. [20]	$N=94$, YA and OA	ALD	30-day stretching intervention with 10% of bodyweight, animals were examined after 7, 14 and 30 days in both ages	MM: YA: 7d: 94.1% ± 7.4%; 14d: 134.7% ± 5.8% OA: 7d: 82.1% ± 4.9%; 14d:102.4% ± 6.5% FL: YA: 7d: 37.7% ± 2.0%; 14d: 28.9% ± 4.0% OA: 7d: 39.8% ± 4.1%; 14d: 21.3% ± 5.3% FN: YA: 14d: 31.6% ± 2.1%; OA:14d: 19.2% ± 2.2% FCSA: YA:14d: 51.6% ± 7%; OA:14d: 39.6% ± 8.5%
Carson et al. [20]	$N=32$, YA $n=16$ vs. OA $n=16$	ALD	Unilateral stretching with 10% of bodyweight, contralateral muscle was control muscle	MM: YA: + 178.7% ± 7.1% OA: + 142.8% ± 7.9% FN: YA: IG: 22.5 ± 0.4 vs. CG: 18.5 ± 0.4 OA: IG: 22.8 ± 1.2 vs. CG:18.4 ± 0.9 MCSA: YA: + 63.8% ± 7.8%; OA: + 49.1% ± 5.4% FN: YA: + 59.6% ± 8%; OA: + 47.2% ± 8.1%
Carson and Alway [19]	$N=30$, YA $n=15$ vs. OA $n=15$	ALD	Unilateral stretching for 7 and 14 days	MM: YA;7d: + 98.7% ± 12% YA;14d: + 141.4% ± 9.5% OA;7d: + 83.9% ± 6.6% OA;14d: + 106.9% ± 11%
Czerwinski et al. [23]	$N=57$, chicken	PAT	11-day intervention, stretched muscle vs. control muscle, banded stretch for one wing	MM: CG: 1.3 ± 0.07 g vs. IG: 1.88 ± 0.09 g
Frankeny et al. [25]	$N=54$	PAT	6 week stretching intervention with several stretching protocols, 8, 4, 2 + 2, 2, 1, 0.5 und 0.25 + 0.25 h of intermittent stretching and 24 h of permanent stretching	MM: 24 h: + 121% MCSA: up to + 111% FCSA: up to + 110%
Matthews et al. [37]	$N=10$	PAT	33-day stretching intervention with 10% of bodyweight	MM: + 247% ± 91% FCSA: IG: 985 ± 291 μm^2 CG: 520 ± 96 μm^2
Roman and Alway 1995 [47]	$N=28$	ALD	21 days stretching intervention, animals examined after 7, 14 and 21 days	MM: 7 days: CG:37.2 ± 1.8 mg IG: 54.6 ± 2.9 mg 14 days: CG:43.5 ± 2.7 mg IG:67.8 ± 4.3 mg 21 days: CG:42.6 ± 3.2 mg IG:71.2 ± 3.7 mg
Sparrow [56]	$N=60$	ALD	30-day stretching intervention, 30 animals examined after 3, 7, 13 and 29 days, remaining animals examined after 5, 13, 25 and 35 days after stretching without intervention to investigate regression	MM: CG:0.928 ± 0.026 g; IG:1.850 ± 0.07 g

ALD anterior latissimus dorsi muscle, *PAT* patagialis muscle, *MM* muscle mass, *MCSA* muscle cross-sectional area, *FCSA* fiber cross sectional area, *FL* fiber length, *FN* fiber number, *YA* young animals, *OA* old animals, *YCG* young control group, *YIG* young intervention group, *OCG* old control group, *OIG* old intervention group

Table 2 Quality Assessment using the Delphi List

Study	Randomization?	Treatment Allocation Concealed?	Groups were Similar at Baseline?	Eligibility Criteria Specified?	Blinding of Outcome Assessor?	Blinding of Care Provider?	Blinding of Patient?	Point Estimates and Measures of Variability Presented?	Intention to Treat Analysis Included?
Czerwinski et al. [23]	Y		Y	Y	DN	DN		Y	Y
Alway et al. [7]	DN		Y	Y	DN	DN		Y	Y
Alway et al. [2]	DN		Y	Y	DN	DN		Y	Y
Alway [3]	DN		DN	Y	DN	DN		Y	Y
Alway [4]	DN		Y	Y	DN	DN		Y	Y
Antonio et al. [10]	DN		Y	Y	DN	DN		Y	Y
Antonio and Gonyea [8]	DN		Y	Y	DN	DN		Y	Y
Antonio and Gonyea [9]	DN		DN	Y	DN	DN		Y	Y
Barnett et al. [14]	DN		DN	Y	DN	DN		Y	Y
Brown et al. [18]	DN		Y	Y	Dn	DN		Y	Y
Carson et al. [20]	DN		Y	Y	DN	DN		Y	Y
Carson et al. [20]	DN		Y	Y	DN	DN		Y	Y
Carson and Alway [19]	DN		Y	Y	DN	DN		Y	Y
Frankeny et al. [25]	DN		Y	Y	DN	DN		Y	Y
Matthews et al. [37]	DN		Y	Y	DN	DN		Y	Y
Roman and Alway [47]	DN		DN	Y	DN	DN		Y	Y
Sparrow [56]	DN		Y	Y	DN	DN		Y	Y

For "treatment allocation concealed?" and "blinding of patient" an assessment was not possible

the SMD was calculated for the comparison of the post-treatment experimental and a respective control group.

Results

Muscle Mass

The included studies show that in animal experiments a significant increase in muscle mass can be achieved by stretching intervention over several weeks. The effect size across all studies was $d = 8.51$, $P < 0.001$, 95% CI 7.11–9.91. Stretching was performed with varying durations per day (minimum 2×15 min) up to 24 h stretching over up to 6 weeks [25]. There were positive effects found on muscle mass in most studies, expect for one intervention performed by Brown et al. [18], see Table 3. The highest increases in muscle mass in the listed studies were obtained by Antonio and Gonyea [8] with a 37-day stretching intervention and an increase of $318\% \pm 39.1\%$ and $d = 7.01$, 95% CI 3.77–10.24. Other high percentage increases were obtained by Antonio and Gonyea [9] with an increase of $294.3\% \pm 39.1\%$ with $d = 11.96$, 95% CI 7.27–16.66 in muscle mass, Alway [2] with an increase of $161.5\% \pm 7.9\%$ with $d = 6.64$, 95% CI 5.43–7.85, and Carson et al. [20, 21] with $178.7\% \pm 7.1\%$ $d = 20.82$, 95% CI 15.44–26.32.

Table 3 Forest plot for muscle mass

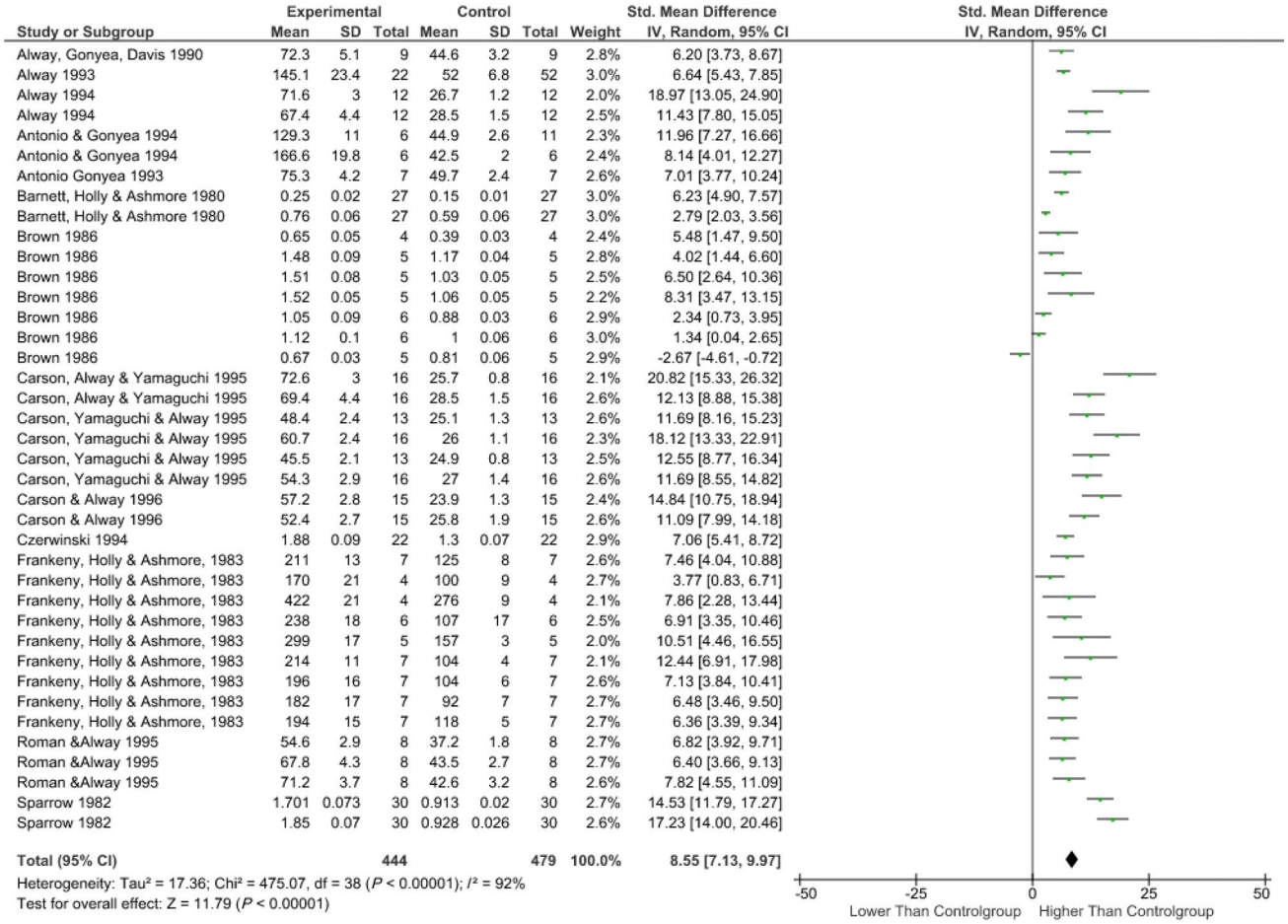
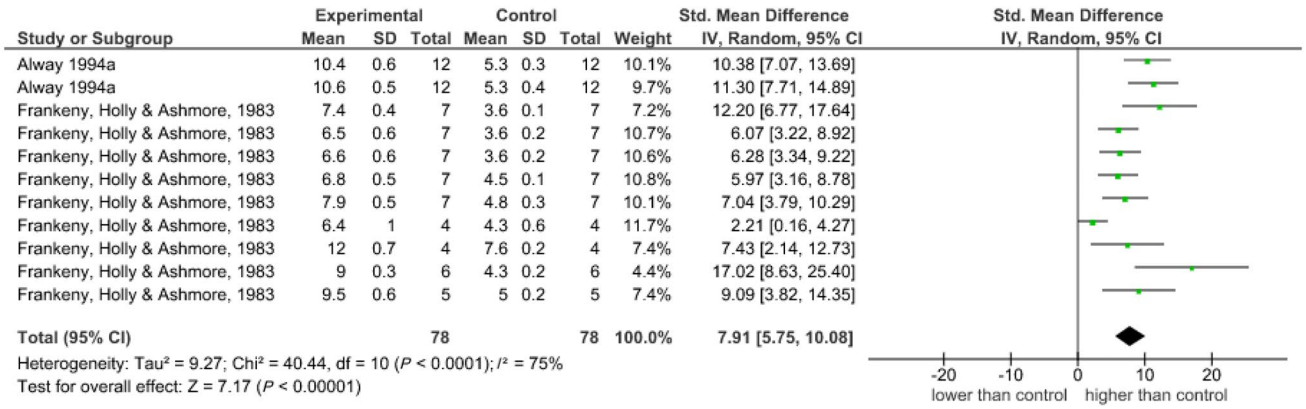


Table 4 Forest plot for muscle cross-sectional area



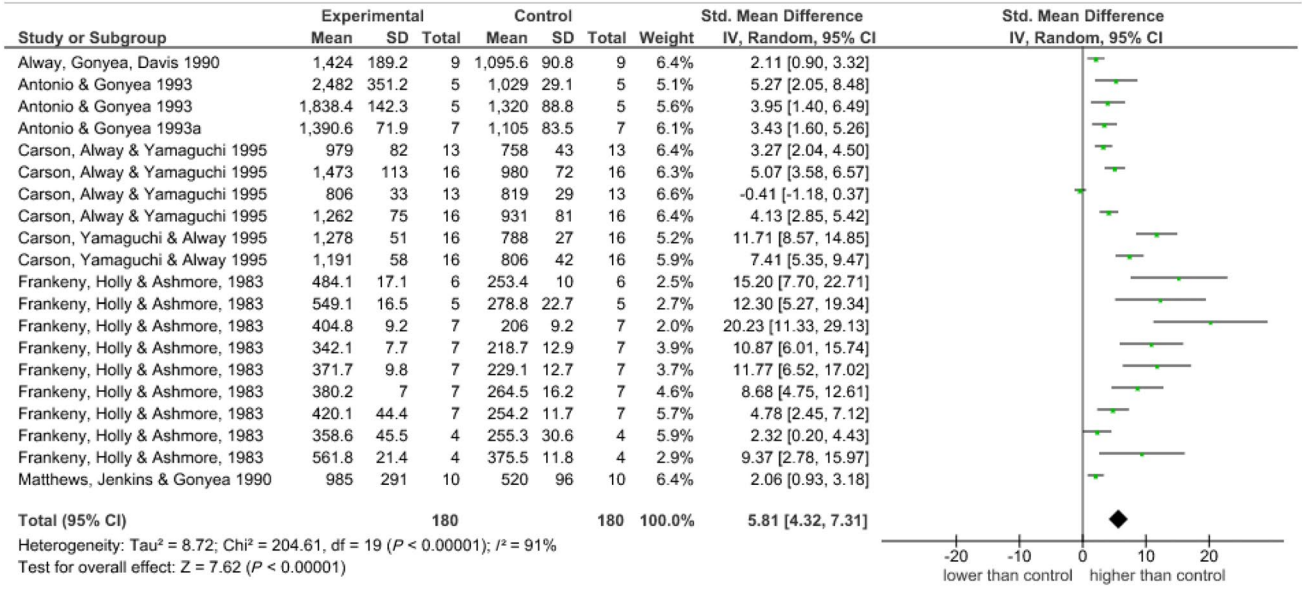
Muscle Cross-Sectional Area

Changes in muscle cross-sectional were all positive. Here, an effect strength of $d = 7.91$, $P < 0.001$, 95% CI 5.75–10.08 was recorded. Frankeny et al. [25] measured an increase in muscle cross-section of 111% compared to the control muscle. Alway [3] also recorded muscle cross-sectional increases of 100% (see Table 4).

Fiber Cross-Sectional Area

For the effects on fiber hypertrophy, an increase due to the stretching intervention was also determined (almost)

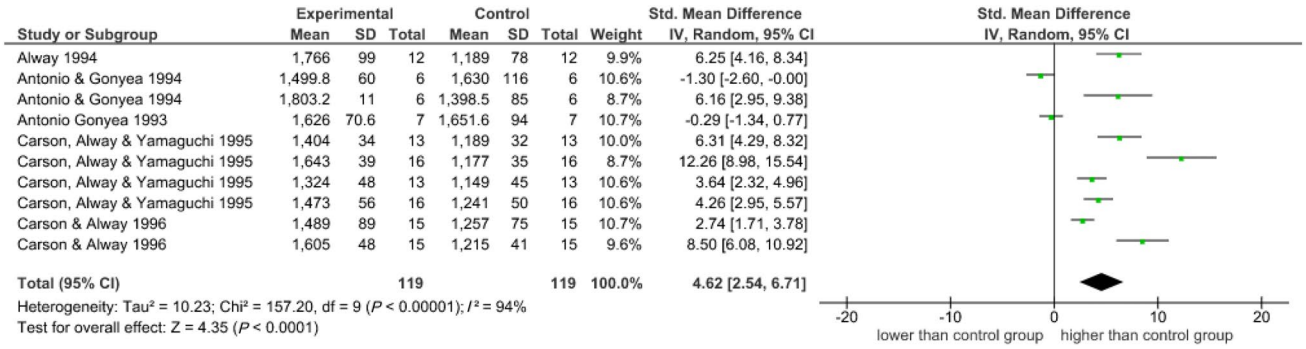
Table 5 Forest plot for fiber cross-sectional area



Study or Subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
Alway, Gonyea, Davis 1990	1,424	189.2	9	1,095.6	90.8	9	6.4%	2.11 [0.90, 3.32]
Antonio & Gonyea 1993	2,482	351.2	5	1,029	29.1	5	5.1%	5.27 [2.05, 8.48]
Antonio & Gonyea 1993	1,838.4	142.3	5	1,320	88.8	5	5.6%	3.95 [1.40, 6.49]
Antonio & Gonyea 1993a	1,390.6	71.9	7	1,105	83.5	7	6.1%	3.43 [1.60, 5.26]
Carson, Alway & Yamaguchi 1995	979	82	13	758	43	13	6.4%	3.27 [2.04, 4.50]
Carson, Alway & Yamaguchi 1995	1,473	113	16	980	72	16	6.3%	5.07 [3.58, 6.57]
Carson, Alway & Yamaguchi 1995	806	33	13	819	29	13	6.6%	-0.41 [-1.18, 0.37]
Carson, Alway & Yamaguchi 1995	1,262	75	16	931	81	16	6.4%	4.13 [2.85, 5.42]
Carson, Yamaguchi & Alway 1995	1,278	51	16	788	27	16	5.2%	11.71 [8.57, 14.85]
Carson, Yamaguchi & Alway 1995	1,191	58	16	806	42	16	5.9%	7.41 [5.35, 9.47]
Frankeny, Holly & Ashmore, 1983	484.1	17.1	6	253.4	10	6	2.5%	15.20 [7.70, 22.71]
Frankeny, Holly & Ashmore, 1983	549.1	16.5	5	278.8	22.7	5	2.7%	12.30 [5.27, 19.34]
Frankeny, Holly & Ashmore, 1983	404.8	9.2	7	206	9.2	7	2.0%	20.23 [11.33, 29.13]
Frankeny, Holly & Ashmore, 1983	342.1	7.7	7	218.7	12.9	7	3.9%	10.87 [6.01, 15.74]
Frankeny, Holly & Ashmore, 1983	371.7	9.8	7	229.1	12.7	7	3.7%	11.77 [6.52, 17.02]
Frankeny, Holly & Ashmore, 1983	380.2	7	7	264.5	16.2	7	4.6%	8.68 [4.75, 12.61]
Frankeny, Holly & Ashmore, 1983	420.1	44.4	7	254.2	11.7	7	5.7%	4.78 [2.45, 7.12]
Frankeny, Holly & Ashmore, 1983	358.6	45.5	4	255.3	30.6	4	5.9%	2.32 [0.20, 4.43]
Frankeny, Holly & Ashmore, 1983	561.8	21.4	4	375.5	11.8	4	2.9%	9.37 [2.78, 15.97]
Matthews, Jenkins & Gonyea 1990	985	291	10	520	96	10	6.4%	2.06 [0.93, 3.18]
Total (95% CI)			**180**			**180**	**100.0%**	**5.81 [4.32, 7.31]**

Heterogeneity: Tau² = 8.72; Chi² = 204.61, df = 19 ($P < 0.00001$); $I^2 = 91\%$
Test for overall effect: Z = 7.62 ($P < 0.00001$)

Table 6 Forest plot for fiber number



Study or Subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
Alway 1994	1,766	99	12	1,189	78	12	9.9%	6.25 [4.16, 8.34]
Antonio & Gonyea 1994	1,499.8	60	6	1,630	116	6	10.6%	-1.30 [-2.60, -0.00]
Antonio & Gonyea 1994	1,803.2	11	6	1,398.5	85	6	8.7%	6.16 [2.95, 9.38]
Antonio Gonyea 1993	1,626	70.6	7	1,651.6	94	7	10.7%	-0.29 [-1.34, 0.77]
Carson, Alway & Yamaguchi 1995	1,404	34	13	1,189	32	13	10.0%	6.31 [4.29, 8.32]
Carson, Alway & Yamaguchi 1995	1,643	39	16	1,177	35	16	8.7%	12.26 [8.98, 15.54]
Carson, Alway & Yamaguchi 1995	1,324	48	13	1,149	45	13	10.6%	3.64 [2.32, 4.96]
Carson, Alway & Yamaguchi 1995	1,473	56	16	1,241	50	16	10.6%	4.26 [2.95, 5.57]
Carson & Alway 1996	1,489	89	15	1,257	75	15	10.7%	2.74 [1.71, 3.78]
Carson & Alway 1996	1,605	48	15	1,215	41	15	9.6%	8.50 [6.08, 10.92]
Total (95% CI)			**119**			**119**	**100.0%**	**4.62 [2.54, 6.71]**

Heterogeneity: Tau² = 10.23; Chi² = 157.20, df = 9 ($P < 0.00001$); $I^2 = 94\%$
Test for overall effect: Z = 4.35 ($P < 0.0001$)

consistently. The effect size here was $d = 5.81$, $P < 0.001$, 95% CI 4.32–7.31. The changes in fiber cross-section ranged from − 0.75% to 141.6% (± 32.6%), with these two values being more of an outlier, as all other results ranged from + 27.8% to + 63.8% (see Table 5).

Fiber Number (Hyperplasia)

With regard to the number of fibers, the studies also show significant increases as an adaptation to permanent stretching. Here, the calculated effect size across the studies is $d = 4.62$, $P < 0.001$, 2.54–6.71. In two studies, a decrease in the number of fibers − 0.7% ± 3.6% with $d = -0.29$, 95% CI − 1.34–0.77 in Antonio and Gonyea [8] and − 6.7% ± 4.6% with $d = -1.3$, 95% CI − 2.6–0.0 in Antonio and Gonyea [9] was initially determined after a certain intervention period, which, however, was no longer present at a later test in the same study, so that an increase in the number of fibers was also recorded in this study (see Table 6).

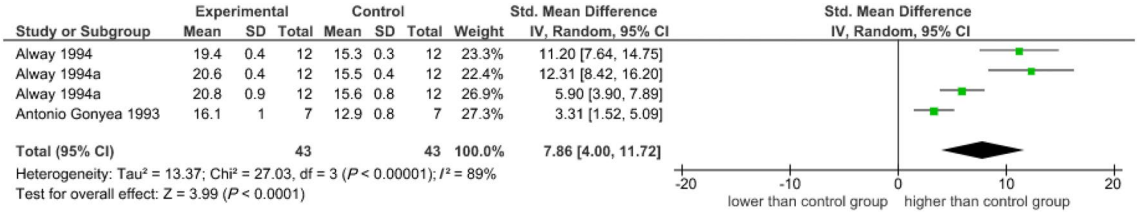
Fiber Length

The fiber length was only taken into account in three studies. The effect size determined was $d = 7.86$, $P < 0.001$, 95% CI 4.00–11.72. Here, percentage increases were 26.1% ± 7.3% ($d = 3.31$, 95% CI 1.52–5.09 [8]. Studies by Alway [3] determined muscle length changes of approx. 25% compared to the control muscle due to the stretching intervention (see Table 7).

Discussion

Based on the studies and the effect sizes determined in this meta-analysis, it can be assumed that (continuous) stretching (from 30 min to 24 h per day in a longitudinal section

Table 7 Forest plot for fiber length



over several days to weeks) induces muscular tension in animal muscles, which leads to the following morphological adaptations of the stretched muscles: an increase in muscle mass, muscle cross-section, fiber cross-section, fiber length, and/or number of muscle fibers. This is confirmed by the results of other studies whose experimental investigations were similar to the analyzed studies, but which could not be included in the statistical analyses due to exclusion criteria or missing information in the method description [6, 12, 15, 35, 69].

Several studies show that there seems to be a correlation between stretching time and stretching intensity with achieved muscle mass increase [15, 25, 35], assuming an upper limit or optimum of stretching duration. In studies by Frankeny et al. [25] and Bates [15], although further increases due to an increase in stretching duration can be detected, the stretching optimum (effort relative to return) seems to be 30 min: "We conclude that daily stretching for as little as 30 min per day is a powerful inducer of growth in normal and dystrophic muscle" [25]. Antonio et al. [10] achieved maximal muscle mass gains of 318% with a progressively increased stretching load and an intermittent stretching protocol. The increases in muscle mass is consistent in almost all studies listed in this meta-analysis except for one measured parameter by Brown et al. [18] due to stretching the PAT for 16 days in old female chicken (28 month old).

The muscle mass gains are attributed by most authors to muscle fiber hypertrophy and muscle fiber hyperplasia. For muscle hyperplasia, uninterrupted continuous stretching seems to be the initiating stimulus, since the muscle fiber is not given sufficient time to regenerate. This stimulates increased satellite cell activation, which leads to the formation of new muscle fibers [8]. Another explanation is that reaching a critical muscle fiber size by hypertrophy effects leads to the splicing of the muscle fiber into several muscle fibers. This could be responsible for hyperplasia [8, 10].

Hypertrophy

Induced tension or mechanical stress on the individual sarcomeres are thought to be responsible for the hypertrophy effects achieved by stretching, such that the mechanical stimulus on the muscle is the adaptation-inducing stressor and thus the crucial stimulus for muscle mass gains [49, 67]. The muscle responds to this stimulus by increasing its serial sarcomere number [66] and the accumulation of myofibrils triggers an increase in cross-sectional area [4, 8, 20, 25]. The increase in muscle mass due to long duration stretching interventions has been clearly demonstrated in animal studies. Various studies with animals have also demonstrated an increased rate of protein synthesis by stretching [16, 28, 29]. Whether and to what extent the results of this study are transferable to humans have not yet been adequately investigated. Several of the studies integrated in this meta-analysis specifically request this step [15, 29]. Critically, protein synthesis differs between humans and animals. Garibotto et al. [27] and Tessari et al. [58] list protein synthesis rates of 2% and 1.5%, respectively, for leg muscles. Early experiments made by Williams and Goldspink indicate 2–3 days for length adaptation of muscle in mice, but 2–3 weeks in cats and humans [67]. For the species primarily studied in this meta-analysis (chickens/quail), Sayegh and Lajtha [50] indicate a lower protein synthesis rate compared to mice. However, the protein synthesis rate is dependent on the species, but also on other factors such as gender or hormones (e.g., testosterone) [60], age, and muscle fiber distribution or the expression of myosin heavy chains [42, 52]. The highest increases in muscle length reported in the literature were found to be up to 60% depending on the duration of stretching by Antonio and Gonyea [8] or up to 77% by Antonio et al. [9].

With regard to fiber hypertrophy in animal experiments, no uniform statement can be made. Antonio et al. [10] found an increase in the cross-section of FT as well as ST fibers, whereas Alway, [3] and Roman and Alway [47], for example, do not highlight any increase in the muscle cross-section of FT fibers. The hypertrophy of ST fibers seems to be regulated by the calcineurin/NFAT signal transduction pathway [48]. This is significant as the studies listed in this meta-analysis are primarily concerned with prolonged exercise leading to ST fiber adaptations [10, 20, 21, 25, 29, 30].

Hyperplasia

Referring to the finding of Antonio et al. [10] that the amount of increase in fiber count is related to the duration as well as the amount of the stretching stimulus, it can be hypothesized that traditional strength training methods do not achieve adequate stretching of the muscles. A stretching intervention lasting several hours to several days, as performed in animal experiments, has of course not been carried out. The proliferation and activation of satellite cells is held responsible for the hyperplasia effects [8, 10, 29]. This seems to occur—at least in animal experiments—when a muscle is seriously damaged by mechanical stress [36, 57].

Maximum Strength and Speed Strength

Studies by Alway [3, 4] found a significant increase in muscle cross-sectional area (approximately 100%), in muscle mass (260%) and in maximum strength (95%) in animal muscles. Stretching the muscle can be assumed to lengthen the muscle fiber through serial accumulation of sarcomeres [4, 10]. In animal experiments, muscle lengthening of up to 60% depending on the stretch duration was found by Antonio and Gonyea [8] or up to 77% by Antonio and Gonyea [9]. According to Goldspink and Harridge [29], this can lead to a faster contractile capacity of the muscle and thus an increase in fast or explosive power capacity. This hypothesis is confirmed by Medeiros and Lima [39] who identified 14 studies with a positive influence on "muscle performance" through chronic stretching. Muscle performance was recorded in the studies by functional tests such as jumps or sprints or by isometric or isotonic contractions. This is contradicted by data on the change in myosin heavy chain expression in stretched muscle as demonstrated in the animal experiment by Roman and Alway [47]. Myosin isoform SM2 increased from a level of 43.1% (± 1.7%) in the control muscle to 55% (± 1.2%) in the stretched muscle. It was shown that sustained stretching resulted in increased expression of SH2 myosin heavy chains and decreased expression of SH1 myosin heavy chains. Thus, due to the decreased ATPase activity in hypertrophied type I fibers after stretching, a negative effect on muscle contraction speed can be assumed, which was confirmed by Alway [2].

Contraction time increased significantly from 149 ms (± 9 ms) to 162 ms (± 7 ms) in young animals and from 174 ms (± 16 ms) to 215 ms (± 14 ms) in old animals by continuous stretching with 12% of their own body weight. "Overload increased twitch contraction time by 36% in muscles from … birds" [5]. There was a measurable shift from SM1 myosin isoform to SM2 myosin isoform. "Nevertheless, the slowing of V, and Vmax in the ALD was related to the decrease in SMl and slow muscle fibers. The explanation for a shift in fiber type or myosin isoforms is unable to explain all of the 60% decline in shortening velocity, unless ATPase activity also declined in SM1 or slow-p fibers. Our preliminary data suggest that Ca^{2+} activated ATPase activity was − 20% lower in the SM2 isoform than the SM1 isoform, and ATPase activity decreased in both isoforms after stretch overload" [4]. If these results are transferable to humans, it can be assumed that an increase in the ST-fiber content and thus a reduction in high-speed power output (e.g. jumps, sprints) is due to muscle plasticity and a reduced ATPase activity.

For the investigated parameters MM, FCSA and FN, heterogeneity was relatively large ($I^2 > 90\%$), suggesting that moderator variables could explain some of the differences between the true effect sizes of the included individual studies. The forest plots for MCSA, FCSA, FN and FL provide graphical information of which effect sizes differ the most from the weighted averages, but systematic subgroup analyses where studies are grouped with respect to moderators, such as muscle group or fiber distribution within the muscle, gender of the test animal, age of the animal or stretching duration, does not seem feasible due to the (still) relatively small number of effect sizes. Using only birds as experimental animals and including ALD and PAT in the analysis of this meta-analysis, we already tried to account for potential heterogeneity by controlling these variables in the selection of studies (in contrast to Kelley [33].

Practical Implications

Although the results from animal experiments presented here are conclusive, they may not be directly transferable to humans. First evidence that stretching training can induce micro-traumatization in humans if appropriate intensity of the stimulus is given was provided by Smith et al. [55]. Schoenfeld [51, p. 2862] also refers to the possibility to induce sufficient mechanical tension to induce morphological adaptations using stretching training: "Mechanically induced tension produced both by force generation and stretch is considered essential to muscle growth, and the combination of these stimuli appears to have a pronounced additive effect". Consequently, there are some studies pointing out improvements in sport-specific parameters as jumping and sprinting [34, 44], maximal strength [41, 43, 70] and muscle thickness [44, 53] using stretching durations of up to 6 × 5 min [70] for up to 12 weeks [44]. However, there is still a lack of human studies on the effects of long-lasting stretching interventions for many weeks on muscular hypertrophy, hyperplasia, and force development. Because frequency, magnitude, and especially intensity of stretching appear to play an important role in adaptive responses, further studies need to focus on load controls via these load normatives. Apostolopoulos et al. [11] hypothesized that below the pain threshold stretches in the muscle are compensated via the

elastic components and only stretches above the pain threshold lead to inflammation, which is normal after a fatiguing load [32] and/or delayed onset muscle soreness. In addition to intensity, a minimum amount and duration of stretching is essential, as Fowles et al. [24] showed that a single bout of stretching does not seem to be sufficient to increase protein synthesis. In accordance, Freitas et al. [26] pointed out that interventions of less than 8 weeks with a stretching duration of less than 20 min per week would not be expected to produce statistically significant structural changes in humans. Therefore, stretching duration may play an important role, too. Only one study using daily long-lasting stretching training for the plantar flexors could be determined, showing significant increases in maximal strength, muscle thickness and flexibility [64]. Since in animal studies, apparatuses were used to achieve long-lasting stretching durations, stretching devices (as used by Warneke et al. [64]) could also be recommended to achieve long-lasting stretching durations in humans. Otherwise, it can be assumed that stretching durations lasting several hours are not feasible. If a certain degree of transferability to humans is assumed, the studies analyzed here can be seen to have particular relevance in rehabilitation [29], as immobilization due to injury is known to lead to significant muscle atrophy [45]. If the hypertrophy effects from animal studies are assumed to be transferable to humans, aid-based continuous stretching for several hours could counteract atrophy and, if necessary, support muscle mass gain. "The therapeutic applications of stretch should therefore be borne in mind when designing regimens for rehabilitation or improved athletic performance" [29]. Furthermore, if voluntary muscle activation is not possible, stretching intervention would already be applicable. This could minimize muscle atrophy and loss of strength through immobilization due to injuries or illnesses [65, 68].

For an examination of the results in humans, moderator variables should be taken into account to be able to examine their influence.

If transferability of our results to humans is given, we see a high potential in using long-lasting stretching to achieve muscle hypertrophy. But it remains controversial whether hyperplasia effects occur in humans as a result of a training intervention. MacDougall notes, "One possible explanation is that hyperplasia occurs only in response to a significant stretch overload that also causes muscle lengthening, and that conventional resistance training does not impose such a stimulus" [36].

Limitations

In all studies included in the meta-analysis, the control values were provided by non-stretched animals because collecting pre- and post-measures from the same animals is not possible. This is different in studies using human participants. With regard to the conducted quality assessment, an important limitation appears to be the fact that in most studies, the assessors (of the outcome parameters) were not blinded with regard to which animals were assigned to the experimental or control group. Also, visual inspection of the funnel plots performed for each outcome parameter suggested slight deviations from a symmetric distribution in some cases. However, this could be due to the rather small effect sizes and should be interpreted with caution. Furthermore, also due to the rather small number of studies, it was not possible to reliably investigate the potential influence of moderator variables, such as duration of stretching, for instance. Finally, it needs to be highlighted that most studies were performed about 30–40 years ago.

Funding Open Access funding enabled and organized by Projekt DEAL.

Data Availability The datasets generated and analyzed during the current study are available from the corresponding author upon request.

Declarations

Conflict of Interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Consent for publication All authors have read and agreed to the published version of the manuscript.

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International Journal of EXERCISE SCIENCE

Original Research

Improvements in Flexibility Depend on Stretching Duration

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ABSTRACT

International Journal of Exercise Science 16(4): 83-94, 2023. To improve flexibility, stretching is most commonly used and in training interventions duration-dependent effects are hypothesized. However, there are strong limitations in used stretching protocols in most studies, particularly regarding documentation of intensity and performed procedure. Thus, aim of this study was to compare different stretching durations on flexibility in the plantar flexors and to exclude potential biases. Eighty subjects were divided into four groups performing daily stretching training of 10min (IG10), 30min (IG30) and 1h (IG60) and one control group (CG). Flexibility was measured in bended and extended knee joint. Stretching was performed with a calf muscle stretching orthosis to ensure long-lasting stretching training. Data were analysed with a two-way ANOVA for repeated measures on two variables. Two-way ANOVA showed significant effects for time (η^2 = 0.557-0.72, p < 0.001) and significant interaction effects for time x group (η^2 = 0.39-0.47, p < 0.001). Flexibility in the knee to wall stretch improved with 9.89-14.46% d = 0.97-1.49 and 6.07-16.39% with d = 0.38-1.27 when measured via the goniometer of the orthosis. All stretching times led to significant increases in flexibility in both tests. While there were no significant differences measured via the knee to wall stretch between the groups, the range of motion measurement via the goniometer of the orthosis showed significantly higher improvements in flexibility depending on stretching duration with the highest increase in both tests with 60 minutes of stretch per day.

KEY WORDS: Long-lasting stretching, range of motion, plantar flexors, stretching device

INTRODUCTION

In literature there is evidence that improved muscle flexibility (extensibility of the muscle) is associated with higher joint mobility, which is the ability to cover higher ranges of motion (ROM), better performance and reduced injuries (42). Gymnasts and dancers are known for having great flexibility (1, 26, 38), while in team sports, athletes are usually characterised by poor mobility (8, 9, 11), especially in the calf muscles and hamstrings (32). Consequently, it seems beneficial to enhance flexibility as a measure to improve performance and decrease the

risk of injury. Thomas et al. (39) showed that higher gains in flexibility are related to higher weekly stretching volume, especially by performing long stretching durations with high frequency and would therefore result in higher ROM. Apostolopoulos et al. (3) demonstrated that stretching intensity is of particular importance regarding physiological adaptations. Therefore, a dose-response relationship is hypothesized. There are several studies showing effects of long-term stretching interventions on flexibility (12, 23, 24). While there are increases in flexibility in the plantar flexors of 18.8% (d = 1.90) performing a 60 sec stretching on three days per week (15), Simpson et al. (37) pointed out significant increases in ROM of 14.94 % (d = 2.05) by performing stretches for the plantar flexors of three minutes on five days per week for five weeks. Besides, investigation performing long-lasting stretching training for one hour per session showed significant increases of 13.2% (d = 1.49) in the knee to wall test (41). In general, maximum heterogeneity in study protocols can be observed, e.g. through the use of different types of equipment to induce stretching stimulus (stretching board (27, 31, 33, 43) leg press (37), stretching device (40, 41), or without equipment (5). Furthermore, different training conditions regarding training frequency, stretching duration and therefore weekly volume were used, ranging from 4x30 sec on three days per week (25, 29) to one hour per day on seven days per week (41). Based on this, comparability of results from different studies is limited considering discrepancies in stretching duration or weekly stretching volume. Furthermore, there was no quantification of stretching intensity in any study. To improve comprehension of stretching training and its effects on flexibility, the aim of this study was to compare different stretching durations from ten min to 60 min per day and to investigate the role of stretching duration on improvements in flexibility. Therefore, participants stretched their plantar flexors on seven days per week for six weeks. It is hypothesized that stretch training induces significant improvements in ROM depending on training duration.

METHODS

Participants

Eighty active subjects (m = 45, f = 35, age: 26.4 ± 4.6 years, height: 176.3 ± 8.1 cm and weight: 74.3 ± 5.5 kg) were recruited from sports study programs and local sports clubs. The training level of participants was evaluated by self-reported time spent with training. Participants were classified as active athletes with moderately trained flexibility when they performed two or more training sessions per week in a gym or a team sport continuously for the previous six months. However, participants were excluded if they perform stretch training in their training routines like additional separated stretching sessions or Yoga training or if they reported injuries within the last six months leading to immobilization of one limb. Furthermore, participants had to declare no increased risk of thrombosis to be included to the investigation. Included subjects were randomly divided into three stretching groups and one control group and instructed not to start any further flexibility training while participating in this study. Characteristics of subjects are shown in Table 1.

All participants were informed about the experimental risks and provided written informed consent to participate in the present study. Furthermore, approval for this study was obtained

from the institutional review board (Carl von Ossietzky Universität Oldenburg, No.121-2021). The study was performed with human participants in accordance with the Helsinki Declaration and in accordance with ethical policies (28).

Table 1. Characteristics of participants for overall sample size and divided into IG10, IG30, IG60 and CG

Group	*N*	Age (in years)	Height (in cm)	Weight (in kg)
total	80 (f = 35, m = 45)	26.4 ± 4.6	176.3 ± 8.1	74.3 ± 5.5
IG10	20 (f = 9, m = 11)	25.5 ± 5.5	177.2 ± 6.4	76.9 ± 4.1
IG30	20(f = 10, m = 10)	26.7 ± 2.5	175.2 ± 8.4	77.7 ± 7.0
IG60	20 (f = 8, m = 12)	24.9 ± 2.9	174.6 ± 4.9	73.9 ± 4.2
CG	20 (f = 8, m = 12)	26.1 ± 3.3	176.6 ± 3.7	74.7 ± 2.3

IG10 = intervention group 10 with a stretching with a daily stretching duration of 10 minutes, IG30 = intervention group 30 with a stretching with a daily stretching duration of 30 minutes, IG60 = intervention group 60 with a stretching with a daily stretching duration of 60 minutes, CG = control group

Protocol

Since Arampatzis et al. (4) and Signorile et al. (34) described different involvements of the gastrocnemius and soleus in muscle performance depending on the knee angle, there were two testing procedures performed to investigate the ROM in the upper ankle with bended and extended knee joint. ROM with bended knee joint was assessed via the goniometer on the orthosis (ORTH) and ROM with extended knee joint was assessed by using the knee to wall stretch (KtW) as a commonly performed flexibility test for the plantar flexors (7, 35, 41). Participants were instructed to perform the testing procedure without wearing shoes.

The KtW was used to examine flexibility in dorsiflexion in the upper ankle joint by trying to maximize the distance of the foot from the wall and pushing the knee forward to the wall until the heel lifts off. Afterwards, the distance between the foremost point of the toes and the wall was measured. To improve objectivity and reliability, a sliding device was used. The participants were instructed to place the foot on the marker while stabilizing the body with their hands inside a doorframe (Fig. 1) as they pushed the board of the sliding device forward until the heel of the standing leg lifted off. To check this, the investigator pulled on a sheet of paper which was placed under the subject's heel. The test procedure was stopped when the sheet could be removed. The reached value was read off in cm from the attached measuring tape (see Fig. 2). The KtW measurement can be seen as a screening tool for ankle flexibility with a bended knee. Three valid trials were performed per leg, and the maximal value was used for evaluation. Reliability in ROM assessment with comparable methods can be classified as high with ICC > 0.97 (36). Moreover, due to a ICC of 0.987-0.992 and a CV of 0.94-1.74% this procedure can be assumed to be reliable (41).

Figure 1. Sliding device for the knee to wall test to evaluate flexibility in the ankle joint with bended knee joint.

As a second test to determine the effects of the stretching training on ROM, ORTH was used. While sitting on a chair, participants had to place their foot on an object of same height as the hip. From the starting position (neutral 0 position), the investigator pushed the foot carefully into maximal dorsiflexion. The angle, which was reached by pressing the foot of the participant in the maximally dorsiflexed position determined as either the participant's maximal tolerable pain or the inability to further increase the angle, in the upper ankle joint was measured via the goniometer on the orthosis. Each big indentation of the goniometer corresponds to an increase of 5°, and each little indentation corresponds to an increase of 2.5°. While performing a stretch with extended knee joint, the achieved angle was read off. This procedure was also performed in previous studies with high reliability (ICC = 0.990-0.997) (41).

Figure 2. Orthosis for stretching the calf muscles with included goniometer to determine the range of motion in the ankle joint with extended knee joint.

The intervention groups were instructed to perform a daily stretching training of the calf muscles lasting ten min (IG10), 30 min (IG30) and one hour (IG60) each session for six weeks, respectively. To realize described long-lasting stretching training, a calf muscle stretching orthosis was provided (see Fig.2). Subjects were instructed to wear the orthosis without shoes with extended knee joint and the stretch intensity was controlled by a goniometer which was also used to determine the angle representing the starting value during the pre-test. To achieve high intensity and muscle tension during the stretching training, subjects were asked to reach a maximally dorsiflexed position with an individual stretching pain by using the VAS at 8 on a scale one to ten, which is commonly used in stretching research (14, 27). To achieve a constant, high stretching tension, the participants were instructed to aim for a stretching pain of 8 on the VAS throughout the study. Therefore, the angle of the orthosis was progressively increased when stretching pain was perceived as being below 8. The stretching was performed seven days a week in a standardized body posture: the subjects were instructed to sit with their backs as straight as possible against the backrest of a chair and place their feet on a support plate at the same height as their chair. All subjects in the intervention groups borrowed one orthosis for the duration of the intervention and had to complete a stretching diary in which the daily stretching duration as well as the set angle were written down to record stretching duration and to provide a homogenous stretching stimulus as well as the possibility to document progression in ROM. If subjects were not able to perform their stretching routine on more than five days within the intervention period or were not able to perform stretching on three consecutive days, the values of these participants were excluded.

Statistical Analysis

The analysis was performed with SPSS (Version 28.0., IBM Corp., USA). Reliability was evaluated by calculating ICC and CV between the best and the second-best value in the KtW and ORTH, providing intra-session reliability. Descriptive data are provided with mean (M) ± standard deviation (SD). Normal distribution was checked via Shapiro Wilk test, whereas the Levene test showed homogeneity in variance. One-way ANOVA was used to rule out significant differences between groups of the pre-test values. A mixed model ANOVA using two factors was performed for the collected parameters. The Scheffé test was used as post-hoc for mean differences. Effect sizes were presented as Eta squares (η^2) and categorized as: small effect $\eta^2 < 0.06$, medium effect $\eta^2 = 0.06$-0.14, large effect $\eta^2 > 0.14$ as well as Cohen's d. (10). Effect sizes with Cohen's d were categorized as: small effects $d < 0.5$, medium effect $d = 0.5$-0.8, large effect $d > 0.8$. Post-hoc Power (1-β) was calculated via G-Power (Version 3.1, Düsseldorf, Germany).

RESULTS

High intra-session reliability for KtW with ICC = 0.942 and a CV of 1.01% and for ORTH with ICC = 0.991 and CV of 0.83% were calculated. A one-way ANOVA for pre-test values shows no significant differences between groups with $F(79,3) = 0.22$, $p = 0.881$. Descriptive statistics as well as evaluation of two-way ANOVA are provided in Table 2.

Table 2. Descriptive statistics and results of two-way ANOVA for both flexibility tests

Parameter	Pre-test (M ± SD) in *N*	Post-test (M ± SD) in *N*	Pre- post Differences in %	Time effect	Time x group
IG10KtW	11.71 ± 3.33	12.88 ± 3.44	+10.02	$p < 0.001$ $F(77.3) = 196.58$ $\eta^2 = 0.72$	$p < 0.001$ $F(77.3) = 22.5$ $\eta^2 = 0.47$
IG30KtW	12.39 ± 3.8	13.61 ± 4.0	+9.89		
IG60KtW	11.96 ± 2.37	13.69 ± 2.19	+14.46		
CGKtW	12.29 ± 1.81	12.36 ± 1.9	+0.57		
IG10ORTH	8.65 ± 2.02	9.18 ± 1.9	+6.07	$p < 0.001$ $F(77.3) = 96.7$ $\eta^2 = 0.557$	$p < 0.001$ $F(77.3) = 16.55$ $\eta^2 = 0.392$
IG30ORTH	8.23 ± 1.75	8.93 ± 1.48	+8.51		
IG60ORTH	9.00 ± 1.5	10.48 ± 1.33	+16.39		
CGORTH	8.74 ± 1.55	8.83 ± 1.69	+1.12		

IG10 = intervention group 10 with a stretching with a daily stretching duration of 10 minutes, IG30 = intervention group 30 with a stretching with a daily stretching duration of 30 minutes, IG60 = intervention group 60 with a stretching with a daily stretching duration of 60 minutes, CG = control group, KtW = Range of motion measurement via knee to wall test, ORTH = Range of Motion measurement via the goniometer of the orthosis

Figure 3 and Figure 4 illustrate the progression in flexibility from pre- to post-test in all four groups.

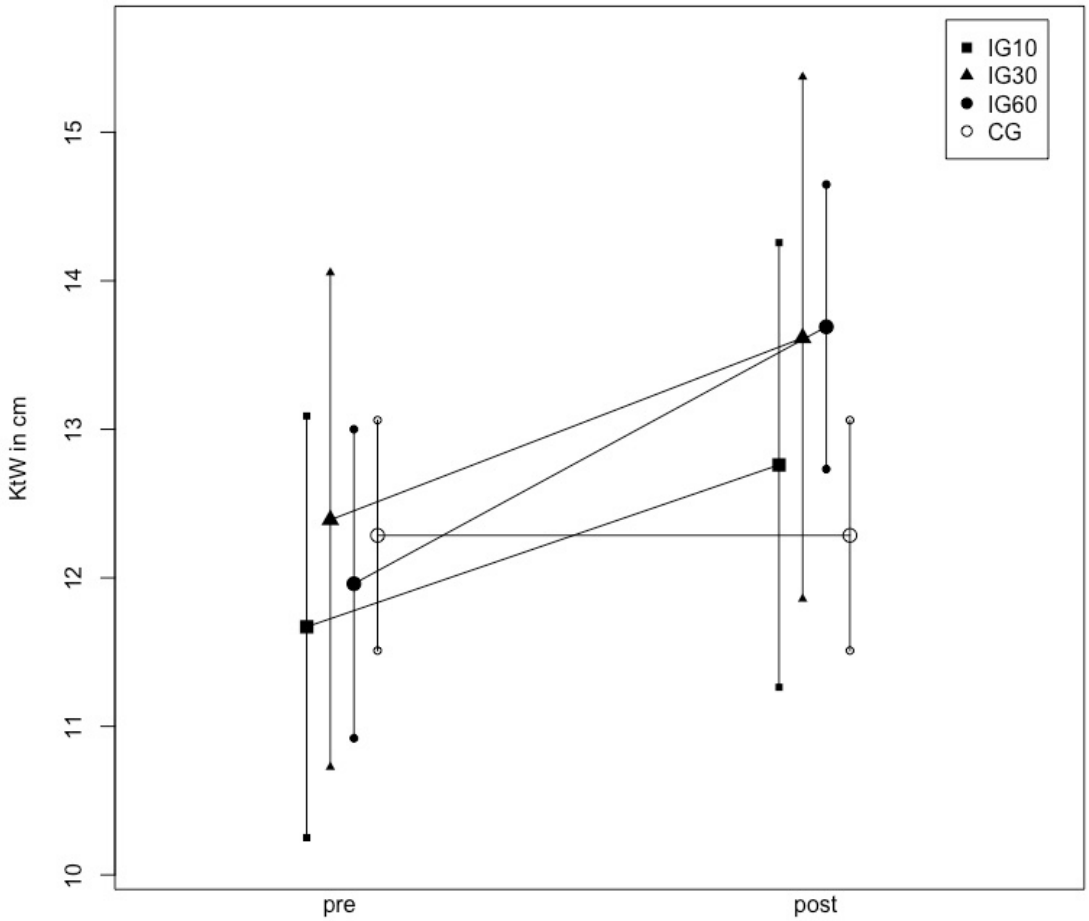

Figure 3. Comparison of progressions measured via the knee to wall stretch between all groups, IG10 = intervention group 10 with a stretching with a daily stretching duration of 10 minutes, IG30 = intervention group 30 with a stretching with a daily stretching duration of 30 minutes, IG60 = intervention group 60 with a stretching with a daily stretching duration of 60 minutes, CG = control group

For results of KtW (see Fig. 3), a mixed model ANOVA demonstrated high effects for the time-dependent effect ($\eta^2 = 0.7$, $p < 0.001$) and for the time x group interaction ($\eta^2 = 0.46$, $p < 0.001$).

The Scheffé test determined no significant difference for the mean differences between pre- and post-test values between IG10, and IG30 ($p = 0.996$) as well as between IG10 and IG60 ($p = 0.09$) and between IG30 and IG60 ($p = 0.14$). Whereas there were significant differences between CG and IG10 with $d = 0.97$, $p < 0.001$, CG and IG30 with $d = 1.03$, $p < 0.001$ as well as IG60 and CG $d = 1.49$, $p < 0.001$.

Post-hoc analysis of G-Power calculated 1-β = 100% with $\eta^2 = 0.33$ for the within effects and 1-β = 93.1% with $\eta^2 = 0.46$ for the interaction for $\alpha = 0.05$ for 4 groups and two measuring time points.

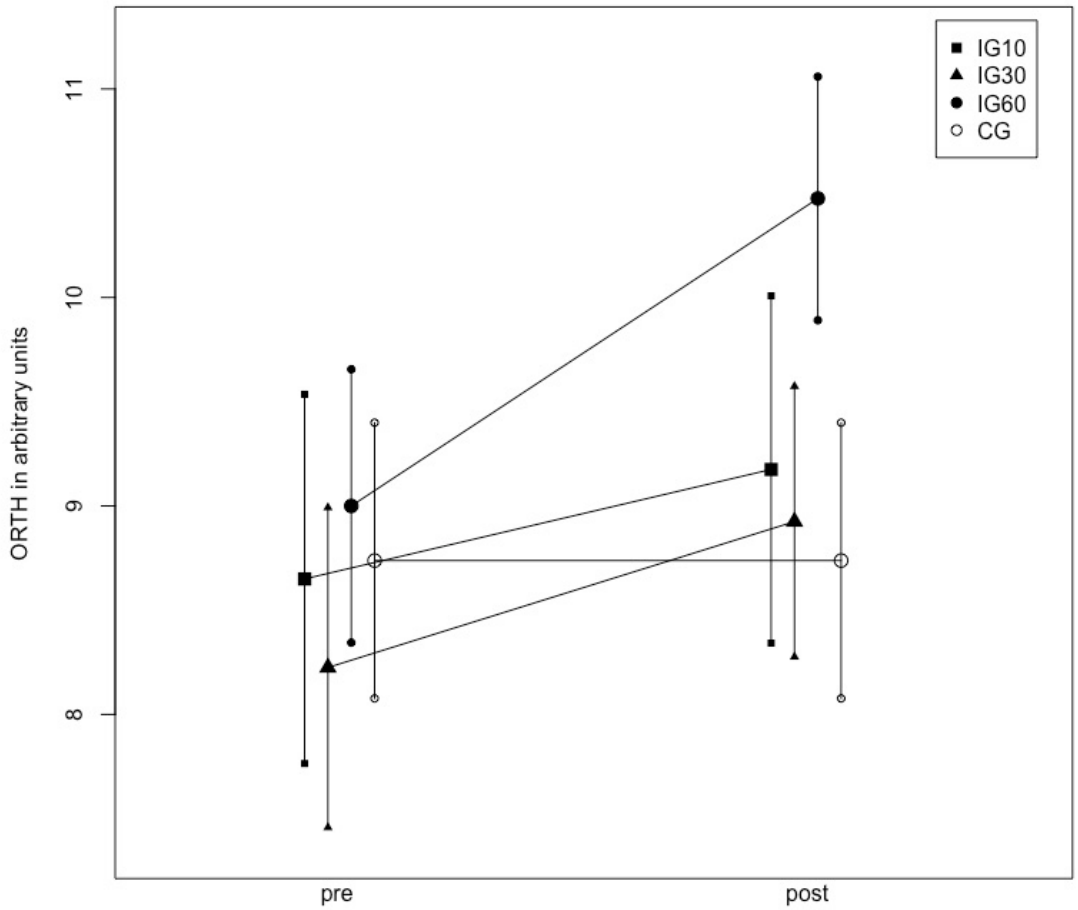

Figure 4. Comparison of progressions measured via the goniometer of the Orthosis between all groups, IG10 = intervention group 10 with a stretching with a daily stretching duration of 10 minutes, IG30 = intervention group 30 with a stretching with a daily stretching duration of 30 minutes, IG60 = intervention group 60 with a stretching with a daily stretching duration of 60 minutes, CG = control group

For results of ORTH (see Fig. 4), a mixed model ANOVA demonstrated high effects for the time-dependent effect ($\eta^2 = 0.56$, $p < 0.001$) and for the time x group interaction ($\eta^2 = 0.39$, $p < 0.001$).

The Scheffé test determined no significant difference for the mean differences between pre- and post-test values between IG10 and IG30 (p = 0.86, d = 0.16) as well as between IG10 and CG (p = 0.21, d = 0.38). However, there were significant differences between IG60 and IG10 (p < 0.001, d = 0.88), IG60 and IG30 (p = 0.004, d = 0.71) as well as IG60 and CG (p < 0.001, d = 1.27) and between IG2 and CG (p = 0.03, d = 0.55).

Post-hoc analysis of G-Power calculated 1-β = 100% with η^2 = 0.33 for the within effects and 1-β = 93.1% with η^2 = 0.46 for the interaction for α = 0.05 for 4 groups and two measuring time points for KtW.

Summary of results: All three stretching durations led to significant increases in ROM in both testing routines. The results show that stretching duration of 60 minutes per day increased flexibility to a higher magnitude than 30 minutes (p = 0.004) and ten minutes (p < 0.001) when measuring via ORTH, but not via KtW.

DISCUSSION

The aim of this study was to investigate if there is a duration- and volume-dependent effect of stretching training for several weeks regarding the improvements in flexibility. Results of the present study compared different stretching durations from ten min to one hour per day and showed significant improvements in flexibility in all three intervention groups with d = 0.97-1.49, p < 0.001 via KtW and d = 0.38-1.27, p < 0.001 via ORTH compared to the control group. However, results showed no significant differences between the intervention groups (p = 0.09-0.99) in the KtW, but significant differences when ROM was measured via ORTH (p = 0.001-0.03, d = 0.16-1.27).

As mentioned before, there is maximum heterogeneity in study designs when comparing the procedure of different studies examining effects of stretching training on flexibility. Therefore, comparability of results of the present study with other investigations seems to be limited since there are differences in study designs, e. g. regarding the way in which the stretching stimulus was generated as well as weekly stretching volume. In this study, the training volume of IG30 and IG60 was significantly higher compared to other studies (19, 25, 29, 33). Mizuno (25) performed stretching intervention on three days per week with 4x30 sec of stretching gaining 12.7% (d = 1.0) in flexibility, Kokkonen et al. (19), showed significant increases of 18.1% (d = 1.15) performing stretching for 3x15 sec per session on three days per week for 12 weeks. Compared to the results of this study showing increases of 10.02% with stretching durations of ten and 30 min per day, listed results of studies using short-time stretching interventions seem to be comparatively high (19, 25, 29, 31). It seems that different factors influence results of stretching training, e.g. training status, stretching frequency and intensity (3, 13). Only one investigation with a comparable study design investigated the influence of a one-hour daily static stretching training which was performed by this research group leading to comparable results of 13.2% (d = 1.49) in the KtW.

While increases in ROM measured via KtW showed no significant differences depending on stretching time, improvements in flexibility measured via the goniometer at the orthosis showed time dependent increases in ROM. While there were no significant differences between stretching time of ten and 30 minutes, there were significant differences to other groups when performing one hour stretching per day. Higher increases in ROM measured via ORTH may be attributed to the identical execution to the stretch training of the intervention. Since an influence of the knee angle of used muscles in the lower extremity can be assumed (4, 34) and the KtW (partially) examines the dorsiflexion with bended knee joint, results show that there is high specificity in effects of stretching training on ROM. It can be noticed that while there is a significant influence of time on flexibility when ROM is measured via ORTH, no clear influence can be seen when measuring the KtW. Between different knee joints influencing the results, influence of hysteresis effects (21) possibly play a major role when longer stretching duration are used (6, 20). It could be hypothesized that stretching stimulus decreases when intensity is not readjusted. Consequently, further studies could possibly investigate the influence of constant torque stretching and compare different stretching durations. Furthermore, poor flexibility values in many sports (8, 9, 11) could possibly be attributed to the intensity being too low or inadequate volume of the training stimuli.

There are several hypotheses explaining increased ROM following stretching training. While some authors hypothesize an increased tolerance of stretching tension via reduced pain threshold instead of structural adaptations (13), other authors demonstrated changes in stiffness, viscosity and elasticity of the muscle-tendon unit. Furthermore, animal models show evidence of structural adaptations by a serial accumulation of sarcomeres (2). From the authors' point of view, there is no conflict with the hypothesis of a reduced pain threshold in a given angle if a serial accumulation in humans can be hypothesized. Based on the hypothesis that pain is present by reaching higher degrees of stretching in sarcomeres, a serial accumulation of sarcomeres would lead to an occurrence of pain in a higher joint angle. Consequently, an increased number of serial sarcomeres would lead to a later occurrence in pain. However, although neither in this study nor in other listed studies a serial sarcomere accumulation could be investigated, it should not be excluded as an explanation.

Limitations: We used a stretching orthosis to induce long-lasting stretching stimuli. Since in other studies comparatively low stretching durations showed higher increases in ROM, investigations using comparable study design are requested to replicate comparable high results from other studies (25, 29) and examine the influence of higher stretching durations by using the same stretching procedure as it was used in listed studies. As a common tool to quantify stretching intensity, VAS was used in this study (14, 18, 27). However, subjective perception may influence comparability between subjects (22). To improve the quality of intensity documentation, a torque measurement should be included to stretching interventions in further studies. Examining flexibility in the plantar flexors by performing the knee to wall stretch, depending on flexibility, knee joint must be bended to a higher extent by increasing flexibility. Since Arampatzis et al.(4) showed that depending on the knee joint angle different parts of the plantar

flexors are used, it can be hypothesized that there are also differences in used muscles when reaching higher values in the KtW.

Practical Application: In many sports, there are athletes with poor flexibility values leading to many problems, e.g. reduced performance or a higher risk of injury (16, 30, 42). The results of this study show that daily stretching durations between ten minutes and 60 minutes induce significant improvements in ROM. However, there are stretching routines performed in other studies with much lower stretching durations producing comparable results. Since doubling daily stretching time (30 min to one hour) in present study does not lead to doubling the effects of stretching training, further studies should investigate the optimal stretching duration to achieve flexibility gains which seems to be lower than ten minutes to achieve higher economy of training, even if a dose-response relationship by comparing percentage increases can be assumed (9.89%-14.46%). Based on this, further studies should enhance the intervention period to at least eight to twelve weeks or longer to consider that effects of morphological adaptation may occur more recently. Consequently, increases in ROM are influenced by many other factors than the stretching duration, e.g., stretching intensity, training status and the way the stretch is induced. Even if one questions the practical application of seven hours of stretch per week, stretching durations used in this study can be understood as an extreme situation provided by 60 minutes stretch per day. This, in turn, can be seen as an appropriate way to investigate general principles of a training method (17). Consequently, this study provides valuable information on the dose-response relationship and demonstrates that regarding adaptations in flexibility, the more time spent in a stretched position, the higher the assumed adaptations will be.

ACKNOWLEDGEMENTS

We thank all participants for performing the stretching interventions. Further, we thank PD Dr. Martin Hillebrecht for providing software and equipment. Additionally, we thank Carina Mählmeyer for assistance with data collection.

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ORIGINAL RESEARCH
published: 25 May 2022
doi: 10.3389/fphys.2022.878955

Influence of Long-Lasting Static Stretching on Maximal Strength, Muscle Thickness and Flexibility

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Background: In animal studies long-term stretching interventions up to several hours per day have shown large increases in muscle mass as well as maximal strength. The aim of this study was to investigate the effects of a long-term stretching on maximal strength, muscle cross sectional area (MCSA) and range of motion (ROM) in humans.

Methods: 52 subjects were divided into an Intervention group (IG, $n = 27$) and a control group (CG, $n = 25$). IG stretched the plantar flexors for one hour per day for six weeks using an orthosis. Stretching was performed on one leg only to investigate the contralateral force transfer. Maximal isometric strength (MIS) and 1RM were both measured in extended knee joint. Furthermore, we investigated the MCSA of IG in the lateral head of the gastrocnemius (LG) using sonography. Additionally, ROM in the upper ankle was investigated *via* the functional "knee to wall stretch" test (KtW) and a goniometer device on the orthosis. A two-way ANOVA was performed in data analysis, using the Scheffé Test as post-hoc test.

Results: There were high time-effects ($p = 0.003$, $\eta^2 = 0.090$) and high interaction-effect ($p < 0.001$, η^2=0.387) for MIS and also high time-effects ($p < 0.001$, η^2=0.193) and interaction-effects ($p < 0.001$, η^2=0,362) for 1RM testing. Furthermore, we measured a significant increase of 15.2% in MCSA of LG with high time-effect ($p < 0.001$, η^2=0.545) and high interaction-effect (p=0.015, η^2=0.406). In ROM we found in both tests significant increases up to 27.3% with moderate time-effect ($p < 0.001$, η^2=0.129) and high interaction-effect ($p < 0.001$, η^2=0.199). Additionally, we measured significant contralateral force transfers in maximal strength tests of 11.4% ($p < 0.001$) in 1RM test and 1.4% (p=0.462) in MIS test. Overall, there we no significant effects in control situations for any parameter (CG and non-intervened leg of IG).

Discussion: We hypothesize stretching-induced muscle damage comparable to effects of mechanical load of strength training, that led to hypertrophy and thus to an increase in maximal strength. Increases in ROM could be attributed to longitudinal hypertrophy effects, e.g., increase in serial sarcomeres. Measured cross-education effects could be explained by central neural adaptations due to stimulation of the stretched muscles.

Keywords: static stretching, muscle cross sectional area, maximal strength, range of motion, hypertrophy

OPEN ACCESS

Edited by:
Gregory C. Bogdanis, National and Kapodistrian University of Athens, Greece

Reviewed by:
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João Pedro Nunes, Edith Cowan University, Australia

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Specialty section:
This article was submitted to Exercise Physiology, a section of the journal Frontiers in Physiology

Received: *18 February 2022*
Accepted: *28 April 2022*
Published: *25 May 2022*

Citation:
Warneke K, Brinkmann A, Hillebrecht M and Schiemann S (2022) Influence of Long-Lasting Static Stretching on Maximal Strength, Muscle Thickness and Flexibility. Front. Physiol. 13:878955. doi: 10.3389/fphys.2022.878955

INTRODUCTION

Regular stretch training over several weeks improves flexibility and range of motion (ROM) (Young et al., 2013; Medeiros et al., 2016). Reduced pain perception due to habituation effects in humans (Freitas et al., 2018) and muscle fiber lengthening due to serial accumulation of sarcomeres following intensive stretch training could be determined in animals, which could be responsible for enhanced flexibility (Williams et al., 1988; Alway S. E., 1994). A transfer to human training studies can be hypothesized, as Damas et al. (2018) demonstrated increased serial sarcomere accumulation in humans in general. To maximize ROM, stretch training should include a long stretching duration with a high training frequency (Thomas et al., 2018). In addition to stretching duration and frequency, stretch intensity has a crucial influence on muscular adaptations. At low stretching intensities, it can be assumed that the tension is compensated primarily by the elastic components so that effects on the contractile tissue are only achieved at a certain minimum intensity (Apostolopoulos et al., 2015).

Long-term stretching of a muscle can also increases muscle mass due to muscular hypertrophy in animals. A variety of studies have been investigated in birds for this purpose, in which a wing of the test animal was stretched from 30 min daily to a 24-h continuous stretch over a period of 1 month (Frankeny et al., 1983; Williams et al., 1988; Antonio et al., 1993; Alway S. E., 1994; Czerwinski et al., 1994). In animal examination, Antonio & Gonyea (1993) achieved an enhancement in muscle mass of 318% with an intermittent stretching protocol by increasing stretching intensity from 10% of the bodyweight to 25% over 33 days. Stretching one wing of quails and chickens for different stretching durations demonstrated an increase in muscle mass depending on stretching duration (Bates, 1993; Frankeny et al., 1983; J.; Lee & Alway, 1996). Furthermore, gains in muscle mass in listed studies can be related to longitudinal hypertrophy and increases in muscle cross-sectional area of over 100% (Frankeny et al., 1983; Matthews et al., 1990; Alway S. E., 1994). Improvements in maximal strength are often related to enhanced muscle cross sectional area (Seitz et al., 2016). In quail, Alway S. E., 1994 found increments of maximal strength of 95% by continuous stretching for 30 days compared to the contralateral control muscle by *in vitro* studies.

Since authors investigated significant muscular hypertrophy in quail and chicken wings due to long lasting stretching interventions of several hours, which are in correlation with improvements in maximal strength (Alway S. E., 1994), question arises whether effects in maximal strength as well as in muscle cross-sectional area are transferable to humans. In a meta-analysis, Medeiros & Lima (2017) determined a positive effect of stretching on muscular performance measured *via* functional tests and isotonic contractions in humans. In addition, literature shows significant improvements in maximal strength up to 32.4% in leg extension by stretching the lower extremity. For this, a 40-min stretching workout was performed three times per week which was divided into 15 different stretching exercises for lower extremities, each hold for 3 × 15 s (Kokkonen et al., 2007). Highest stretching duration was performed by (Yahata et al., 2021) by stretching the plantar flexors with a specific stretching board for 30 min per session, each session twice a week for 5 weeks. While Yahata et al. (2021) reported improvements in maximal strength of 6.4%, Mizuno (2019) showed increases in maximal strength of 20.2% in maximal strength with a stretching intervention for 8 weeks. However, other studies failed to point out any significant changes in MCSA or maximal strength after several weeks of stretching training (Sato et al., 2020; Longo et al., 2021; Nakamura et al., 2021).

Furthermore, Panidi et al. (2021) and Kokkonen et al. (2007) demonstrated improvements in jumping performance of up to 22% (Panidi et al., 2021). While Nunes et al. (2020) point out that low intensity stretching intervention is not a sufficient stimulus to induce muscular hypertrophy, Panidi et al. (2021) examined an enhancement in muscle thickness of 23% due to a stretching training for 12 weeks in volleyball players. Moreover, Simpson et al. (2017) showed increments of 5.6% in muscle thickness due to 3 minutes stretching stimulus on 5 days a week.

In addition to improved maximal strength of 29% in the stretched leg, Nelson et al. (2012) showed significant increases in maximal strength in the contralateral leg of 8%. Panidi et al. (2021) also point out contralateral improvements in muscular performance measured in unilateral CMJ. To this point, cross-education effects are mostly known from strength training when conducted unilaterally (Andrushko et al., 2018a; Andrushko et al., 2018b; M.; Lee et al., 2009; Lee & Carroll, 2007). We were not able to find other studies investigating long-term effects of stretching durations lasting at least 1 hour per day on maximal strength as well as muscle thickness.

Consequently, no statement about transferability of results from animal studies can be given, so the aim of the present work is to investigate the adaptive responses to a daily one-hour stretching training in maximal strength, muscle cross-sectional area as well as ROM. In addition, single-leg stretching is used to investigate cross education effects by using the non-stretched leg as an intra-inidividual control condition. We hypothesize, that 1 hour of stretching over 6 weeks lead to enhanced maximal strength, muscle thickness and ROM in the stretched leg. Furthermore, we suggest improvements in maximal strength in the not intervened control leg.

METHODS

Subjects

G-Power analysis was performed to estimate the required sample size showing a minimal total sample size of 36. 52 athletically active subjects were recruited from sports study programs, sports clubs, and fitness studios. Participants were classified as active athletes if they performed two or more training sessions per week in a gym or a team sport continuously for the previous 6 months. Subjects performing daily stretching training or similar activities like yoga as well as untrained subjects were excluded from the study. Included subjects were randomly divided into an intervention group (IG) and a control group (CG). One participant was dropped out, because of a sports related injury

TABLE 1 | Characteristics of test subjects.

Group	N	Age (in years)	Height (in cm)	Weight (in kg)
Total	52 (f = 21, m = 31)	27.0 ± 3.1	175.9 ± 5.2	80.5 ± 7.3
IG	27 (f = 11, m = 16)	27.4 ± 3.1	176.2 ± 5.6	81.0 ± 6.2
CG	25 (f = 10, m = 15)	26.8 ± 2.9	175.6 ± 4.9	79.3 ± 5.3

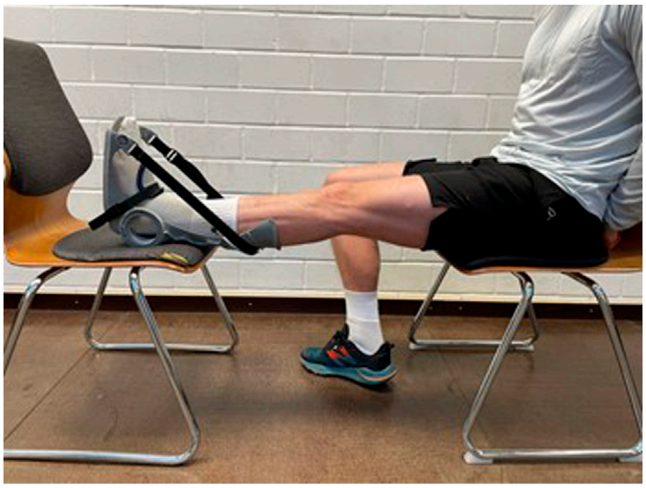

FIGURE 1 | Orthosis used for calf muscle stretching.

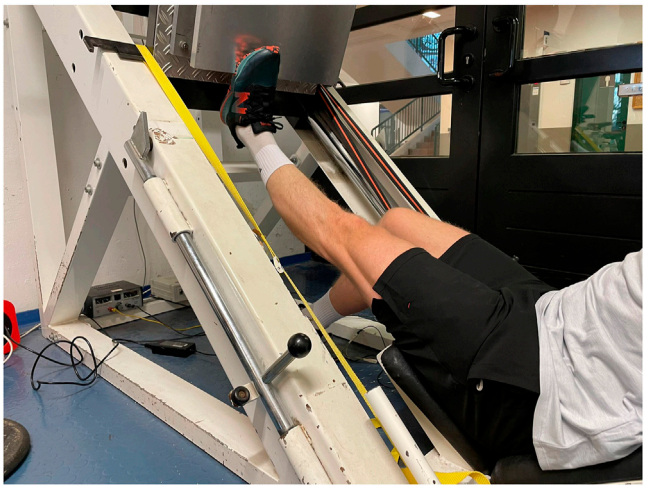

FIGURE 2 | Testing device for maximal isometric strength in extended knee using leg press (LP).

of his knee joint. Characteristics of subjects are displayed in **Table 1**.

All participants were informed about the experimental risks and provided written informed consent to participate in the present study. Furthermore, approval for this study was obtained from the institutional review board (Carl von Ossietzky Universität Oldenburg, No.121-2021). The study was performed with human participants in accordance with the Helsinki Declaration.

Intervention

The intervention consisted of daily stretching training of the calf muscles lasting 1 hour each session for 6 weeks, which was realized by wearing an orthosis designed for this purpose (**Figure 1**). The intervention was performed with the dominant leg only to give the opportunity to evaluate potential cross-sectional effects. To define the dominant leg, participants were asks about which leg they use when perform single-leg jumps. Subjects were instructed to wear the orthosis with extended knee joint and the stretch Intensity was controlled by an goniometer which was also used to determine the angle representing the starting value during the pre-test. To achieve high intensity and muscle tension during the stretching training, subjects were asked to reach maximal dorsiflexed position with an individual stretching pain at eight on a scale 1 to 10. The angle of the orthosis had to be set on corresponding angle to ensure sufficient intensity. Consequently, set angle of the orthosis should improve with enhanced ROM. The stretching was to be performed 7 days a week in a standardized body posture: the subjects were instructed to sit with their backs as straight as possible and place their feet on a support plate at the same height as their chair. All subjects in the intervention group borrowed one orthosis for the duration of the intervention and had to complete a stretching diary in which the daily stretching duration as well as the set angle were written down to record stretching duration and intensity. The control group did not perform any stretching interventions.

Testing Procedure

Before testing a five-minute warm up routine consisting of 5 min with a 130-bpm heart rate ergometer cycling was performed.

Maximal Strength Measurement

All subjects participated in the pre- and post-test. Maximal isometric and dynamic strength were assessed using single-leg testing in extended as well as in flexed knee joint. A 45° leg press was used to measure maximal strength in the extended knee joint. A force plate was attached to the footpad to record the maximal strength in the calf muscles with extended knee joint. We used an 50 × 60 cm force plate with a measuring range of ± 5000N and a 13-bit analog-to-digital converter. To measure maximal isometric strength, the subject was instructed to place the feet on the attached force plate such as that the metatarsophalangeal joints of the feet were placed on the edge flush (**Figure 2**). The starting position was chosen to give a 90° ankle joint

TABLE 2 | Reliability for the pre-test values. ICC = intraclass correlation coefficient, CV = coefficient of variance, SD = Standard deviation.

Parameter	ICC	CV (%)	SD
LPisoil	0.954	1.68	24.29
LPisocl	0.971	1.82	25.58
LPisoCGR	0.968	2.21	35.28
LPisoCGL	0.964	1.83	27.27
SONOil	0.947	2.99	4.6
SONOcl	0.971	1.93	7.07
KtWil	0.987	1.74	0.21
KtWcl	0.992	0.94	0.13
KtWCGR	0.979	1.81	0.24
KtWCGL	0.991	1.40	0.16
ORTil	0.997	0.64	0.38
ORTcl	0.997	0.62	0.38
ORTCGR	0.989	0.78	0.7
ORTCGL	0.990	1.16	0.8

LP, leg press; iso, isometric maximal strength; il, intervened leg; cl, control leg; Wt, weight in dynamic maximal strength; CG, control group; R, right; L, left.

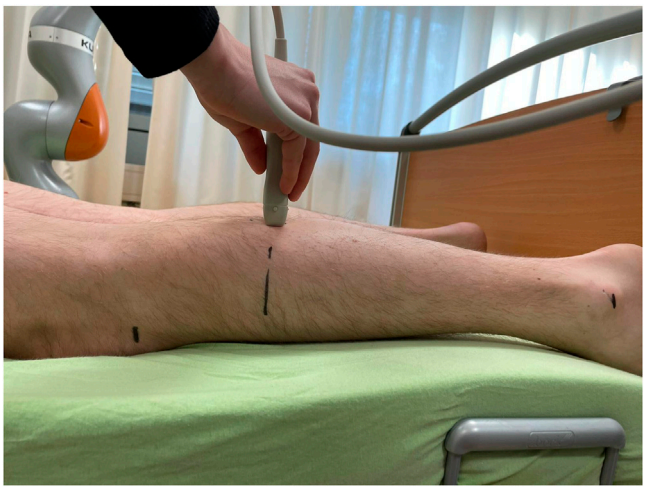

FIGURE 3 | Sonography to investigate muscle thickness in the calf muscle.

angle, which was controlled *via* the placement of an angle template. The force plate was fixed to form an impassable resistance from this position. The subject was instructed to perform a maximal voluntary contraction with a plantarflexion in response to an audible signal. Participants had to hold maximal contraction for at least one second after reaching perceived maximal strength. Force-time curve was recorded for 5 s. After each trial, a one-minute rest was observed to avoid fatigue. Measurements were conducted until no improvement in maximal strength was recorded but for a minimum of three trials. Reliability was determined between best trial and second-best trial, for which a high reliability can be considered **Table 2**. In the following, after taking a recovery break of 5 min, the maximal dynamic strength of the calf muscles was tested with the knee joint extended. The subject was instructed to assume the starting position (90° ankle joint angle) and to press the applied weight into a maximal plantarflexed position. For this purpose, the covered distance was recorded with a motion sensor from the company "MicroEpsilon" with an accuracy of 0.1 mm. Based on isometric data of the previous testing, we added weight corresponding to 60% of the maximal strength. After each trial, we added weight (first 10 kg, then 5 or 2.5 kg) on the leg press until the participant was no longer able to perform the 1RM for full ROM. The criterion for the end of measurement was the distance measurement *via* the motion sensor. Best trial with full ROM measured was used for further analysis.

Measuring Muscle Thickness

Measures of skeletal muscle architecture were done using two-dimensional B-mode ultrasound (Mindray Diagnostic Ultrasound System). Here, muscle thickness represents the most employed measure of muscle dimension (Sarto et al., 2021) according to its correlation to muscle cross-sectional area, which is proportional to the number of parallel sarcomeres, thereby influencing maximal force production (Lieber & Fridén, 2000; Narici et al., 2016; May et al., 2021). In our examination, ultrasound images from the lateral gastrocnemius were recorded using a linear transducer with a standardized frequency of 12–13 MHz. Each participant was placed prone on a table with the feet hanging down at the end to ensure no contraction in the calf muscles. Then, the sonographer identified the proximal and distal landmark of the lateral gastrocnemius for each participant and measurement (Perkisas et al., 1999). The transducer was placed at 30% of the distance from the most lateral point of the articular cleft of the knee to the most lateral top of the lateral malleolus (see **Figure 3**) (Perkisas et al., 1999). For measuring muscle thickness, the transducer was positioned at the midpoint of the muscle belly perpendicular to the long axis of the leg (Sarto et al., 2021). The muscle belly was determined as the center of the muscle between its medial and lateral borders. This is the point where the muscle's anatomical cross-sectional area is maximal (Fukunaga et al., 1992). In addition, the image plane is best aligned with the muscle's fascicles, including minimal fascicle curvature (Bénard et al., 2009; May et al., 2021; Raj et al., 2012). Before starting the measurement, transmission gel was applied to improve acoustic coupling and to reduce the transducer pressure on the skin. Then, the sonographer ensured that the superficial and deep aponeuroses were as parallel as possible by holding and thereby rotating the transducer around the sagittal-transverse axis to the determined point on the skin without compressing the muscle. Hence, the visibility of the fascicles as continuous striations from one aponeurosis to the other was optimized. Muscle thickness is defined as the linear, perpendicular distance between the two linear borders of the skeletal muscle and was obtained by averaging three measurements across the proximal, central, and distal portions of the acquired ultrasound images (Franchi et al., 2017; Sarto et al., 2021). Two persons independently evaluated muscle thickness using the image processing software GIMP 2.10.28. The objectivity of the evaluators was found to be between 0.85 (control leg) and 0.94 (intervention leg).

FIGURE 4 | Sliding device for the KtW to evaluate flexibility in the ankle.

FIGURE 5 | Measuring device for maximal dorsiflexion *via* goniometer attached to the orthosis.

In the literature, high-reliability values of up to r = 0.9 for determining muscle thickness *via* ultrasound for within-day reliability (Nabavi et al., 2014; Cuellar et al., 2017) and with ICC values of up to 0.88 for between-day reliability are considered high (König et al., 2014; Rahmani et al., 2019a, 2019b).

Reliability was determined between best and second-best value and the "with-in day" reliability determined in this paper can be classified as high with a value of r = 0.98. ICC, CV and SD are listed in **Table 2**, too. Two persons evaluated the ultrasound images independently from each other.

ROM Measurement

ROM in the upper ankle joint was recorded in IG and CG *via* the functional "knee to wall stretch" test (KtW) and the angle-measuring device on the orthosis. A sliding device was used for the KtW. The subject was instructed to place the foot on the attached marker. The contralateral leg was held in the air, and the subject was allowed to hold onto the wall with his hands. To record the range of motion, the subject pushed the board of the sliding device forward until the heel of the standing leg lifted off. For this purpose, the investigator pulled on a sheet of paper placed under the subject's heel. The measurement was finished as soon as this could be removed. The mobility was read in cm from the attached measuring tape (**Figure 4**). Depending on ankle ROM, this measurement can be seen as screening flexibility in bended knee. Three valid trials were performed per leg, and the maximal value was used for evaluation. Reliability was determined between best trial and second-best trial and can be classified as high **Table 2**.

Since we measured maximal strength in extended knee joint, we used the angle measurement device of the orthosis which could be used as goniometer (ORT) to measure maximal dorsiflexion in extended knee joint (see **Figure 5**). For this purpose, the foot of the participant should place his foot on a support plate at the same height as the chair. While wearing orthosis the foot was pushed into maximal dorsiflexed position with extended knee joint. Starting position was neutral 0 position in the ankle. Each big mark of the angle measurement device corresponds to a distance of 5°, and each little mark corresponds to a distance of 2.5°. The achieved marker was read off from the angle measurement device of the orthosis. Reliability was determined between best trial and second-best trial and can be classified as high, **Table 2**.

To improve comprehension of testing procedure, in **Figure 6** the study design is presented graphically.

Data Analysis

The analysis was performed with SPSS 28. We used one-way ANOVA with Scheffé post-hoc test to ensure that there were no differences in pre-test values for any measurement. Thus, two-way ANOVA with repeated measures was performed for the collected parameters. Scheffé test was used as post-hoc for mean differences of one-way ANOVA. *p*-Values for percentage changes were determined with pared t-test between pre- and posttest. Effect sizes were presented as Eta squares (η^2) and categorized as: small effect η^2<0.06, medium effect η^2 = 0.06–0.14, large effect η^2>0.14 as well as Cohen's d. (Cohen, 1988) Effect sizes with Cohen's d were categorized as: small effects d < 0.5, medium effect d = 0.5–0.8, large effect d > 0.8. In addition, Pearson correlations were determined between maximal strength and muscle thickness as well as between changes in maximal strength and muscle thickness.

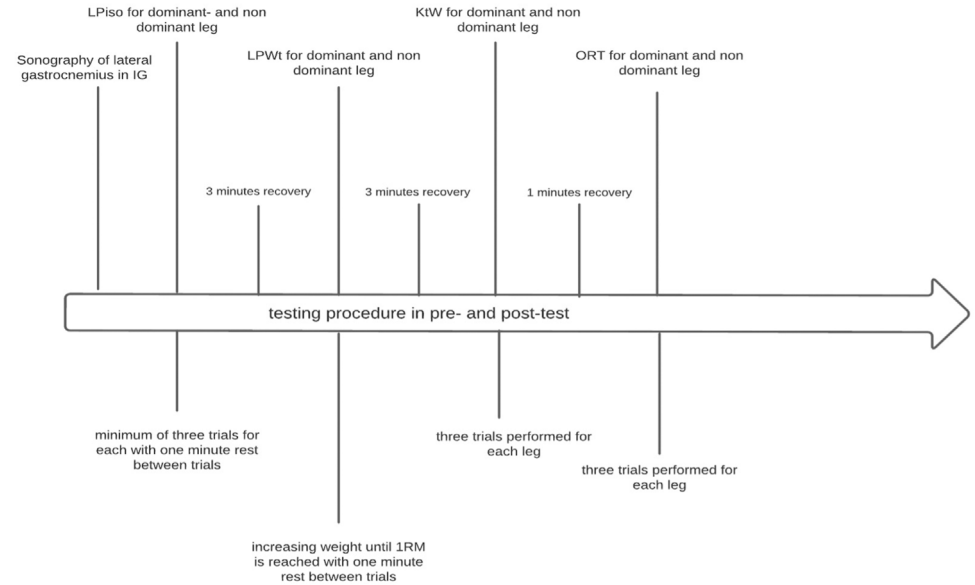

FIGURE 6 | Graphical presentation of study design.

TABLE 3 | Descriptive statistics and two-way ANOVA of maximal strength tests.

Parameter	Pretest (M±SD)	Posttest (M±SD)	Pre-post differences in %	Time effect	Time x group
LPIsoil	1478.4 ± 309.7N	1726.8 ± 315.8N	16.8 ($p < 0.001$)	$p < 0.003$	$p < 0.001$
LPIsocl	1542.3 ± 339.1N	1564.5 ± 300.5N	1.4 ($p = 0.462$)	F = 9.108	F = 19.387
CGR	1585.4 ± 215.1N	1559.0 ± 217.8N	−1.6 ($p = 0.075$)	$\eta^2 = 0.090$	$\eta^2 = 0.387$
CGL	1540.1 ± 184.94N	1518.0 ± 202.55N	−1.4 ($p = 0.164$)	d = 0.629	d = 1.589
LPWtil	91.9 ± 35.0 kg	115.0 ± 32.3 kg	25.1 ($p < 0.001$)	$p < 0.001$	$p < 0.001$
LPWtcl	93.5 ± 32,3 kg	104.2 ± 34.4 kg	11.4 ($p < 0.001$)	F = 22.028	F = 17.434
CGR	96.9 ± 27.6 kg	95.0 ± 28.6 kg	−1.2 ($p = 0.467$)	$\eta^2 = 0.193$	$\eta^2 = 0.362$
CGL	98.6 ± 27.8 kg	95.0 ± 28.4 kg	−3.6 ($p = 0.214$)	d = 0.978	d = 1.506

LP = leg press; iso = isometric maximal strength; il = intervened leg; cl = control leg; Wt = weight in dynamic maximal strength; CG = control group; R = right; L = left.

RESULTS

All subjects who appeared for the pretest completed the examination. No significant problems with the orthosis were reported and the daily wearing durations were adhered to all subjects.

Results of descriptive statistics as well as the two-way ANOVA are presented in **Table 3**. P- and F- Values of the two-way ANOVA as well as effect sizes η^2 for time dependent effect and interaction effects are displayed.

Analysis of Maximal Strength With Extended Knee Joint *via* Leg Press

One-way ANOVA showed no significant differences between pretest values of all parameters ($p > 0.05$).

Progression and comparison of mean values of maximal strength in pre- and post-testing in the stretched and the control leg of the intervention group is presented in **Supplementary Figure S1**.

Two-way ANOVA demonstrated high effects for the time dependent effect ($\eta^2 = 0.09$ and 0.193) and for the time × group interaction ($\eta^2 = 0.387$ and 0.362).

The Scheffé test determined significant differences for the mean differences between pre- and posttest values in the LPisoil and the LPisocl as well as LPisoil and CGR ($p < 0.001$) and LPisoil and CGL ($p < 0.001$). No significant difference could be determined between the control leg and CGR ($p = 0.415$) as well as control leg and CGL (0.812). Between the legs of the CGs, no significant difference could be detected ($p = 0.927$).

For maximal dynamic strength there were significant differences for the mean differences between pre- and posttest values in LPWtil and LPWtcl ($p = 0.026$), LPWtil and CGR ($p < 0.001$), LPWtil and CGL ($p < 0.001$) as well as LPWtcl and CGR ($p = 0.026$) and LPWtcl and CGL ($p = 0.014$). No significant difference could be determined between CGR and CGL ($p = 0.987$).

TABLE 4 | Descriptive statistics and two-way ANOVA of muscle thickness *via* sonography.

Parameter	Pretest (M±SD) in mm	Posttest (M±SD) in mm	Pre-post differences in %	Time effect	Time x group
SONOil	14.31 ± 2.42	16.5 ± 2.78	15.3 ($p < 0.001$)	$p < 0.001$	$p = 0.015$
SONOcl	14.54 ± 2.32	14.85 ± 2.08	2.1 ($p = 0.03$)	F = 33.588	F = 19.166
				$\eta^2 = 0.545$	$\eta^2 = 0.406$
				d = 2.189	d = 1.653

SONO, sonography; il, intervened leg cl, control leg.

TABLE 5 | Descriptive statistics and two-way ANOVA of ROM tests.

Parameter	Pretest (M±SD)	Posttest (M±SD)	Pre-post differences in %	Time effect	Time x group
KtWil	12.1 ± 3.0 cm	13.7 ± 2.6 cm	13.2 ($p < 0.001$)	$p = 0.011$	$p < 0.001$
KtWcl	12.7 ± 3.9 cm	12.6 ± 3.7 cm	–0.8 ($p = 0.701$)	F = 6.674	F = 16.925
CGR	12.6 ± 1.1 cm	12.3 ± 2.0 cm	–2.4 ($p = 0.007$)	$\eta^2 = 0.068$	$\eta^2 = 0.356$
CGL	12.2 ± 1.8 cm	12.1 ± 1.5 cm	–0.8 ($p = 0.506$)	d = 0.54	d = 1.487
ORTil	6.7 ± 1.9	8.4 ± 2.0	27.3 ($p < 0.001$)	$p < 0.001$	$p < 0.001$
ORTcl	6.8 ± 1.9	7.2 ± 2.1	7.5 ($p = 0.211$)	F = 13.527	F = 7.613
CGR	7.6 ± 1.4	7.6 ± 1.3	0.7 ($p = 0.724$)	$\eta^2 = 0.129$	$\eta^2 = 0.199$
CGL	7.6 ± 1.6	7.6 ± 1.6	0 ($p = 1.000$)	d = 0.77	d = 0.997

KtW, knee to wall stretch; il, intervened leg; cl, control leg; CG, control group; ORT, angle measuring device of the orthosis; R, right; L, left.

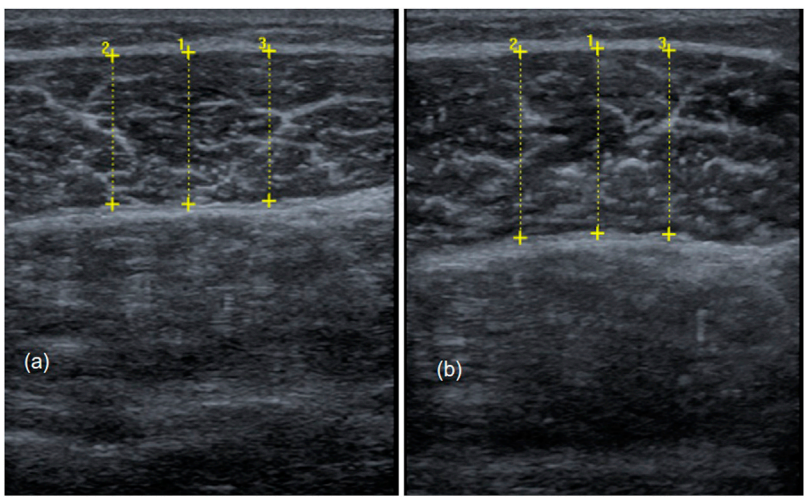

FIGURE 7 | Comparison of muscle thickness from pre-to posttest in the non-stretched control leg **(A)** and the intervened leg **(B)**.

Analysis of Muscle Thickness *via* Sonography

Table 4 shows descriptive statistics as well as time dependent effect and interaction effects of tow-way ANOVA for determining muscle thickness in the calf muscle.

Figure 7 shows examples of sonography measurements from pre to posttest of the control leg and the intervened leg.

Progression and comparison of mean values of muscle thickness in pre- and post-testing in the stretched and the control leg of the intervention group is presented in **Supplementary Figure S2**.

Two-way ANOVA demonstrated high effects for the time dependent effect ($\eta^2 = 0.545$) and for the time × group interaction ($\eta^2 = 0.406$).

Analysis of ROM Values

Progression and comparison of mean values of ROM tested *via* KtW and the angle measurement device of the orthosis (ORT) in pre- and post-testing in the stretched and the control leg of the intervention group is presented in **Table 5** and in **Supplementary Figure S3**.

Two-way ANOVA demonstrated high effects for the time dependent effect (η^2 = 0.068 and 0.129) and for the time × group interaction (η^2 = 0.356 and 0.199). The Scheffé test determined significant differences for the mean differences between pre-to posttest KtWil and KtWcl ($p < 0.001$) as well as between KtWil and CGR ($p < 0.001$) and CGL ($p < 0.001$). No significant difference was found between the control leg and CGR (p = 0.941) and CGL (p = 1.000). Furthermore, no significant difference was found between CGR and CGL (p = 0.959).

Significant differences were found for the mean differences between pre-to posttest for ORTil and the ORTcl (p = 0.019) and ORT and CGR (p = 0.002) and CGL (0.002). No significant differences were found between ORTcl and CGR (p = 0.838) and CGL (p = 0.783), as well as CGR and CGL (p = 1.000) measured *via* angle measuring device of the orthosis.

Pearson correlations determined for muscle thickness and maximal strength show correlations of r = 0.594 in the pre-test as well as 0.74 for post-test values. However, Pearson correlation for increases from pre-to post-test show no significant relationship with r = 0.02 (p = 0.935).

DISCUSSION

In previous research, we already compared effects of one hour vs. two hours static stretching on maximal isometric strength in bended knee joint. Significant differences in required muscle groups in maximal strength testing between bended and extended knee joint (Signorile et al., 2002; Arampatzis et al., 2006) as well as type of contraction—isometric vs. dynamic testing condition—(Murphy & Wilson, 1996; Feeler et al., 2010) can be assumed.

In this work, a significant improvement in maximal strength in the calf muscles was achieved by daily one-hour stretching training. There was a significant improvement in maximal isometric strength production determined in the extended knee joint by approximately 16.8% from 1478.4 ± 309.7N in pretest to 1726.8 ± 315.8N in the stretched leg. In comparison, an average maximal strength increase of 1.4% from 1542.3 ± 339.1N to 1564.5 ± 300.5N was determined in the non-stretched control leg while no significant increase was determined between legs of CG. Furthermore, we determined enhanced maximal dynamic strength *via* 1RM testing by 25.1% and 11.4% from 91.9 ± 35 kg to 115 ± 32.3 kg and 93.5 ± 32.3 kg to 104.2 ± 34.4 kg in the stretched and non-stretched control leg, respectively. In both legs in CG no significant change in 1RM could be determined. For all maximum strength measurements, large effect sizes were shown for interaction effect in ANOVA (η^2>0.14 and d > 0.8). In addition, we measured significant hypertrophy effects in the lateral head of the gastrocnemius of 15.2% from in the intervention leg vs. 2.1% in the control leg. In the intervened leg, we determined and increase 14.31 ± 2.42 mm to 16.5 ± 2.78 mm. In control leg muscle thickness, we found muscle thickness of 14.54 ± 2.32 in pretest and 14.85 ± 2.08 mm in posttest. Furthermore, moderate correlations between maximal strength values in the extended knee joint and muscle thickness in the pre-test (r = 0.594; p = 0.012) and between maximal strength values and muscle thickness in the post-test (r = 0.74; $p < 0.001$) were determined but no correlation was found for increases in maximal strength and muscle thickness from pre-to post-test. From this, it can be assumed that maximal strength increases are not related to increases in muscle thickness so that further investigations are required to examine the origin of maximal strength increases. The initial hypothesis can be accepted to a large extent. We examined high interaction effects (η^2>0.14 and d > 0.8) in the extended knee joint in isometric and dynamic conditions. In both maximum strength tests there were significant increases in maximum strength values in the intervened leg. However, Scheffé test showed no significant differences between maximal strength increases in non-stretched control leg and both legs of the control group. Although the changes in maximal strength of the control leg are not significantly different from the control group under isometric conditions, while Scheffé test showed significant differences between the non-stretched control leg of the intervention group compared to both legs of CG.

In the present work, a stretching duration of 1 hour per day and a weekly volume of 7 hours was realized, which led to comparable results in maximal strength as can be expected from strength training performed two to three times per week (Aube et al., 2020; Pearson et al., 2021). The recorded maximal strength gains can possibly be attributed to muscular adaptations to the mechanical stimuli. A mechanical tension can be seen as an initiating stimulus to induce various cellular processes or signal transduction and induce changes in muscle morphology (Tatsumi, 2010; Mohamad et al., 2011; Riley & van Dyke, 2012; Boppart and Mahmassani, 2019). This so-called mechanotransduction can induce tension-induced muscle hypertrophy (Aguilar-Agon et al., 2019). Smith et al. (1993) and Jacobs & Sciascia (2011) previously showed that stretching tension of sufficient intensity can lead to DOMS and associated inflammation. After this microtraumatization of muscle tissue, the repair processes are related to stimulation of protein synthesis rate (Goldspink & Harridge, 2003; Brentano & Kruel, 2011). Because maximal strength production is closely related to the muscle cross-sectional area of the force-generating muscle, we assume that the muscle tension generated by the one-hour stretching intervention was sufficient to produce muscle hypertrophy and maximal strength gains. We determined muscle thickness *via* ultrasound measurement to investigate structural adaptations of the one-hour stretching training. A similar procedure has already been used by Simpson et al. (2017). The authors investigated the adaptive responses of a three-minute stretching training performed five times per week on maximal strength, muscle thickness, and muscle architecture. Although there were no significant improvements in maximal strength while authors showed muscular hypertrophy (+5.6% in muscle thickness) in addition, Panidi et al. (2021) were also able to determine an enhanced muscle cross-sectional area of 23 ± 14% in the intervention leg vs. 13 ± 14% in the control leg by a 12-week stretching intervention. The cause of the structural change on the control leg seems questionable here due to stretching intervention and possibly are attributed to regular training of the included participants. While central nervous

adaptations may be responsible for the contralateral force transfer, which was also recorded in this study, the source of hypertrophic effects on the contralateral leg of 13% must be considered critically, especially since no control group was included in the study. Thus, habituation effects and associated performance gains cannot be ruled out to improve maximal strength production in the non-stretched control leg either.

Another possible explanation for enhanced maximum strength production can be seen in possible changes in muscle architecture, e.g., changes in pennation angle and fascicle length (Cormie et al., 2011a; 2011b). The enhanced maximal strength due to a larger pennation angle is achieved by allowing more sarcomeres to be arranged parallel. In contrast, a higher fascicle length results in optimizing the muscle's tension-length relationship. While we did not examine muscle architecture and fascicle length, Simpson et al. (2017) found a decrease in pennation angle and an increase in fascicle length in addition to muscle hypertrophy. Normally, a bigger muscle cross sectional area is correlated to an increased pennation angle (Cormie et al., 2011a, 2011b; Suchomel et al., 2018). Consequently, further studies should investigate the influence of long-lasting stretching interventions on muscle architecture as a potential factor for improved maximal strength values. In addition, the changes in muscle architecture recorded by Simpson et al. (2017) suggest an influence on the contraction velocity of the stretched muscle. In addition, study by (Möck et al., 2019) established moderate to high correlations between maximal strength in the calf muscles and sprint performance. Because of achieved significant increase in maximal strength due to one-hour stretching intervention, the influence on sport-specific parameters as jumping and sprinting performance should be investigated in further investigations. Therefore, Panidi et al. (2021) provide first results by recording jumping performance after a twelve-week intervention and examined 27% enhanced vertical jumping heights due to one legged counter movement jump.

While there are studies showing positive effects of stretching interventions on maximal strength (Kokkonen et al., 2007; Nelson et al., 2012; Mizuno, 2019; Yahata et al., 2021) and muscle thickness (Abdel-Aziem & Mohammad, 2012; Moltubakk et al., 2021), there are also studies showing no effects on strength capacity (Sato et al., 2020; Nakamura et al., 2021), hypertrophy and muscle architecture (Nunes et al., 2020; Yahata et al., 2021). Assuming significant influence of stretching intensity on adaptations of the muscle-tendon unit (Apostolopoulos et al., 2015; Nakamura et al., 2021) partially differences in results may be explainable due to heterogeneity in study design of these studies. Most studies did not quantify stretching intensity (Kokkonen et al., 2007; Nelson et al., 2012; Mizuno, 2019) and stretching duration varied to a high degree from 4 × 30 s on 3 days per week (Nelson et al., 2012; Mizuno, 2019) to 6 × 5 min on 2 days per week (Yahata et al., 2021) with very different exercises. Consequently, comparability of results must be questioned and quantification in particular regarding is requested.

Previous studies showing significant increases in maximal strength and/or muscle thickness used shorter stretching duration. Highest stretching volume found in literature was 6 × 5min per session with a weekly volume of 1 h, which was used in our study within 1 day. Compared to Yahata et al. (2021) determining a mean enhancement in maximal isometric strength of 6.4% and 7.8% in maximal dynamic strength with no improvement in muscle thickness, our results show higher increases in maximal strength capacity as well as an improvement in muscle thickness. Considering that we used seven times of the stretch volume compared to Yahata et al. (2021), we demonstrated that increasing the stretching duration leads to increased adaptations as well. Further investigations should examine the most economic stretching duration to improve maximal strength.

Since a contralateral force transfer could be recorded, especially in 1RM measurement, increments in MSt cannot be exclusively attributed to tension-induced hypertrophy effects. After performing intensive strength training, improved distribution of anabolic hormones can be hypothesized, which also have an anabolic effect on the non-stretched calf muscle. However, it seems questionable whether a stretching of the calf muscles of 1 h can result in such a deflection, since especially the amount of hormonal change seems to depend on the size of the involved muscles (Fleck & Kraemer, 2004) and the calf can be considered a relatively small muscle group. In addition to hypertrophy effects in the stretched leg, we hypothesize neuromuscular adaptation through stretching as an additional reason for the effect on maximal strength, since contralateral force transfer due to strength training is also primarily explained by neuromuscular adaptations (Green & Gabriel, 2018; M.; Lee et al., 2009; M.; Lee & Carroll, 2007). Therefore, the inclusion of EMG studies is necessary to clarify neuromuscular adaptations. Since neuromuscular deficits, as well as a loss of muscle mass and cross-sectional area (sarcopenia), lead to reduced balance ability and thus an increased risk of falls (Gschwind et al., 2013; Lacroix et al., 2017), the influence of long-term stretching on balance ability can be investigated in future studies. The calf muscles can be considered relevant, especially in this context (Stolzenberg et al., 2018; Reynoldsid et al., 2020).

Significant improvements in ROM, determined *via* the KtW, were also found to average 13.2% from 12.1 ± 3.0 cm to 13.7 ± 2.6 cm in the intervention leg, while the values for the control leg did not change significantly with −0.8% from 12.7 ± 3.9 cm to 12.6 ± 3.7 cm. ROM values in both control legs measured with KtW did not change significantly. Measurement of ROM by the orthosis revealed a significant improvement of 27.3% in intervened leg from 6.7 ± 1.9 to 8.4 ± 2.0 which corresponds to an angle of 33.5 ± 9.5°–42.5 ± 10°. The contralateral control leg improved flexibility measured *via* the angle measurement device of the orthosis by 7.5% from 6.8 ± 1.9 to 7.2 ± 2.1 with corresponding angle improvement from 34 ± 9.5° to 36 ± 10.5°. No significant changes in ROM could be determined for both legs of the control group.

The influence of stretch training on ROM has already been extensively studied (Medeiros et al., 2016; Medeiros & Martini, 2018). Improvements in ROM in the present study of 13% in the KtW and 27% measured *via* orthosis can possibly be attributed to an increase in serial sarcomere number. In animal experiments,

this so-called longitudinal hypertrophy has already been demonstrated by a long-lasting stretch intervention (Antonio et al., 1993; Alway S., 1994, Alway, S. E. 1994). Freitas et al. (2018) and Magnusson (1998)point to an altered pain tolerance at high stretch levels, rather than morphological muscle adaptation, as the cause of expansions in ROM.

Highest effects of stretching the plantar flexors with the orthosis on maximal strength and ROM were determined in testing conditions in extended knee joint. This is explainable as stretching was performed in extended knee joint as well. However, there were significant improvements in maximal strength measured in previous examination of our group and ROM in bended knee joint, too. For listed testing conditions there were significant increases in maximum strength and for 1RM testing significant improvements of the non-stretched control leg. In ROM, no significant effect of the daily 1 h stretching training could be determined in the non-stretched control leg in regard to both control legs.

In conclusion, increases in maximum strength can be commonly attributed to changes in innervation of the central nervous system, changes in muscle architecture or, independently from that, muscle hypertrophy (Loenneke et al., 2019)

Limitations

Several studies could be found in which ultrasound measurement was used to determine muscle cross-sectional area (Nabavi et al., 2014; Cuellar et al., 2017; Simpson et al., 2017; Messina et al., 2018; Albano et al., 2020; Panidi et al., 2021). In particular, investigating muscle cross-sectional area *via* sonography offers advantages over MRI examinations in terms of cost and time (Sergi et al., 2016). However, stronger or weaker pressure of the ultrasound probe on the muscle belly can influence muscle thickness, so there is a subjective influence on the result. To counteract this, in this study, we took three image acquisitions in succession per leg for each measurement and had the same examiner perform the pretest and posttest of one subject. From a measurement methodology perspective, sonography can be used to investigate structural changes in the muscle, if investigators and evaluators are experienced but the use of MRI images must be considered the gold standard for determining muscle cross-section (Messina et al., 2018; Albano et al., 2020), especially because all subjective factors can be excluded. No randomization could be performed for the present study because not all included subjects agreed to wear the orthosis for 1 h per day.

PRACTICAL APPLICATIONS

The effects of the training method of long-term stretching on maximal strength, muscle cross-sectional area, and flexibility were investigated in this study can be used in diverse areas. "The therapeutic applications of stretch should therefore be borne in mind when designing regimes for rehabilitation or improved athletic performance" (MacDougall, 2003). Its use in the rehabilitation of orthopedic conditions or lower extremity injuries that result in immobilization seems particularly relevant. A stretching intervention would already be applicable if, due to immobilization or corresponding injuries and diseases, voluntary activation of the musculature in the context of strength training is not (yet) feasible. This could minimize muscle atrophy and loss of strength. Prostheses and cartilage transplants (in the knee and hip) result in long periods of immobilization. This is associated with muscular atrophy (Stevens et al., 2004; Perkin et al., 2016).

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the medical ethics committee of Carl von Ossietzky University of Oldenburg. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

KW carried out the experiment, performed the analytic calculations and took the lead in writing the manuscript with support from AB, SS, and MH. AB supervised and directed the analysis of ultrasound images and helped and assisted in writing the manuscript. MH conceived the main conceptual ideas and planned the experiments in consultation with KW and SS. SS supervised the project and provided critical feedback to the design of the study and the statistical analysis. All authors discussed the results and contributed to the final version of the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2022.878955/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Influence of One Hour versus Two Hours of Daily Static Stretching for Six Weeks Using a Calf-Muscle-Stretching Orthosis on Maximal Strength

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Abstract: Rebuilding strength capacity is of crucial importance in rehabilitation since significant atrophy due to immobilization after injury and/or surgery can be assumed. To increase maximal strength (MSt), strength training is commonly used. The literature regarding animal studies show that long-lasting static stretching (LStr) interventions can also produce significant improvements in MSt with a dose–response relationship, with stretching times ranging from 30 min to 24 h per day; however, there is limited evidence in human studies. Consequently, the aim of this study is to investigate the dose–response relationship of long-lasting static stretching on MSt. A total of 70 active participants (f = 30, m = 39; age: 27.4 ± 4.4 years; height: 175.8 ± 2.1 cm; and weight: 79.5 ± 5.9 kg) were divided into three groups: IG1 and IG2 both performed unilateral stretching continuously for one (IG1) or two hours (IG2), respectively, per day for six weeks, while the CG served as the non-intervened control. MSt was determined in the plantar flexors in the intervened as well as in the non-intervened control leg to investigate the contralateral force transfer. Two-way ANOVA showed significant interaction effects for MSt in the intervened leg ($\eta^2 = 0.325$, $p < 0.001$) and in the contralateral control leg ($\eta^2 = 0.123$, $p = 0.009$), dependent upon stretching time. From this, it can be hypothesized that stretching duration had an influence on MSt increases, but both durations were sufficient to induce significant enhancements in MSt. Thus, possible applications in rehabilitation can be assumed, e.g., if no strength training can be performed, atrophy could instead be reduced by performing long-lasting static stretch training.

Keywords: plantar flexors; stretch training; rehabilitation; immobilization-related strength deficit; physical therapy

Citation: Warneke, K.; Keiner, M.; Hillebrecht, M.; Schiemann, S. Influence of One Hour versus Two Hours of Daily Static Stretching for Six Weeks Using a Calf-Muscle-Stretching Orthosis on Maximal Strength. *Int. J. Environ. Res. Public Health* **2022**, *19*, 11621. https://doi.org/10.3390/ijerph191811621

Academic Editors: Paul B. Tchounwou and Pantelis T. Nikolaidis

Received: 2 August 2022
Accepted: 14 September 2022
Published: 15 September 2022

1. Introduction

Increasing strength capacity is of high importance to the prevention [1] and rehabilitation of orthopedic indications [2,3]. Mechanical tension is an important stimulus to achieving an increase in maximal strength (MSt), which is commonly induced by strength training [4–6]. While low intensities seem to be sufficient to induce hypertrophy [7,8], a load intensity of 60–80% of the one-repetition maximum (1RM) is generally recommended as an appropriate method to achieve both MSt and muscle hypertrophy [4,9]. From this, it can be hypothesized that high intensities seem to be more beneficial to achieve improvements in MSt [8,10]. Results regarding animal research from between 1970–1995 show that long-lasting static stretching can induce sufficient mechanical stress, leading to muscle hypertrophy and increases in MSt [11–14], too. Assuming a transferability to humans, static stretching could be used in the rehabilitation of injury-related immobilization of lower limb muscles, as this usually results in significant atrophy and strength loss [15,16]. To date, there are some studies that have investigated the effects of short duration stretch training for several weeks on the maximal strength of humans [17–19]. Many of these studies show

a significant increase in MSt due to a long-term static stretching intervention [17,20,21]. Yahata et al. [22] found a significant increase in MSt of 6.4 ± 9.9% in the calf muscles within a five-week period by stretching twice a week with durations of 6 × 5 min using a stretching board. In addition, Kokkonen et al. [20], Nelson et al. [17] and Mizuno et al. [23] were able to achieve significant MSt increases of 15.3% to 32.4% due to stretch training for up to ten weeks, while other studies investigated the effects of a daily stretching routine on concentric peak torque and found significant increases of 9.3% [24] and 11% [16], respectively.

Cross-education effects are known from unilateral strength training [25–27]. Zhou [28] and Zhou et al. [29] suggested that adaptations in motor learning are a preferred explanation of cross-education effects by showing that the electrical stimulation on one leg results in strength increases in the contralateral leg. The authors hypothesized that the cross-training effects could be attributed to afferent modulations. Based on these suggestions, Nelson et al. [17] hypothesized that static stretching could also provide a sufficient stimulus to induce a contralateral force transfer due to its activation of afferents and the impact on MSt in the stretched limb. The authors confirmed their hypothesis by demonstrating an increase in maximal strength of up to 29% (d = 1.24) in the stretched leg, which was accompanied by a contralateral force transfer of 11% to the contralateral leg (d = 0.46) by inducing 4 × 30 s of stretching for three days per week for ten weeks. Furthermore, Caldwell et al. [30] showed that stretching the quadriceps twice daily for two weeks resulted in significant increases in MSt of 7.1% (d = 0.8) in the stretched leg and of 6.6% (d = 0.45) in the contralateral leg, while stretching once per day did not lead to a contralateral force transfer. Warneke et al. [31] also showed cross-educational effects in dynamic strength testing of 11.4% ($p < 0.001$) after stretching the plantar flexors for one hour, while no effects were obtained in isometric strength testing (1.4%, $p = 0.46$). Furthermore, jumping height was also increased in the stretched as well as in the contralateral leg with 13.7%, d = 1.28 and 13%, d = 1.01, respectively. This was confirmed by Panidi et al. [32] who showed improvements in jumping height of 27%, d = 0.78 and 17%, d = 0.46 in the stretched and unstretched control leg, respectively. However, Handel et al. [33] found no effects in the contralateral side from unilateral training.

Only one investigation has examined the long-term effects of long-lasting static stretching on MSt, showing significant improvements in MSt of about 16% in the plantar flexors by using a one-hour stretching protocol daily [31]. However, some investigations could not point out any significant changes in MCSA or maximal strength after several weeks of stretch training [18,22,34]. Inconsistencies in intensity and volume could explain the heterogeneity of the results in the listed investigations in regard to MSt gains following a stretching stimulus. Furthermore, Apostolopoulos et al. [35] pointed out the high relevance of high intensity in stretching interventions: at lower intensities, tension is compensated via elastic tissue instead of generating adequate muscle tension. Considering a dose–response relationship of stretching in animal experiments [36,37] and the results from Warneke et al. [31], it can be assumed that long-lasting stretching could lead to improved MSt capacity in humans, which can be seen as highly relevant in designing rehabilitation programs: "The therapeutic application of stretch should therefore be kept in mind when designing regimes for rehabilitation" [38]. Therefore, the aim of this longitudinal study was to investigate the effects of a stretching stimuli lasting one or two hours per day, respectively, on MSt in the plantar flexors with a bended knee joint, and to investigate whether a contralateral strength transfer can be induced via long-lasting stretching interventions.

2. Materials and Methods

To answer the research question, MSt in the plantar flexors was examined with a bended knee joint via unilateral isometric strength testing for both legs in pre- and post-test. Afterwards, a daily unilateral stretching intervention was performed for one or two hours using a stretching orthosis for six weeks.

2.1. Participants

The study procedure was approved by the ethics vote 2019-016 of the ethics committee of the Carl von Ossietzky University Oldenburg. Seventy active participants were recruited from sports study programs, sports clubs and gyms. They were divided into three groups (two experimental groups (IG1 and IG2), as well as one control group (CG). Characteristics of subjects are provided in Table 1. The differences in group size can be explained by the willingness of active participants to partake in the long-lasting intervention groups; especially in IG2 with a stretching duration of two hours per day. Participants were categorized as active if they performed two to three training sessions in a gym or team sport continuously for the past six months. They were instructed to continue with their previous training routine throughout the six-week intervention period; consequently, long-lasting stretching was performed in addition to existing routines. However, participants were not allowed to perform any separated calf muscle training within the six-week training intervention.

Table 1. Characteristics of subjects.

Group	N	Age (in Years)
Total	70	24.1 ± 3.5
IG1	25 (f = 7; m = 18)	23.4 ± 4.7
IG2	15 (f = 3; m = 12)	27.2 ± 5.3
CG	30 (f = 14; m = 16)	24.6 ± 3.8

2.2. Testing Procedure

2.2.1. Maximum Strength Measurement

All participants performed a pre- and post-test. For all participants, the maximum isometric strength (MStiso) in the bended knee joint was recorded for both legs using unilateral testing (see Figure 1). For this purpose, the participant was instructed to perform a plantar flexion for three seconds with a maximum voluntary contraction against the pad of the measuring device in response to an acoustic signal. The seated calf raise machine was adjusted for each participant to achieve a 90° angle in the participant's ankle and knee joints. Testing was performed until the force values stopped increasing with a minimum of five trials. The MStiso was determined in each case using a 10 × 10 cm force measurement platform in which force sensors Kistler Element 9251A with a resolution of 1.25 N, a pull-in frequency of 1000 Hertz and a measurement range of ±5000 N were installed. The vertical forces (Fz) were recorded. A Typ5009 Charge Amplifier and a 13-bit AD converter NI6009 were used. A calculation program (Carl von Ossietzky University Oldenburg) was used to illustrate the force–time curves of the vertical forces from the unfiltered raw data to provide an objective determination of MSt for further evaluation, and to rule out any artifacts that could affect the results. Reliability was determined with an ICC between the best and second-best value reached in each test, which were classified as high when the ICC = 0.994 and CV = 1.89%.

Figure 1. Calf-muscle-testing device (CMD) measuring the maximum isometric strength in pre- and post-test with force plates.

2.2.2. Intervention

The intervention consisted of daily stretch training of the calf muscles, induced by wearing an orthosis developed for this purpose, which comprised an angle-measuring apparatus to quantify the stretching intensity (see Figure 2). Stretching was performed on only one leg to be able to investigate the cross-education effects. Thereby, the intervention was performed on the dominant leg. Group IG1 stretched for one hour per day and group IG2 stretched for two hours per day for seven days per week over a period of six weeks. Thus, a weekly stretching volume of seven hours in IG1 and fourteen hours in IG2 was performed. Daily stretch training was performed because similar animal experiments exhibited significant positive effects on MSt gains [11,12]. The orthosis had to be worn with the knee extended. Participants were instructed to sit with their back straight against a backrest while wearing the orthosis and to place their foot on an object that was the same height as the chair they were sitting on. In order to address the general problem of documenting the training intensity in stretching interventions, the angle of the ankle joint was set and controlled via the goniometer on the orthosis. The intensity was to be set by the participant so that the individual stretching pain corresponded to an eight on a scale of 1–10, where 1 represented no stretching pain and 10 was determined as the maximal tolerable stretching pain. The angle set for this purpose was read off by the participants and documented in the stretch diary.

Figure 2. Orthosis used for calf-muscle stretching.

2.3. Data Analysis

The analysis was carried out with SPSS 28. As previously performed by Caldwell et al. [30], a mixed-model ANOVA was performed for the collected parameters with variables tested for group, time and interaction group * time. Separate two-way ANOVAs with repeated measures were used for the three groups (IG2, IG1 and CG) to analyze the influence of static stretching on the intervened and the non-intervened leg. Another separate analysis was performed which included four groups, investigating the contralateral force transfer (intervened leg of IG1, non-intervened leg of IG1 and both legs of the CG; and intervened leg of IG2, non-intervened leg of IG2 and both legs of the CG). The Scheffé test was used as a post-hoc test. Effect sizes are presented as Eta squared (η^2) and categorized as: small effect $\eta^2 < 0.06$, medium effect $\eta^2 = 0.06–0.14$, large effect $\eta^2 > 0.14$ [39]. Furthermore, effect sizes for increases in MSt were calculated and provided [39]. Power analysis was performed by using post-hoc power analysis via G-Power.

3. Results

The normal distribution of data was ensured by using the Shapiro–Wilk test. All participants who participated in the pre-tests completed the study. No problems were reported on the intervention and the use of the orthosis. The prescribed wearing durations were fulfilled by all participants. Levene's test for variance homogeneity yielded $p > 0.05$.

3.1. Overall Statistics

Table 2 provides the mean (M) and standard deviations (SD) for the pre- and post-test values of all included groups.

Table 2. Descriptive statistics of the maximum strength values in intervention groups as well as in control group in pre- and post-testing for the stretched leg.

Group	Pre-Test (M ± SD) in N	Post-Test (M ± SD) in N
IG1il	1195.3 ± 321.09	1364.54 ± 355.43
IG1cl	1210.6 ± 371.8	1277.2 ± 343.2
IG2il	1144.2 ± 244.7	1397.9 ± 366.5
IG2cl	1151.7 ± 306.5	1277.2 ± 380.8
CGl	1076.3 ± 364.5	1056.0 ± 332.7
CGr	1100.9 ± 346.1	1088.9 ± 364.8

The results showed no significant group effect for the pre-test in the intervened leg with $F_{2.72} = 0.96$, $p = 0.39$ with $\eta^2 = 0.026$, as well as in the non-stretched leg with $F_{2.72} = 0.72$, $p = 0.49$, $\eta^2 = 0.02$. In the intervened groups, a significant post-test effect was observed with $F_{2.72} = 8.08$, $p < 0.001$ and $\eta^2 = 0.18$, while there was no significant group effect for post-test values in the contralateral leg $F_{2.72} = 2.54$, $p = 0.086$, $\eta^2 = 0.07$.

In the overall statistics, the two-way ANOVA revealed high effects for the time effect ($F_{1.69} = 48.48$; $\eta^2 = 0.2.75$), as well as for the interaction effect group * time ($F_{2.68} = 10.06$; $\eta^2 = 0.28$) with $p < 0.001$. The mean value in IG1 increased by 14.2% ($p < 0.001$, d = 0.51) from pre-test to post-test, and by 22.3% ($p < 0.001$, d = 0.91) in IG2; the CG did not change significantly, changing by 1.9% ($p = 0.45$). The mean value in IG1 increased by 5.5% ($p = 0.024$, d = 0.18) from pre-test to post-test, and by 10.9% ($p = 0.011$, d = 0.36) in IG2; the control group did not change significantly, changing by 1.1% ($p = 0.45$).

The group differences determined by the Scheffé test showed significant differences between IG1il and the CG ($p = 0.003–0.004$), as well as between IG2il and the CG ($p < 0.001$). No significant differences could be determined between IG1il and IG1cl ($p = 0.392$), or between IG2il and IG2cl ($p = 0.41$). Furthermore, the Scheffé test showed no significant differences for IG1cl and the control group ($p = 0.56–0.60$), or between IG2cl and the control group ($p = 0.14–0.16$).

For a more precise analysis that considers the differences between separate legs, further analysis was performed for the intervened legs of IG1 and IG2 compared to CGl, and the non-intervened leg compared to CGr.

3.2. Analysis of Maximum Strength Tests of the Intervened Leg

The two-way ANOVA revealed high effects for the time effect ($F_{1.69}$ = 54.245; η^2 = 0.430, d = 1.74), as well as for the interaction effect group * time ($F_{2.68}$ = 18.494; η^2 = 0.325, d = 1.39) with $p < 0.001$. The mean value in IG1 increased by 14.2% ($p < 0.001$, d = 0.51) from pre-test to post-test, and by 22.3% ($p < 0.001$, d = 0.91) in IG2; the CG did not change significantly, changing by 1.9% ($p = 0.45$). The group differences determined by the Scheffé test exhibited significant differences between the mean of IG1 and the CG ($p < 0.001$), and IG2 and the CG ($p < 0.001$). No significant differences were found between IG1il and IG2il ($p = 0.23$).

3.3. Analysis of Maximum Strength Tests of the Non-Intervened Leg

Descriptive data of MStiso testing of the intervened legs of IG1il and IG2cl, as well as the left legs of the control group are provided in Table 2.

The two-way ANOVA revealed medium effects for the time effect ($F_{1.69}$ = 10.761; $p = 0.002$; η^2 = 0.130, d = 0.77) and the interaction effect group * time ($F_{2.68}$ = 5.063; $p = 0.009$; η^2 = 0.123, d = 0.749). The mean value in IG1cl increased by 5.5% ($p = 0.024$, d = 0.18) from pre-test to post-test, and by 10.9% ($p = 0.011$, d = 0.36) in IG2; the control group did not change significantly, changing by 1.1% ($p = 0.45$). The Scheffé test showed a significant difference in mean differences, only for IGcl2 vs. the CG ($p = 0.014$).

Figure 3 shows the mean value curve of the maximum strength values in intervention groups, as well as in the control group in pre- and post-testing for the stretched leg. Values in IG1 and IG2 represent the stretched leg. Values in the CG represent the left leg of the control group.

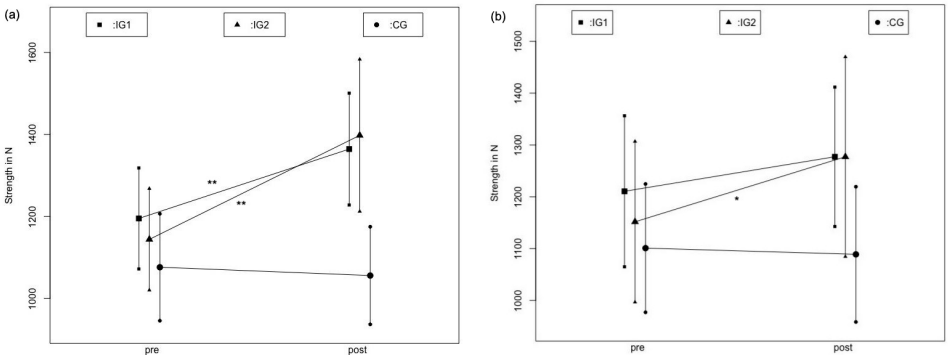

Figure 3. Comparison of maximum strength in pre- to post-test between IG1il, IG2il and CG (**a**) as well as between IG1cl, IG2cl and CGr (**b**). ** = $p < 0.001$, * = $p < 0.05$ for difference to control group.

3.4. Analysis of the Stretched Leg versus the Non-Stretched Leg within One Group to Examine the Contralateral Force Transfer

For IG1, the two-way ANOVA revealed a significant moderate time effect with $F_{1.116}$ = 17.78, $p < 0.001$, η^2 = 0.13 and a significant high interaction effect group * time with $F_{3.116}$ = 12.84, $p < 0.001$, η^2 = 0.25. The Scheffé test showed significant differences between IG1il and both legs of the control group ($p < 0.001$), but no significant difference between IG1il and IG1cl ($p = 0.062$). No significant differences between IG1cl and both legs of the CG were determined ($p = 0.96$–0.156). For IG2, the two-way ANOVA revealed a significant moderate time effect with $F_{1.98}$ = 28.95, $p < 0.001$, η^2 = 0.23 and a significant high interaction effect group * time with $F_{3.78}$ = 15.48, $p < 0.001$, η^2 = 0.32. The Scheffé test showed significant differences between IG2il and both legs of the control group ($p < 0.001$), but no significant difference between IG2il and IG2cl ($p = 0.14$). A significant difference between IG2cl and both legs of the CG were also determined ($p = 0.02$–0.033).

The results are graphically illustrated in Figure 4, presenting changes in MStiso from the pre- to post-test for IG1il and cl, and both test groups for the CG and for IG2il and cl, and both test groups for the CG.

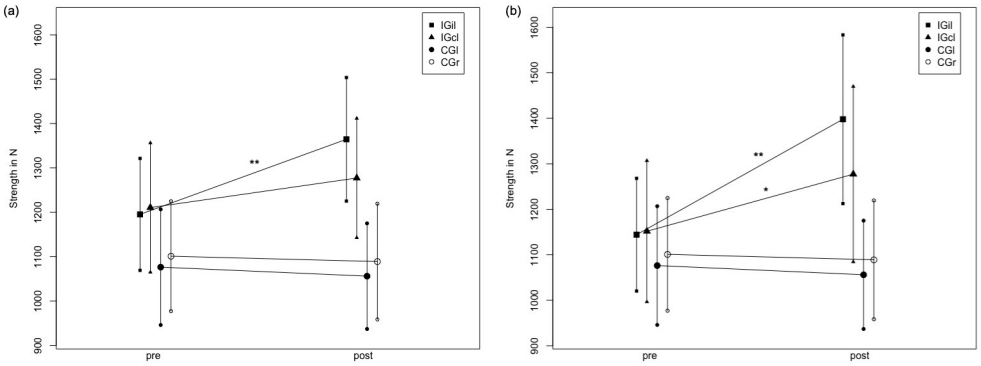

Figure 4. Illustrating the mean value curve of the maximum strength values in IG1il, IG1cl and both groups of CG (CGl and CGr) (**a**) as well as the mean value curve of the maximum strength values in IG2il, IG2cl and both groups of CG (**b**). ** = $p < 0.001$, * = $p < 0.05$ for difference to control group.

Post-hoc analysis for F-tests of G-Power were calculated as $1 - \beta = 42.00\%$ for the lowest effect size with $\eta^2 = 0.123$, and $1 - \beta = 99.99\%$ for the highest effect size with $\eta^2 = 0.430$, and with $\alpha = 0.05$ for the three groups and two time points for the interaction.

4. Discussion

Stretch training of one and two hours, respectively, resulted in significant increases in MStiso ($p < 0.001$ in both groups), while no significant increases in MStiso were measured in the CG ($p = 0.45$). Two hours of daily stretch training resulted in an average MStiso increase of 22.3% in the intervened leg, and a contralateral force transfer to the non-intervened leg of 10.9%, $p = 0.011$ in the control leg. Stretching the calf muscle for one hour daily resulted in a 14.2% ($p < 0.001$) increase in average MSt in the calf muscles of the intervened leg and a 5.5% ($p = 0.024$) increase in MSt in the control leg. However, the statistical analysis revealed no significant difference between IG2il and IG1il ($p = 0.23$). Furthermore, no statistically significant difference was determined between IG1cl and IG2cl ($p = 0.489$), but it was determined between IG2cl and the CG. The results were confirmed by investigating the contralateral force transfer within the intervention groups, showing no significant difference between IG1il and IG1cl ($p = 0.062$) and between IG1cl and CGs ($p = 0.156$–0.96). However, IG2 showed significant differences between IG2il and CGs ($p < 0.001$) and between IG2cl and CGs ($p = 0.02$–0.033). The results indicate that stretching one leg for two hours per day resulted in significant strength increases in the stretched as well in the non-stretched leg (i.e., contralateral force transfer), while one hour of daily stretching showed significant improvements in the stretched leg without a statistically significant force transfer.

The aim of this study was to investigate if doubling the stretching time would also lead to significantly higher increases in MSt capacity; however, although there were increases in MSt of 22.3% due to two-hour daily stretching compared to 14.2% due to one-hour daily stretching for six weeks, the difference failed to be statistically significant. Thus, this hypothesis must be declined. It is known from animal studies that longer stretching times per training session led to higher increases in muscle mass with a dose–response relationship [37]. Previous research on daily long-lasting static stretching showed significant increases in MSt measured in the extended knee joint, comparable to those in the present study. Since Warneke et al. [31] showed significant hypertrophy in the plantar flexors due to long-lasting stretching intervention, which could possibly be attributed to stretch-induced mechanical tension, leading to hypertrophy and MSt increases, a general transferability of the effects observed in animal studies to humans could be hypothesized. The hypothesis of

mechanical-induced structural changes are confirmed by the results of a variety of animal studies [40], showing significant increases in muscle mass, the muscle cross-sectional area and the fiber cross-sectional area due to chronic stretching interventions: "It is Stretch that causes the Hypertrophy of Muscle" ([41] p. 93). However, increased MSt in the first weeks of training are commonly related to neuronal aspects [42,43], while morphological changes might be of minor relevance. The measured contralateral force transfer can also be seen as confirmation of the inclusion of neuronal aspects in the stretching-induced MSt increases. Zhou et al. [29] referred to different methods of peripheral stimulation, e.g., electrical stimulation or vibration training to induce cross-educational effects; however, the underlying mechanisms remain unclear. Based on the current literature, the authors hypothesize that peripheral sensory inputs might play an important role in inducing contralateral force transfer effects. Consequently, it is possible that long-lasting stretching provides sufficient peripheral stimulation in the intervened leg (e.g., via nociception) that leads to cross-education effects. Caldwell et al. [30] also referred to tension as the mechanism behind MVC improvements following stretching interventions. However, the contralateral force transfer may be attributed to changes in neuronal activity, which could possibly be attributed to afferences [28] due to the involvement of muscle spindles [33], while Caldwell et al. [30] referred to the possibility of active contraction against the stretching device. A high involvement of neuronal activity due to stretching can be assumed as many non-local effects can be observed via stretching interventions [44,45]. The results show that there was a statistically significant contralateral strength transfer exclusively in the two-hour stretching group, while one hour of static stretching did not reach statistical significance when compared to the control group.

Nelson et al. [17] found a contralateral force transfer from stretching intervention over a ten-week training period in humans. They were able to determine an 11% increase in the control leg when stretching three times a week. While Nelson and colleagues [17] assumed the cause of the MSt increase in the contralateral leg to be the stabilization of the body during the stretch training, such an effect can be excluded in the present work because the stretching intervention was performed in a seated position. The comparatively high MSt increases of 29% in the intervention leg as well as 11% in the control leg reported by Nelson et al. [17], as well as in Kokkonen et al. [20], can possibly be attributed to the conditional training status of the participants, if the authors attribute the MSt increases in the non-intervened leg to the stabilization of the body during the stretch training. In trained participants, no MSt increase would be expected due to the stabilizing activity during stretch training. Additionally, there are investigations showing that training with low load intensities, but with the addition of blood-flow restriction, led to muscular hypertrophy as well as increases in MSt [7,46]. Because participants in our investigation reported initial numbness after approximately 10–15 min, blood hypoxia combined with a mechanical stretch stimulus could be hypothesized to be the underlying mechanism for adaptations in MSt, similar to blood flow restriction training. In animal studies, Hotta et al. [47] demonstrated that 30 min of stretching per day resulted in a significant enhancement in blood supply to the stretched musculature as during the stretch, the blood inflow to the muscle was highly inhibited. In further studies, the influence of blood flow and VO_2 to the working muscle should be investigated regarding the improvement in strength capacity.

In general, the results were limited because gender was not balanced in each group. Therefore, gender specific differences could not be examined in the context of this study, especially because there were just three females in IG2. However, the aim of this study was not to investigate gender-related differences regarding stretching-induced improvements in MSt. Since it is well accepted that stretch training could lead to increases in flexibility in general [48,49], and Warneke et al. [31] showed significant improvements in flexibility due to a similar stretching intervention, we did not examine the influence of stretching on flexibility in this study. It was not possible to include equal sample sizes in the study as not all participants were willing to join the two-hour stretching group. It could be hypothesized that differences between IG1 and IG2 did not reach the level of significance because of

unequally distributed groups and a comparably low sample size in IG2, including high SDs. Further research should include higher sample sizes. Furthermore, to investigate the dose–response relationship, which is known from animal studies, the investigation of different stretching durations is requested. Since neither imaging techniques (such as sonography or magnetic resonance imaging) were used to assess muscle hypertrophy, nor were EMG measurements conducted to quantify changes in neuronal innervation, the physiological factors of the increased MSt, as well as the cross-education effects, remain unclear. As Manca et al. [50] highlighted a variety of possible explanations and Zhou et al. [29] referred to the need for clarification regarding the underlying mechanisms of contralateral effects due to unilateral peripheral stimulation, further studies should include additional measuring procedures to gain more insights.

5. Conclusions

In general, increases in MSt due to long-lasting static stretching of one or two hours in both the stretched and the non-stretched contralateral muscle can possibly be attributed to neuronal adaptations via changes in innervation of the central nervous system, or morphological adaptations due to changes in muscle architecture and muscle mass [31,51]. Since the intervention period lasted six weeks, and observed increases in MSt can be majorly attributed to changes in neuronal adaptations in the first few weeks of a strength training [42] and a contralateral strength transfer by using two hours of static stretching, improvements in MSt are suggested to be primarily attributed to neuronal adaptations. Further research is needed to obtain deeper insights (e.g., EMG measurements and magnetic resonance imaging) on the increases in MSt due to long-lasting static stretching interventions.

6. Practical Application

The results show that stretching can lead to an improvement in MSt when performed for a sufficient duration and under a sufficient intensity, leading to adequate muscle tension. If the aim is to induce increases in MSt in the contralateral leg, a long stretching duration (>1 h) seems to be required. Especially in the case of lower extremity injuries that lead to immobilization, and thus to a loss of MSt, mobility and the muscle cross-sectional area, this training method and implementation via a calf-stretching orthosis appears to be useful. The early use of this training method could counteract immobilization-related loss of strength and mobility. Therefore, the use of long-lasting stretching interventions in the early phase of immobilization should be tested. In addition to applications in rehabilitation, there is a potential use for astronauts, since time in a weightless environment results in atrophy effects and conventional strength training with external weights seems unfeasible in implementation [52,53].

Author Contributions: Conceptualization, K.W. and M.H.; methodology, K.W. and M.K.; software, M.H.; validation, K.W., M.K., M.H. and S.S.; formal analysis, K.W. and M.K.; investigation, K.W.; resources K.W. and S.S.; data curation, K.W. and M.H.; writing—original draft preparation, K.W.; writing—review and editing, K.W., M.K. and S.S.; visualization, K.W. and M.H.; supervision, S.S.; project administration, M.H. and K.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of Carl von Ossietzky University Oldenburg (No. 2019-016).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patient(s) to publish this paper.

Data Availability Statement: Original data can be provided from corresponding author due to reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

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European Journal of Applied Physiology
https://doi.org/10.1007/s00421-023-05184-6

ORIGINAL ARTICLE

Comparison of the effects of long-lasting static stretching and hypertrophy training on maximal strength, muscle thickness and flexibility in the plantar flexors

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Received: 24 October 2022 / Accepted: 16 March 2023

Abstract

Maximal strength measured via maximal voluntary contraction is known as a key factor in competitive sports performance as well as injury risk reduction and rehabilitation. Maximal strength and hypertrophy are commonly trained by performing resistance training programs. However, literature shows that long-term, long-lasting static stretching interventions can also produce significant improvements in maximal voluntary contraction. The aim of this study is to compare increases in maximal voluntary contraction, muscle thickness and flexibility after 6 weeks of stretch training and conventional hypertrophy training. Sixty-nine (69) active participants ($f = 30$, $m = 39$; age 27.4 ± 4.4 years, height 175.8 ± 2.1 cm, and weight 79.5 ± 5.9 kg) were divided into three groups: IG1 stretched the plantar flexors continuously for one hour per day, IG2 performed hypertrophy training for the plantar flexors (5 × 10–12 reps, three days per week), while CG did not undergo any intervention. Maximal voluntary contraction, muscle thickness, pennation angle and flexibility were the dependent variables. The results of a series of two-way ANOVAs show significant interaction effects ($p < 0.05$) for maximal voluntary contraction ($\eta^2 = 0.143$–0.32, $p < 0.006$), muscle thickness ($\eta^2 = 0.11$–0.14, $p < 0.021$), pennation angle ($\eta^2 = 0.002$–0.08, $p = 0.077$–0.625) and flexibility ($\eta^2 = 0.089$–0.21, $p < 0.046$) for both the stretch and hypertrophy training group without significant differences ($p = 0.37$–0.99, $d = 0.03$–0.4) between both intervention groups. Thus, it can be hypothesized that mechanical tension plays a crucial role in improving maximal voluntary contraction and muscle thickness irrespective whether long-lasting stretching or hypertrophy training is used. Results show that for the calf muscle, the use of long-lasting stretching interventions can be deemed an alternative to conventional resistance training if the aim is to increase maximal voluntary contraction, muscle thickness and flexibility. However, the practical application seems to be strongly limited as a weekly stretching duration of up to 7 h a week is opposed by 3 × 15 min of common resistance training.

Keywords Maximum strength · Resistance training · Mechanical tension · Range of motion · Calf muscle

Abbreviations

MVC	Maximal voluntary contraction
ROM	Range of motion
MSt	Maximal strength
mTOR	Mammalian target of rapamycin
p70S6k	Protein S6 kinase
IG1	Intervention group 1
IG2	Intervention group 2
CG	Control group

Communicated by William J. Kraemer.

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Published online: 08 April 2023

ICC	Intraclass correlation coefficient
CMD	Calf muscle device
ORTH	Goniometer of the orthosis
KtW	Knee-to-wall test
M	Mean
SD	Standard deviation
ANOVA	Analysis of variance
MTh	Muscle thickness
LP	Leg press

Introduction

While stretch training in humans is commonly used to improve flexibility, a meta-analysis of animal studies showed significant hypertrophic effects (Warneke et al. 2022b) with increases in muscle cross-sectional area of up to 141.6% with $d = 5.85$ as well as an increase in maximal strength of up to 95% with $d = 12.34$ following chronic stretching for six weeks. However, evidence for stretch-mediated hypertrophy and strength increases in humans is contradictory and scarce. On the one hand, Nunes et al. (2020) reviewed current literature pointing out that mostly used stretching durations in humans of up to two min per session seem not to be sufficient to induce hypertrophy. This might be explained by large differences regarding training durations (two min per session vs. chronic 24 h of stretch) as well as muscle protein synthesis between animals and humans (Garibotto et al. 1997; Sayegh and Lajtha 1989). On the other hand, there are conflicting results regarding stretch-induced maximal strength increases in humans probably based on high heterogeneity between studies regarding the way in which the stretch was induced combined with a lack in stating stretching intensity. While some studies showed significant increases in maximal strength in response to long-term stretching interventions of up to 30 min per training session (Mizuno 2019; Yahata et al. 2021), others were not able to induce significant changes in strength capacity following stretching interventions (Nakamura et al. 2021; Sato et al. 2020). All listed studies were performed including participants with a low training status or even with untrained participants. Since in animal model stretching durations of up to 24 h per day were used (Warneke et al. 2022b), a comparison to human studies performed previously seems not to be adequate. Thus, it could be assumed that previous studies in humans may not have used sufficient stretching volume (stretching duration × training frequency per week) or intensity leading to inconsistent significant increases (Nakamura et al. 2021; Nunes et al. 2020; Yahata et al. 2021). Based on this, Warneke et al. (2022a, d) investigated the effects of long-lasting static stretching interventions of up to two hours per day on seven days per week in the plantar flexors of physically active humans to improve comparability to stretching durations used in animal studies. The authors determined significant maximal strength improvements of up to 22% while—in a different study—significant stretch-mediated hypertrophy of approximately 15.3% ($d = 0.84$) could be induced by using long-lasting static stretch training of one hour per day, seven days a week (Warneke et al. 2022a, d). To date, increases in maximal strength and muscle thickness are commonly associated with resistance training routines (Ralston et al. 2017; Refalo et al. 2021; Schoenfeld et al. 2017). Different authors found maximal strength increases of 11.9% ($d = 0.47$) up to 17.0 ± 8.75% ($d = 1.0$) (Green and Gabriel 2018) as well as hypertrophic effects via magnetic resonance imaging of up to 5.2 ± 2.7% ($d = 0.3$) in young, recreationally active to moderately trained participants in the lower extremities within six weeks (Souza et al. 2014). To achieve improvements in maximal strength, on the one hand, inducing metabolic stress (Millender et al. 2021) via high training volume and frequency (Grgic et al. 2018; Ralston et al. 2017) seems to be beneficial. On the other hand, intensity regulated by mechanical loading seems to be of crucial importance to achieve maximal strength increases and hypertrophy (Krzysztofik et al. 2019; Schoenfeld et al. 2015). In resistance training, the morphological and functional adaptations are accompanied by stimulation of anabolic signaling pathways such as mTOR/p70S6k (Lamas et al. 2010; Vissing et al. 2013). Interestingly, Sasai et al. (2010) as well as Tatsumi (2010) showed the activation of this pathway due to muscle stretching. Based on very similar adaptations and underlying physiological responses, the question arises whether long-lasting stretch training could be used as an alternative to commonly used resistance training to induce significant increases in maximal strength and muscle thickness.

Consequently, the aim of the present study was to investigate the effects of long-lasting stretching interventions on maximal strength, muscle thickness and the pennation angle and compare the effects with a commonly used hypertrophy training program for the calf muscle. Since enhanced flexibility can be assumed when performing stretch training (Medeiros et al. 2016) and literature leads to the assumption that a resistance training using full range of motion (ROM) could also lead to improvements in flexibility (Afonso et al. 2021), the effects on ROM of both training interventions will be investigated as done by Warneke et al. (2022a, d). It was hypothesized that both interventions, daily long-lasting stretching and a commonly used resistance training to achieve hypertrophy, would lead to significant increases in maximal strength, hypertrophy and flexibility gains, independent of the respective intervention group.

Methods

To compare the effects of a one-hour daily stretch training with those of a commonly used hypertrophy training, recreationally active participants were recruited from the university sports program. They were divided into a stretch training group (IG1) and a hypertrophy training group (IG2) performing either a daily long-lasting stretch training or a resistance training routine which is commonly used to induce hypertrophy in the plantar flexors. Therefore, a pre–post-design with a six-week intervention period, incorporating two maximal strength tests with extended and flexed knee joint for the plantar flexors, two flexibility tests for the range of motion in dorsiflexion of the ankle joint as well as a sonography assessment to examine changes in muscle thickness and the pennation angle were performed. Before testing, a warm-up routine consisting of five minutes of bodyweight ergometer cycling with 1 Watt/kg was performed.

Participants

Ad hoc sample size calculation was performed using $d = 0.7$ for F-tests with repeated measures and within–between interaction, based on previous studies (Warneke et al. 2022a) pointing out a total sample size of at least 36 participants (12 per group). To increase the power of the investigation and counteract potential dropouts 69 recreationally active and non-competitive participants from sports study programs and local sports clubs were recruited. Participants were classified as novice to recreationally active when they performed either two or more training sessions per week in a gym or a team sport in addition to their physical education classes if they were physical education students or completing at least three resistance training sessions continuously for the previous six months. Therefore, participants had some training experience in resistance training with commonly used intensity and volume to induce hypertrophy (5×10–12 repetitions) as well as in team sports, such as soccer, basketball, tennis or handball. Participants with an increased risk for thromboses or serious injury in the lower extremities entailing surgery and immobilization within the past year were excluded from the study. Consequently, training status was classified as moderately trained as no untrained participants as well as no elite sport athletes were included. The participants were randomly allocated to the three groups (IG1, IG2 and CG). If participants had skipped more than three stretch training sessions or more than two resistance training sessions, respectively, data would not have been considered for further evaluation. This was, however, not the case. All participants were instructed to continue performing their previous training routines to avoid a decrease in performance in any group by stopping training. Therefore, the stretching and hypertrophy training intervention was accompanied by either the university sports program or the training routine in the gym the participants were used to. This was also the case in the control group. Characteristics of the participants are shown in Table 1.

All participants were informed about the experimental risks and provided written informed consent to participate in the present study. Furthermore, approval for this study was obtained from the university's institutional review board (Carl von Ossietzky University of Oldenburg, No. 121-2021). The study was performed in accordance with the Helsinki Declaration.

Testing procedure

Figure 1 illustrates the measuring procedure used in both the pre- and post-test. The study was conducted from March to August 2022. The post-test was performed at the same time of day as the pre-test. All testing sessions were performed between 9 am and 5 pm. Participants were instructed to eat a meal latest two hours before testing.

Maximal strength testing

It can be assumed that there are differences in muscle innervation of the triceps surae dependent on the knee joint angle (Warneke et al. 2022c). Thus, the isometric maximal voluntary contraction was assessed using single-leg testing with extended and flexed knee joint.

Table 1 Characteristics of participants for overall sample size and divided into IG1, IG2 and CG

Group	N	Age (in years)	Height (in cm)	Weight (in kg)
Total	69 ($f = 30$, $m = 39$)	27.4 ± 4.4	175.8 ± 2.1	79.45 ± 5.9
IG1	23 ($f = 10$, $m = 14$)	27.4 ± 3.1	176.2 ± 5.6	81.0 ± 6.2
IG2	23 ($f = 9$, $m = 13$)	26.3 ± 2.6	175.6 ± 4.9	79.3 ± 5.3
CG	23 ($f = 11$, $m = 12$)	27.9 ± 6.1	174.4 ± 6.3	79.1 ± 7.0

IG1 stretching group, *IG2* hypertrophy group, *CG* control group

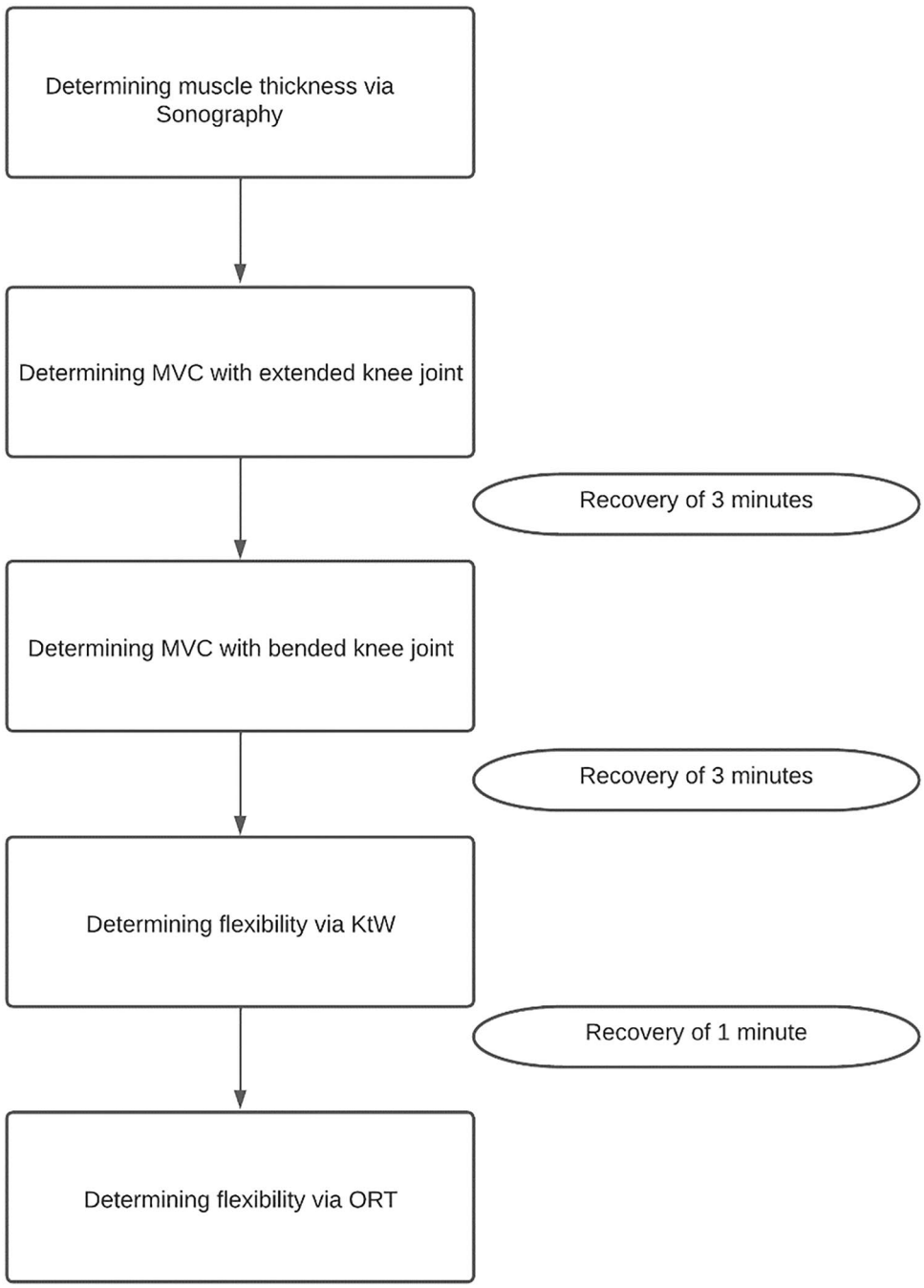

Fig. 1 Flow chart of the testing procedure used in pre- and post-test

Maximal isometric strength testing with extended knee joint

A 50 × 60 cm force plate with ± 5000N and a 13-bit analog-to-digital converter attached to a 45° leg press was used to measure the maximal isometric force production with an extended knee joint. In the starting position (see Fig. 2) the ankle joint angle was set to be 90°. The participants were instructed to perform a maximal plantar flexion in response to an acoustic signal and hold the maximal voluntary contraction for three seconds. After each trial, participants rested for one minute to avoid fatigue. Measurements were conducted until no improvement in maximal strength was recorded with a minimum of three trials. For isometric strength measurements, high reliability (intraclass correlation coefficient = 0.99) can be assumed (Warneke et al. 2022a).

Fig. 2 Leg press testing device for maximal isometric strength with extended knee joint (MVC180)

Fig. 3 Calf muscle testing device equipped with force plates to measure maximal isometric strength with flexed knee joint (MVC90)

Maximal isometric strength testing with flexed knee joint

A calf muscle testing device was used to assess maximal isometric strength with a flexed knee joint. The maximal strength was determined using a 10 × 10 cm force plate with force sensors "Kistler Element 9251" with a resolution of 1.25N, a pull-in frequency of 1000 Hertz and a measurement range of ± 5000N. The vertical forces (Fz) were recorded via a charge amplifier "Typ5009 Charge Amplifier" and a 13-bit analog-to-digital converter NI6009 (see Fig. 3). The participants were instructed to perform maximal plantar flexion for three seconds in response to an acoustic signal. Testing was performed until participants could not improve the achieved maximal strength values with a minimum of three trials. High reliability can be assumed using maximal isometric strength testing (intraclass correlation coefficient = 0.99) (Warneke et al. 2022d).

Determination of skeletal muscle architecture

Muscle thickness and pennation angle were measured in the lateral and medial gastrocnemius using two-dimensional B-mode ultrasound with a linear transducer (12–13 MHz, Mindray Diagnostic Ultrasound System). The measurement was conducted with the participant laying in a prone position with fully extended legs and their feet hanging down at the end of a table to ensure no contraction in the calf muscles. The transducer was placed at 25% of the distance between the most lateral point of the joint space of the knee and the most lateral tip of the lateral malleolus (Perkisas et al. 2021). By holding and rotating the transducer around the sagittal-transverse axis, it was ensured that the superficial and deep aponeuroses were as parallel as possible to optimize the visibility of the fascicles as continuous striations from one aponeurosis to the other (see Fig. 4). The transducer was positioned at the midpoint of each muscle belly perpendicular to the long axis of the participant's leg (Sarto et al. 2021). Both muscle thickness and pennation angle were obtained by averaging three measurements across the proximal, central and distal portions of the acquired ultrasound images (Franchi et al. 2017; Sarto et al. 2021). Two investigators performed the image processing independently using MicroDicom (Sofia, Bulgaria). With the measurement device stated above, the reliability can be classified as high with an intraclass correlation of 0.88–0.95 (Warneke et al. 2022a).

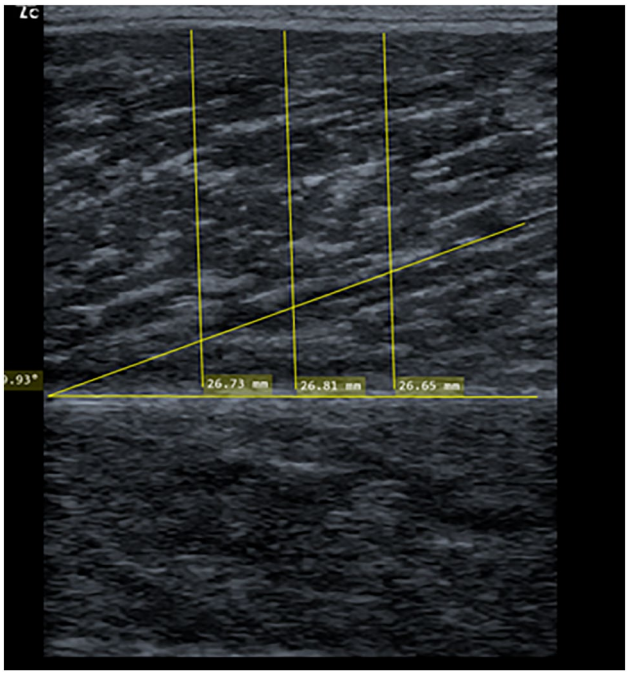

Fig. 4 Sonography to investigate muscle thickness and pennation angle in the calf muscle

Fig. 5 Orthosis used for calf muscle stretching

Range of motion measurement

Range of motion in the upper ankle joint was recorded via the knee-to-wall test and the goniometer on the orthosis.

Range of motion testing via knee-to-wall test

A sliding device was used for the knee-to-wall stretch. Participants were instructed to place the foot on the attached marker. The contralateral leg was held in the air and participants were allowed to stabilize the body with their hands placed on a doorframe. The participants pushed the board of the sliding device forward with their knee until the heel of the standing leg started to lift off. Throughout the test, the investigator pulled on a sheet of paper placed under the heel of each participant. The measurement was stopped as soon as the sheet could be removed. The distance achieved was read off in cm from the attached measuring tape. Depending on ankle range of motion, this measurement can be seen as screening flexibility with a flexed knee joint. Three valid trials were performed per leg and the furthest distance was used for evaluation. Range of motion assessment with comparable methods can be classified as high with an intraclass correlation of 0.99 (Warneke et al. 2022a).

Range of motion testing via goniometer of the orthosis

Range of motion in the ankle with an extended knee joint was measured via goniometer of the orthosis. For this purpose, the foot of the participant was placed on an object with the same height as the chair. While the participants were wearing the orthosis the foot was brought into a maximally dorsiflexed position keeping the knee joint in an extended position. The right angle between the lower leg and foot is classified as neutral 0°. Each big indentation of the goniometer corresponds to an increase in dorsiflexion of 5° and each little indentation corresponds to an increase of 2.5°. Range of motion assessments in the ankle joint using a goniometer can be classified as high with an intraclass correlation coefficient of 0.99 (Warneke et al. 2022a).

Intervention

Stretch training (IG1)

The stretching group (IG1) was instructed to perform a one hour daily stretch training for the calf muscles for six weeks. To realize this long-lasting stretch training, a calf muscle stretching orthosis was provided (see Fig. 5). The intervention was performed with the dominant leg which was determined as the leg used when performing single-leg jumps.

Subjects were instructed to wear the orthosis with an extended knee joint. To improve consistency regarding the used magnitude of stretch, the used ankle angle was quantified by the goniometer of the orthosis. Thus, the stretch could be replicated and better standardized within the six-week training intervention. Participants were instructed to reach a maximally dorsiflexed position with an individual stretching pain of 7–8 on a visual analog scale of 1–10. Participants were instructed to sit with their backs straight against the backrest and place their intervened foot on a support object at the same height as their chair. All subjects completed a stretching diary in which the daily stretching duration as well as the angle of the goniometer were written down to record the stretch duration and intensity (Fig. 5).

Hypertrophy training (IG2)

IG2 was instructed to perform a resistance training routine commonly used to achieve hypertrophy in the plantar flexors. Participants performed calf muscle hypertrophy training with an extended knee joint on a 45° leg press with five sets of 10–12 repetitions on three non-consecutive days per week. Training sessions lasted about 15 min. The inter-set rest was 90 s with the instruction to perform each set over full range of motion until failure. If more than 12 repetitions were accomplished, more weight was added. When a participant was not able to manage ten repetitions, the load was reduced. Participants had to complete a training diary in which training day and load were documented.

Statistical analysis

The analysis was performed with SPSS 28 (IBM, Armonk, New York, USA). Data is provided as mean (M) ± standard deviation (SD) for the pre–post values. The normal distribution of data was checked via Shapiro–Wilk test. Reliability was determined and is provided with intraclass correlation coefficient, coefficient of variability and 95% confidence interval (CI) for aforementioned tests (see Table 2). 95% CI for intraclass correlation coefficients and the coefficient of variability are interpreted considering the general guidelines by Koo and Li (2016): poor reliability ≤ 0.5, moderate reliability = 0.5–0.75, good reliability ≥ 0.75–0.9, excellent reliability ≥ 0.9. Reliability for sonography was determined between best and second-best value as the "within day" reliability (see Table 2). Two investigators evaluated the ultrasound images independently from one another to ensure inter-rater reliability. Moreover, Levene's test for homogeneity in variance was performed. A one-way ANOVA was used to rule out significant differences between groups in pre-test values. A series of two-way ANOVAs with repeated measures was performed for data analyses of the pre-post comparisons. To investigate the differences in increases between the intervention groups and the control group, the Scheffé test was used as post hoc test. Effect sizes are presented as Eta squares (η^2) and categorized as: small effect $\eta^2 < 0.06$, medium effect $\eta^2 = 0.06$–0.14, high effect $\eta^2 > 0.14$ (Cohen 1988). Additionally, effect sizes are reported with Cohen's d (Cohen 1988) and categorized as: small effects $d < 0.5$, medium effect $d = 0.5$–0.8, high effect $d > 0.8$. The level of significance was set to $p < 0.05$. Pearson correlations were calculated for pre–post comparisons in maximal strength and muscle thickness.

Table 2 Reliability for the pre-test values

Parameter	ICC (95%-CI)	CV (95%-CI) in%
MVC180	0.984 (0.978–0.989)	1.72 (1.44–2.01)
MVC90	0.983 (0.976–0.988)	1.97 (1.66–2.33)
KtW	0.991 (0.984–0.995)	0.94 (0.35–1.59)
ORT	0.992 (0.981–0.995)	0.64 (0.22–1.19)
SONOL	0.876 (0.83–0.91)	5.21 (4.4–6.15)
SONOM	0.917 (0.885–0.94)	3.5 (2.96–4.07)
PaL	0.878 (0.833–0.912)	6.64 (5.64–7.74)
PaM	0.81 (0.743–0.861)	6.49 (5.2–7.98)

MVC maximal voluntary contraction, *KtW* knee-to-wall test, *ORT* range of motion measurement with orthosis, *SONO* measurement of muscle thickness via sonography, *Pa* Pennation angle, *180* MVC measured with extended knee joint, *90* MVC measured with flexed knee joint, *L* lateral head of the gastrocnemius, *M* medial head of the gastrocnemius

Results

Results of reliability are shown in Table 2. Descriptive statistics for maximal strength and flexibility are provided in Table 3 and descriptive statistics for muscle thickness and pennation angle are listed in Table 4. All data were normally distributed.

The evaluation of pre-test group differences showed no significance between groups ($F = 0.161$–1.699, $p = 0.191$–0.813).

Table 3 shows the descriptive statistics of the maximal strength and flexibility assessment in plantar flexion.

Maximal strength analysis

Figure 6 illustrates changes in maximal strength using the maximal strength measurement with extended and flexed knee joint for the intervened leg.

Plantar flexor maximal voluntary contraction with extended knee joint

Results for maximal strength measured using the maximum voluntary contraction in the plantar flexors with extended

Table 3 Descriptive statistics and results of two-way ANOVA for maximal strength and ROM

Parameter	Pretest (M ± SD) in *N*	Post-test (M ± SD) in *N*	Pre-Post-Diff. in %	Time effect	Time × group
IG1MVC180	1522.61 ± 310.25	1796.78 ± 368.08	+ 18.00	$p < 0.001$	$p < 0.001$
IG2MVC180	1594 ± 321.78	1807.8 ± 361.11	+ 13.36	$F = 88.26$	$F = 15.49$
CG	1557.05 ± 284.46	1585.57 ± 292.04	+ 1.8	$\eta^2 = 0.57$	$\eta^2 = 0.32$
IG1MVC90	1314.7 ± 305.79	1440.61 ± 332.67	+ 9.58	$p < 0.001$	$p = 0.006$
IG2MVC90	1371.8 ± 289.45	1508.44 ± 258.7	+ 9.96	$F = 25.908$	$F = 5.51$
CG	1334.76 ± 235.36	1340.33 ± 205.81	+ 0.42	$\eta^2 = 0.28$	$\eta^2 = 0.14$
IG1KtW	11.72 ± 2.52	12.98 ± 2.55	+ 10.75	$p < 0.001$	$p = 0.046$
IG2KtW	12.26 ± 2.1	13.36 ± 2.31	+ 8.97	$F = 48.96$	$F = 3.24$
CG	11.71 ± 12.17	12.17 ± 2.0	+ 3.93	$\eta^2 = 0.43$	$\eta^2 = 0.09$
IG1ORT	8.35 ± 2.08	9.39 ± 1.41	+ 12.46	$p < 0.001$	$p < 0.001$
IG2ORT	7.92 ± 1.637	8.64 ± 1.31	+ 9.09	$F = 39.37$	$F = 8.85$
CG	8.17 ± 1.25	8.21 ± 1.03	+ 0.49	$\eta^2 = 0.37$	$\eta^2 = 0.21$

IG1 stretching group, *IG2* hypertrophy training group, *CG* control group, *MVC* maximal voluntary contraction, *KtW* ROM Measurement via knee-to-wall test, *ORT* range of motion measurement via goniometer of the orthosis, *180* MVC testing in extended knee joint, *90* MVC testing in flexed knee joint

Table 4 Descriptive statistics of muscle thickness and the pennation angle

Parameter	Pretest (M ± SD) in *N*	Post-test (M ± SD) in *N*	Pre-Post-Diff. in %	Time effect	Time × group
IG1MThL	14.53 ± 2.43	15.21 ± 2.11	+ 4.68	$p < 0.001$	$p = 0.021$
IG2MThL	14.83 ± 2.91	16.09 ± 3.35	+ 8.5	$F = 15.51$	$F = 4.08$
CG	14.33 ± 2.48	14.40 ± 2.32	+ 0.49	$\eta^2 = 0.19$	$\eta^2 = 0.11$
IG1MThM	19.55 ± 2.59	21.06 ± 2.88	+ 7.72	$p < 0.001$	$p = 0.006$
IG2MThM	19.25 ± 3.47	20.87 ± 3.09	+ 8.42	$F = 19.46$	$F = 5.48$
CG	18.49 ± 3.13	18.41 ± 2.87	− 0.43	$\eta^2 = 0.23$	$\eta^2 = 0.14$
IG1PaL	13.39 ± 2.33	13.49 ± 2.73	+ 0.75	$p = 0.549$	$p = 0.625$
IG2PaL	14.14 ± 2.91	14.59 ± 2.28	+ 3.18	$F = 0.36$	$F = 0.47$
CG	12.67 ± 2.86	12.55 ± 2.76	− 0.95	$\eta^2 = 0.01$	$\eta^2 = 0.02$
IG1PaM	17.32 ± 4.07	19.46 ± 3.24	+ 12.3	$p < 0.001$	$p = 0.077$
IG2PaM	16.92 ± 3.18	19.07 ± 3.04	+ 12.71	$F = 12.81$	$F = 2.66$
CG	16.51 ± 3.92	16.62 ± 3.67	+ 0.67	$\eta^2 = 0.16$	$\eta^2 = 0.08$

IG1 stretching group, *IG2* hypertrophy training group, *CG* control group, *MThL* muscle thickness in the lateral head of gastrocnemius, *MThM* muscle thickness in the medial head of gastrocnemius, *PaL* pennation angle in the lateral head of the gastrocnemius, *PaM* pennation angle in the medial head of the gastrocnemius

knee joint showed high, significant increases with a time effect of $\eta^2 = 0.572$, $p < 0.001$ and a significant time × group interaction ($\eta^2 = 0.319$, $p < 0.001$). Post hoc testing pointed out no significant differences for increases from pre- to post-test between the stretching group (IG1) and the hypertrophy training group (IG2) ($p = 0.387$, $d = 0.4$) but differences in favor of the intervention groups between the stretching group (IG1) and the control group (CG) ($p < 0.001$, $d = 1.17$) as well as between the hypertrophy training group (IG2) and the control group (CG) ($p < 0.001$, $d = 0.9$). Therefore, no change in the control group but significant increases in both intervention groups were obtained.

Plantar flexor maximum voluntary contraction with flexed knee joint

Results for maximal strength in the plantar flexors measured with flexed knee joint also showed a high, significant increase with a time effect of $\eta^2 = 0.282$, $p < 0.001$ and a significant time × group interaction ($\eta^2 = 0.143$, $p = 0.006$). Furthermore, post hoc testing pointed out no significant difference for the increases in maximal strength between the stretching group (IG1) and the hypertrophy training group (IG2) ($p = 0.986$, $d = 0.05$). There were differences in favor of the intervention groups with moderate effect

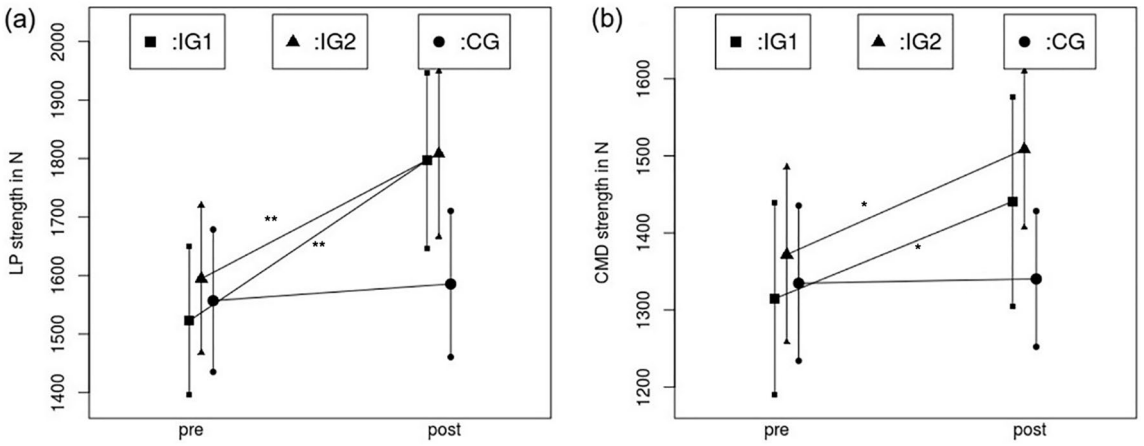

Fig. 6 Comparison of maximal strength from pre- to post-test in the stretching group (IG1), the hypertrophy training group (IG2) and the control group (CG) with extended (**a**) and flexed knee joint (**b**). ** indicates a significant increase compared to the control group of $p < 0.001$, * indicates a significant increase compared to the control group of $p < 0.05$

sizes between the stretch training group (IG1) and the control group (CG) ($p = 0.029$, $d = 0.6$) as well as between the hypertrophy training group (IG2) and the control group (CG) ($p = 0.013$, $d = 0.651$). Therefore, the results show significant increases in both intervention groups without any significant change in the control group.

Range of motion analysis

Range of motion via knee-to-wall stretch

Results of the knee-to-wall test demonstrated high, significant increases with a time effect of $\eta^2 = 0.426$, $p < 0.001$ and a time × group interaction ($\eta^2 = 0.169$, $p = 0.046$). Post hoc testing showed no significant differences between the increases of the stretching (IG1) and hypertrophy training group (IG2) with $p = 0.882$, $d = 0.24$, while there were moderate magnitudes in effect sizes for differences in favor of the intervention groups between the stretching group (IG1) and the control group (CG) ($p = 0.062$, $d = 0.53$) as well as between the hypertrophy training group (IG2) and the control group (CG) ($p = 0.152$, $d = 0.42$), showing increases in all groups without a significant difference between groups..

Range of motion via goniometer of the orthosis

Furthermore, there was a high, significant increase in the flexibility measured with the goniometer of the orthosis with a time effect of $\eta^2 = 0.374$, $p < 0.001$ and a significant, high time × group interaction ($\eta^2 = 0.212$, $p < 0.001$). Post hoc testing determined no significant difference for the increases between the stretching (IG1) and the hypertrophy training group (IG2) ($p = 0.378$, $d = 0.38$). There were significant differences in favor of the intervention groups between the stretching group (IG1) and the control group (CG) ($p < 0.001$, $d = 0.9$) and the hypertrophy training group (IG2) and the control group (CG) ($p = 0.022$, $d = 0.61$), showing no significant change in the control group, while there were significant range of motion increases in both intervention groups.

Muscle thickness and pennation angle analyses

Table 4 shows the descriptive statistics for muscle thickness and the pennation angle in the lateral and medial gastrocnemius.

Muscle thickness in lateral and medial head of the gastrocnemius

Figure 7 illustrates changes in the muscle thickness measured via sonography in the lateral and medial gastrocnemius in all three groups.

Results for muscle thickness measurement in the lateral head of the gastrocnemius showed a significant increase from pre- to post-test with a time effect of $\eta^2 = 0.19$, $p < 0.001$ with a moderate, significant interaction effect (time × group, $\eta^2 = 0.11$, $p = 0.021$). In the medial head of the gastrocnemius, there was a high, significant increase in muscle thickness showing a time effect of $\eta^2 = 0.228$, $p < 0.001$ with a significant time x group interaction ($\eta^2 = 0.142$, $p = 0.006$).

For the lateral head of the gastrocnemius, post hoc testing pointed out significant differences in favor of the intervention group (IG2) with moderate effect sizes between the hypertrophy training group and (IG2)

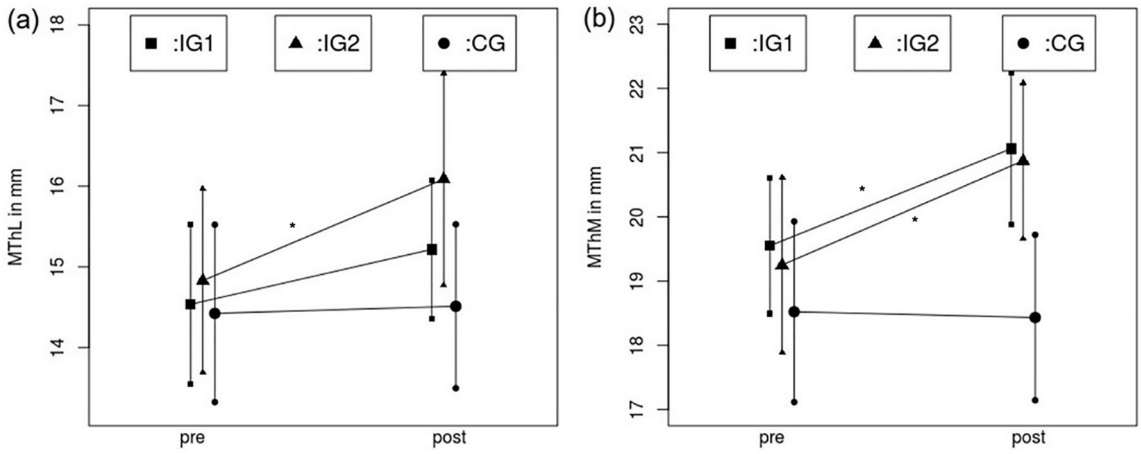

Fig. 7 Muscle thickness comparison from pre- to post-test in the stretching group (IG1), the hypertrophy training group (IG2) and the control group (CG) in the lateral (**a**) and medial (**b**) gastrocnemius. ** indicates a significant increase compared to the control group of $p < 0.001$, * indicates a significant increase compared to the control group of $p < 0.05$

and the control group (CG) ($p = 0.021$, $d = 0.61$) but no significant differences could be observed between the stretching group (IG1) and the control group (CG) ($p = 0.36$, $d = 0.32$) and the stretching group (IG1) and the hypertrophy training group (IG2) ($p = 0.37$, $d = 0.32$). Therefore, no significant increase was found for the stretching group compared with the control group, while there was a significantly greater increase in the muscle thickness of the lateral head in the IG2. In the medial head of the gastrocnemius, no significant difference was found between the stretching (IG1) and the hypertrophy training group (IG2) ($p = 0.979$, $d = 0.03$), however, there were significant differences in favor of the intervention groups with moderate effect sizes between the stretching group (IG1) and the control group (CG) ($p = 0.027$, $d = 0.6$) as well as between the hypertrophy training group (IG2) and the control group (CG) ($p = 0.014$, $d = 0.646$), showing significant hypertrophy in IG1 and IG2 without a difference between the groups, while no significant changes could be obtained in the control condition.

Individual progressions of the listed parameters are illustrated in separate figures in the supplemental material.

Pennation angle in the lateral and medial head of the gastrocnemius

For the pennation angle in the lateral head of the gastrocnemius, no significant increase from pre- to post-test could be observed (time effect of $p = 0.549$, $\eta^2 = 0.006$, time × group interaction $p = 0.625$, $\eta^2 = 0.015$). In the medial head of the gastrocnemius, there was a high, significant time effect ($p < 0.001$, $\eta^2 = 0.163$), however, no significant time × group interaction ($p = 0.077$, $\eta^2 = 0.075$) could be found.

Discussion

The present study compared the effects of a one hour daily stretching intervention in the plantar flexors with a commonly used hypertrophy training routine over a period of six weeks. Results showed an increase in maximal strength with moderate to high effects ($\eta^2 = 0.143$–0.572, $d = 0.6$–1.17, $p < 0.001$–0.006), low to moderate effects for increases in muscle thickness ($\eta^2 = 0.11$–0.228, $d = 0.32$–0.65, $p < 0.001$–0.021) as well as low to high effects for increases in flexibility ($\eta^2 = 0.089$–0.426, $d = 0.42$–0.9, $p < 0.001$–0.046) irrespective of performing a commonly used hypertrophy training or long-lasting stretching for the calf muscle. The control group exhibited no significant changes in any measured value. Results showed that there was no significant difference in adaptations between the stretching and hypertrophy training group regarding increases in maximal strength, muscle thickness and flexibility ($p = 0.37$–0.99, $d = 0.03$–0.4). Therefore, performing stretch training can be assumed to provide a sufficient stimulus to increase maximal strength and hypertrophy in the calf muscle if performed with adequate training volume (stretch duration × weekly frequency), which is comparable to adaptations of commonly used resistance training.

Previous studies were able to show stretch-mediated strength increases as well. Nelson et al. (2012) and Yahata et al. (2021) pointed out improvements in maximal strength of up to 29% ($d = 1.24$) and 6.6% ($d = 0.35$) using lower stretching durations of 4 x 30 s three times per week and 30 min per session two times per week, respectively. Considering a stretch-induced increase in maximal strength of 29% by using 4 × 30 s of stretching, the included participants should be stated as untrained, as listed increases would be higher as expectable effects of resistance training programs.

Since Nelson et al. (2012) described their participants as physically inactive or "minimally recreationally active" by performing training less than five times per months for less than 60 min per session, the training level of the participants included in the present study must be considered as significantly higher. While Nunes et al. (2020) reviewed current literature pointing out no significant influence of stretch training on hypertrophy, the only studies that used long-lasting stretching (> 30 min of stretch per session) with a daily frequency showed significant, stretch-mediated hypertrophy and maximal strength increases (Warneke et al. 2022a), comparable with previous animal studies (Kelley 1996; Warneke et al. 2022b).

In animal studies (Frankeny et al. 1983) and also in human studies (Warneke et al. 2022a, c; Yahata et al. 2021) higher adaptations were found by increasing stretching duration and volume. Since in resistance training, previous authors pointed out increases in strength capacity of about 17.0 ± 8.75% ($d = 1.0$) (Green and Gabriel 2018; Grgic et al. 2018) and Warneke et al. (2022a) showed comparable increases in strength and muscle thickness in response to one hour of daily stretching, these long durations seem to be necessary to achieve an adequate stimulus.

It is well known that mechanical tension (intensity) plays a crucial role in physiological adaptations when aiming to induce hypertrophy but especially for maximal strength improvements, which is accompanied by a stimulation of anabolic signaling pathways (Schoenfeld et al. 2015; Wackerhage et al. 2019). Literature points out the possibility to induce similar mechanical tension and therefore anabolic signaling due to the activation of so-called stretch-activated channels (Suzuki and Takeda 2011), resulting in stimulating mTOR signaling pathways (Tyganov et al. 2019). Therefore, increases in maximal strength are possibly explained by mechanical tension-induced adaptations which one could speculate to be similar to adaptations of a common hypertrophy training, including increases in muscle quality, muscle thickness and architecture and/or elongation of the muscle-tendon unit. Accordingly, in animal studies, Devol et al. (1991) referred to mechanical tension per sarcomere as an important factor to induce stretch-related responses in the muscle, however, in humans the underlying physiological processes of stretch-activated increases in maximal strength and muscle thickness remain unclear. A previous study (Warneke et al. 2022a) found no relationship between increases in muscle thickness and maximal strength.

Noticeable, even though there are several similarities regarding the adaptations over the six-week period following stretching and hypertrophy training in the results reported in this study (regarding maximal strength, muscle thickness and flexibility), it can be assumed that resistance training would lead to further health-related benefits, such as improved cardiovascular function (Schjerve et al. 2008; Yu et al. 2016) and bone mineral density (Westcott 2012). To this point, it remains unclear whether and to which extend long-lasting stretching would be effective concerning health-related parameters.

It is well known that neuronal factors play an essential role in maximal strength increases in the first weeks of training (Del Vecchio et al. 2019) while structural adaptations might play a secondary role (Gabriel et al. 2006). Consequently, it can be assumed that enhanced strength capacity could be primally explained by neuronal changes. The potential neuromuscular adaptations leading to stretch-mediated increases in maximal strength capacity still remain unclear. Holly et al. (1980) pointed out that no significant increase in central nervous activity was found when inducing long-term stretching in animal models, while Sola et al. (1973) pointed out significant stretch-mediated hypertrophy even if the muscle was previously denervated. Therefore, further investigations are requested to clarify the physiological mechanism of stretch-induced maximal strength increases. In contrast, benefits of central nervous innervated muscle contraction such as motor learning effects can be hypothesized to occur in a lower magnitude compared to active training protocols.

However, even though transferability of results from animal research should be considered carefully, in animal model the morphological adaptations are investigated more frequently, pointing out a serial accumulation of sarcomeres in response to chronic stretching interventions even after a few days (Antonio et al. 1993) which could also be responsible for increased muscle mass and, due to optimizing the length–tension relationship, for changes in force production capability of the muscle. Hypothesizing a general transferability to humans, these adaptations could also indicate changes in muscle morphology which could contribute to significant maximal strength increases. Furthermore, since an increased muscle thickness was measured, an enhancement in the pennation angle was reasonably hypothesized (Cormie et al. 2011). Accordingly, the pennation angle seems to increase with enhancement in muscle thickness in both groups. This may also be responsible for improvements in maximal strength as an increase in the number of contractile filaments in parallel and a higher strength capacity can be assumed (Cormie et al. 2011).

However, even without a significant difference between the stretching group (IG1) and the hypertrophy training group (IG2) the comparatively high time-effort of the stretch training should be considered, as the time spent with training for IG1 was long compared with IG2. While IG2 performed their training routine within a weekly duration of about 45 min (3 × 15 min), IG1 had to stretch the plantar flexors for up to seven hours per week. Furthermore, the stretching group performed their training routine more frequently

(seven days per week) than the hypertrophy training group (three times per week). Even with (non-significant) higher increases in maximal strength in the stretching group, the time-effort of this group can be assumed to be unproportionally high compared with the hypertrophy training group. However, the training of IG1 could be integrated in the daily life or prolonged times of immobilization, which was not possible for IG2, as the hypertrophy orientated training protocol required a leg press machine.

It is well accepted that performing stretch training results in improved flexibility (Medeiros and Lima 2017). There are many hypotheses trying to explain increases in range of motion after a stretch training. While authors hypothesize an increased tolerance of stretching tension via a reduced pain sensitivity (Freitas et al. 2018), animal models show evidence of structural adaptations by a serial accumulation of sarcomeres (Antonio and Gonyea 1993). However, when resistance training is performed over full range of motion, improvements in range of motion can be assumed as well (Afonso et al. 2021). There are many theories explaining the increases in muscle flexibility and joint range of motion, pointing out neuromuscular changes (Freitas et al. 2018; Freitas and Mil-Homens 2015) and structural changes in the muscle–tendon unit and reduction in passive peak torque (Moltubakk et al. 2021; Nakamura et al. 2017). The described increased number of serial sarcomeres in animals (Antonio et al. 1993; Warneke et al. 2022b)was, to the best knowledge, not confirmed in humans.

In the supplemental material, the individual progressions were reported for the significant results of this study, showing no difference in consistency of the increment of maximal strength between stretch-mediated hypertrophy and resistance training-induced hypertrophy as well as maximal strength increases (Suppl. Fig. A–F). Since most previous studies were performed with untrained participants, this study was conducted with (recreationally) active participants, showing a comparatively wide range of strength and flexibility level as well as in muscle thickness. Although lower adaptations can be assumed in trained participants, the stretch-mediated hypertrophy was also effective in participants with higher strength levels and/or muscle thickness. However, since the study was conducted over a period of only six weeks, investigations using longer training durations are requested to exclude strong adaptations because of an unfamiliar training stimulus.

Limitations

Since testing of maximal strength was performed under isometric conditions, higher increases in the stretching group might be explained with contraction-specificity because of proximity to the intervention stimulus (Lanza et al. 2019). To improve comparability to dynamic conditions, dynamic one repetition maximum testing should be included in future testing as hypertrophy training of IG2 was performed dynamically but tested under isometric conditions. There is limited transferability of isometric strength to one repetion maximum measurements (Murphy and Wilson 1996). In contrast to maximal strength increases, there was higher hypertrophy in the gastrocnemius in the resistance training compared to the stretching group. This may be explained due to the use of different joint angles and, therefore, used stimuli in different muscle length while stretching used maximal range of motion only. In both groups, the interventions seem to be more effective for increases in muscle thickness of the medial head of the gastrocnemius. To rule out adaptations based on an unfamiliar stimulus or only adaptations in the first phase of training, investigations examining longer intervention periods are requested. As this study compared the effects of a one hour daily stretching routine to the effects of a hypertrophy training using 5×10-12 repetitions performed three times per week, obviously, the time under tension as well as the intensities cannot be compared with one another. However, this was not the aim of this study as the effects of two different training routines are contrasted. Furthermore, inconsistency in the wording to describe the training status of included participants throughout the studies should be considered when interpretating the results of these studies. No statement can be given about the effects in highly trained participants, as no previous research investigated long-lasting stretching in elite athletes.

Furthermore, ultrasound imaging to investigate hypertrophy following training interventions seems to be biased by limited objectivity and a lack of accuracy (Warneke et al. 2022e). Therefore, using magnetic resonance imaging measurements to confirm morphological adaptations should be considered in future study designs.

In general, there is no “real” quantification of stretching intensity in many studies in humans. Using stretching pain as an indicator for stretch intensity seems to be biased, as Lim and Park (2017) pointed limited correlations between stretching pain and passive peak torque. Assuming mechanical tension is of crucial importance for adaptations in maximal strength and hypertrophy, the passive torque of the muscle should be considered as relevant. Therefore, no studies could be found addressing the effects of different intensities which could be of high impact for the practicability of the stretching routine, since it might be hypothesized that using higher intensities could reduce the required stretching duration to reach comparable adaptations.

Lastly, the influence of training level, sex and age was not investigated in this study. However, the sex-dependent adaptations were previously investigated by Warneke et al.

2023. To investigate further independent variables' influence such as age and training level, a more heterogeneous group of participants should have been included to the study.

Practical applications

Results point out long-lasting stretch training (one hour daily, high elongation stress) as a promising alternative to resistance training (e.g., hypertrophy training) in different settings over a six-week period, especially if commonly used resistance training is contraindicated, e.g., after injury and surgery. There are some advantages of long-lasting stretch training for athletes and patients to perform their training routine independent of training equipment like the leg press or calf muscle machines which are required for traditional resistance training of the plantar flexors to achieve hypertrophy.

Outlook

Long-lasting stretching interventions produced significant hypertrophy and maximal strength gains in animal studies (Antonio et al. 1993; Bates 1993; Warneke et al. 2022b). In humans, more evidence regarding long-lasting stretching interventions and its impact on maximal strength and muscle thickness is required. Even though Nunes et al. (2020) showed that short-lasting stretching is not sufficient to induce hypertrophy, previous research shows that long-lasting stretching interventions can induce sufficient tension to improve maximal strength, range of motion and muscle thickness (Warneke et al. 2022a). The present study also showed significant increases over a six-week period in the measured parameters which are comparable to those of a commonly used resistance training in the plantar flexors. Since significant decreases in strength capacity, flexibility as well as muscle thickness due to immobilization (Stevens et al. 2004) after injury and/or surgery can be assumed, the results of this study are promising as a method with high potential in rehabilitation of orthopedic indications. Therefore, studies including clinical trials and older participants should be performed. To investigate the underlying physiological adaptations leading to increased strength capacity as well as hypertrophy, neuromuscular adaptations (for example via EMG) as well as further morphological adaptations should be addressed in further studies.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00421-023-05184-6.

Author contributions KW1 and KW2 developed the idea of the study. KW1 carried out the experiment with the help of LHL and TW. Statistical analysis was performed by KW1 and MK. KW1 and AB determined the muscle thickness from the sonography measurement. MH provided the measurement devices and programs. KW1 wrote the first draft of the manuscript which was discussed and reworked by KW1, KW2, MK, and SS. Results were discussed by all authors. SS supervised the study.

Funding Open Access funding enabled and organized by Projekt DEAL.

Data availability Data can be provided by the corresponding author due to reasonable request.

Declarations

Conflict of interest No involved authors declares a conflict of interest.

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Supplemental Material

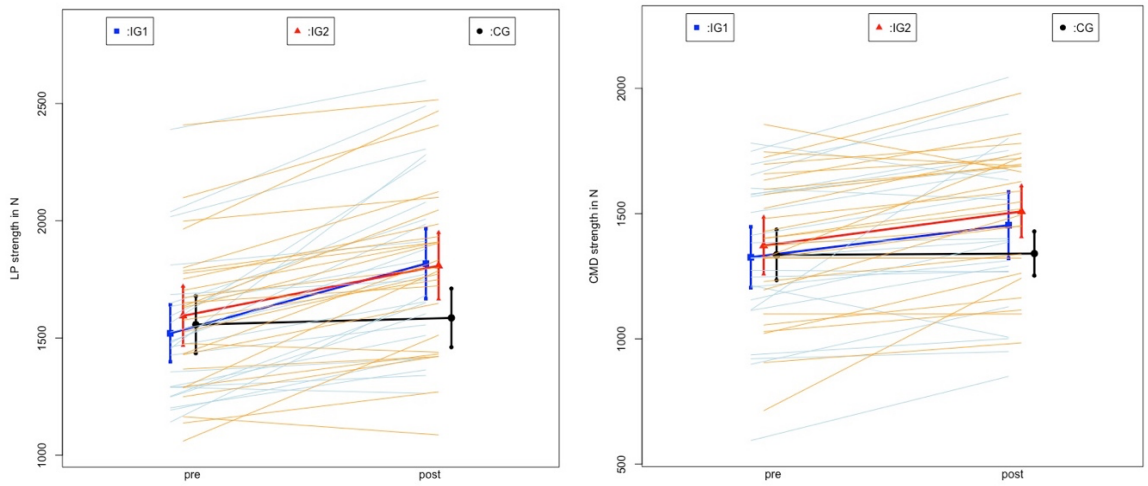

Figure A Comparison of maximal strength from pre- to post-test in the stretching group (IG1), the strength training group (IG2) and the control group (CG) with extended (a) and bent knee joint (b) under consideration of individual progressions

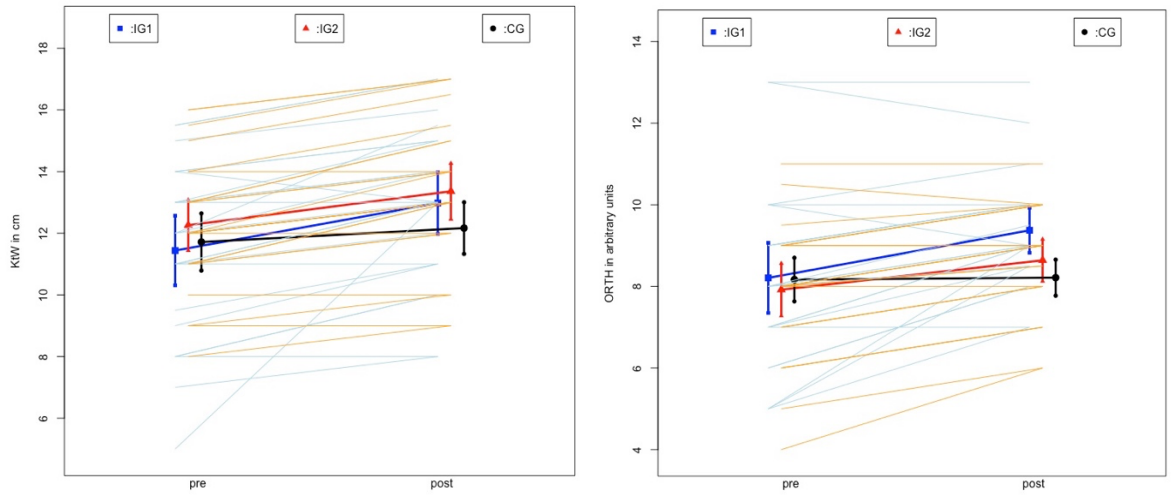

Figure B Comparison of flexibility from pre- to post-test in the stretching group (IG1), the strength training group (IG2) and the control group (CG) in the knee to wall stretch (a) and via the goniometer of the orthosis (b) under consideration of individual progressions

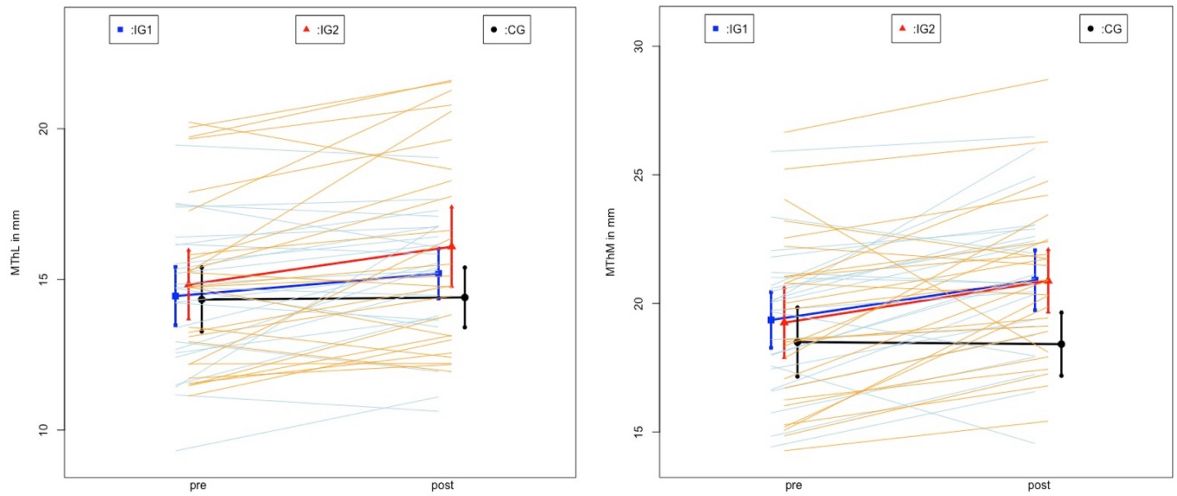

Figure C Comparison of hypertrophy from pre- to post-test in the stretching group (IG1), the strength training group (IG2) and the control group (CG) in lateral head of the gastrocnemius (a) and the medial head of the gastrocnemius (b) under consideration of individual progressions

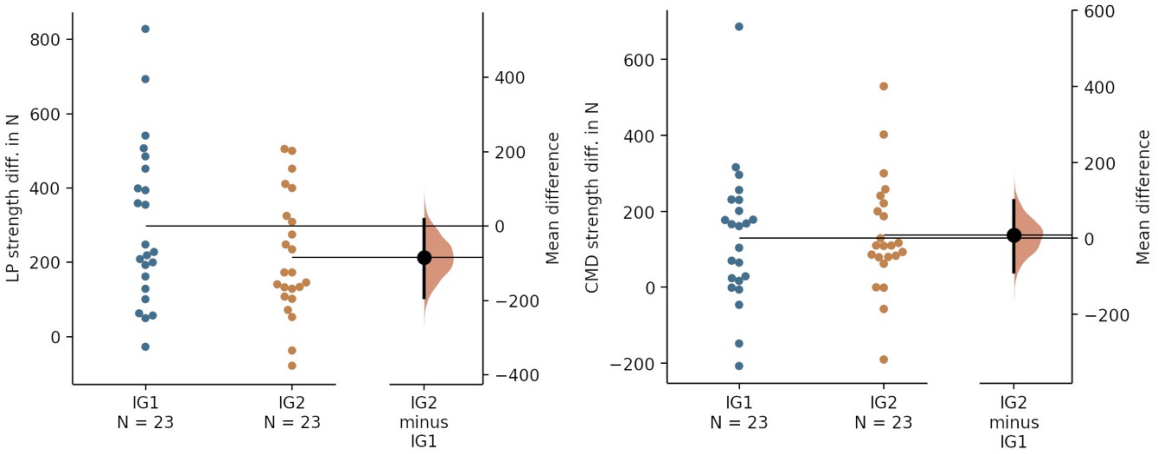

Figure D Comparison of increases in maximal strength in the stretching group (IG1)and the strength training group (IG2) with extended and bent knee joint illustrating the group differences in mean differences

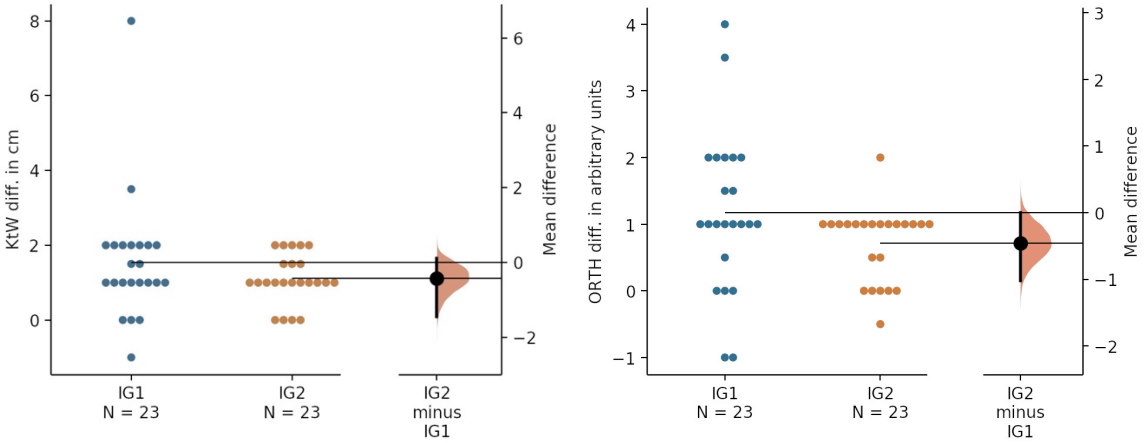

Figure E Comparison of increases in flexibility in the stretching group (IG1)and the strength training group (IG2) in the knee to wall stretch and via the goniometer of the orthosis illustrating the group differences in mean differences

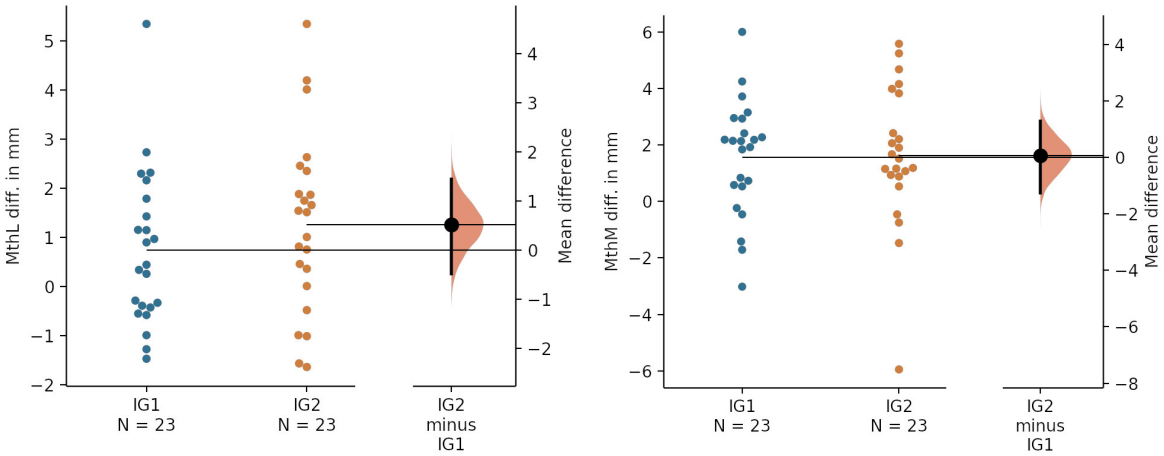

Figure F Comparison of progressions in muscle thickness in the stretching group (IG1) and the strength training group (IG2) in lateral head of the gastrocnemius and the medial head of the gastrocnemius illustrating the group differences in mean differences

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Influence of Long-Lasting Static Stretching Intervention on Functional and Morphological Parameters in the Plantar Flexors: A Randomized Controlled Trial

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Abstract

Warneke, K, Keiner, M, Wohlann, T, Lohmann, LH, Schmitt, T, Hillebrecht, M, Brinkmann, A, Hein, A, Wirth, K, and Schiemann, S. Influence of long-lasting static stretching intervention on functional and morphological parameters in the plantar flexors: a randomised controlled trial. *J Strength Cond Res* XX(X): 000–000, 2023—Animal studies show that long-lasting stretching training can lead to significant hypertrophy and increases in maximal strength. Accordingly, previous human studies found significant improvements in maximal voluntary contraction (MVC), flexibility, and muscle thickness (MTh) using constant angle long-lasting stretching. It was hypothesized that long-lasting stretching with high intensity will lead to sufficient mechanical tension to induce muscle hypertrophy and maximal strength gains. This study examined muscle cross-sectional area (MCSA) using magnetic resonance imaging (MRI). Therefore, 45 well-trained subjects (f: 17, m: 28, age: 27.7 ± 3.0 years, height: 180.8 ± 4.9 cm, mass: 80.4 ± 7.2 kg) were assigned to an intervention group (IG) that stretched the plantar flexors 6 × 10 minutes per day for 6 weeks or a control group (CG). Data analysis was performed using 2-way ANOVA. There was a significant Time × Group interaction in MVC ($p < 0.001$–0.019, $\eta^2 = 0.158$–0.223), flexibility ($p < 0.001$, $\eta^2 = 0.338$–0.446), MTh ($p = 0.002$–0.013, $\eta^2 = 0.125$–0.172), and MCSA ($p = 0.003$–0.014, $\eta^2 = 0.143$–0.197). Post hoc analysis showed significant increases in MVC ($d = 0.64$–0.76), flexibility ($d = 0.85$–1.12), MTh ($d = 0.53$–0.6), and MCSA ($d = 0.16$–0.3) in IG compared with CG, thus confirming previous results in well-trained subjects. Furthermore, this study improved the quality for the morphological examination by investigating both heads of the gastrocnemius with MRI and sonography. Because stretching can be used passively, an application in rehabilitation settings seems plausible, especially if no commonly used alternatives such as strength training are applicable.

Key Words: maximal strength, maximal voluntary contraction, muscle cross-sectional area, muscle thickness, range of motion, long-lasting stretching

14 Supplemental Material: Short Study-Summaries

Content

14 Short Study-Summaries

Findings of the studies included to this work were previously reduced to the very minimum of the results to focus on the own conducted work. However, it seems valuable to review the study design and get more detailed information about what was done before. Therefore, the following sections will resume the main points of the included baseline research by providing information about the theoretical background used by the authors, more detailed information about the study design as well as the outcome of the animal studies in 1.1 and the studies using human model in section 1.2.

14.1 Short Study-Summaries of Animal Research

14.1.1 Regionalized Adaptations and Muscle Fiber Proliferation in Stretch-Induced Enlargement

S.E. Alway, P.K. Winchester, M.E. Davis, W.J. Gonyea, (1989) [12]

The ALD of one wing of 34 adult quails (6 weeks old, 150g body mass) was stretched for 30 days by using a cardboard sleeve (developed by [282]) equivalent to the weight of 10% of the birds' body weight. The bodyweight did not change in the period of intervention. 30 days of stretch resulted in a muscle mass of 147.9 ± 16.8 mg in the intervened muscle compared to 54.1±12.2 mg in the contralateral control muscle corresponding to a difference of 171.8%. Furthermore, authors reported an increase in FCSA of 184.7 ± 6.9%, an increase in fiber length of 23.5 ± 0.8% with 16.9 ± 0.7 mm in the contralateral control muscle to 20.8 ± 0.9mm in the stretched muscle and a difference of 51.8 ± 5.6% in the fiber number (1945 ± 121 in the intervened muscle and 1281 ± 83 in the control muscle). Because of no enhanced electromyographic activity while stretching was performed, authors attribute the enlarged muscle parameters to the stretch stimulus. Authors showed stronger increase in slow twitch fibers (ST-fibers) and therefore they speculate that fiber proliferation due to stretching depends on fiber distribution, which can be seen in differences between responses of the ALD (90% ST fibers) and the PAT (mostly fast twitich (FT) fibers). Hypertrophy and Hyperplasia effects were shown to be larger in the anterior latissimus dorsi. It cannot be ruled out that adaptations of the muscle could occur from voluntary contraction against the added weight. Increases in fiber length were attributed to a serial accumulation of sarcomeres.

14.1.2 Attenuation of CA2+-activated ATPase and Shortening Velocity in Hypertrophied Fast Twitch Skeletal Muscle from Aged Japanese Quail

Stephen E. Alway, (2002) [10]

The PAT of one wing of 35 8-week-old and 35 110-week-old japanese quails was stretched for up to 30 days with 12% of the animal's bodyweight. In addition to morphological assessment, contractile measurements of the muscle were performed in young and old animals. Authors stated an increase of 45% and 24% in the intervened muscles of young and old animals respectively. In young animals, there was an increase of 19% in the first four days. In older animals, authors measured an increase in muscle mass of 8% after 7 days, however, it did not change after 21 days as well. Furthermore, there was an increase in muscle length (17.9 ± 1.3mm vs 15 ± 1.4mm) in young animals and 17.1 ± 1.4mm vs 15.3 ± 1.5mm in old animals. Hypertrophy occurred in both ST and FT fibers, however, FT fibers hypertrophied to a larger extend in young animals. Contraction time of the muscle was higher in the old animals compared to the young animals. Velocity in shortening decreased due to stretching. The authors resumed that stretching overload decreased the velocity of shortening in both, young and old animals and that Ca2+-ATPase activity is able to be changed in myosin isoforms of twitching muscles. In young muscles there was a shift from fastest myosin isoform FM1 to slowest myosin isoform FM3 due to stretching, while there was no change of myosin expression in old animals. Myosin shift cannot be the only explanation of reduced shortening velocity.

14.1.3 Stretch-Induced Growth in Chicken Wing Muscles Myofibrillar Proliferation

C.R. Ashmore & P.J. Summers, (1981) [25]

The PAT of the right wing of ten chickens was stretched for up to seven days. Authors investigated myofibrillar protein changes and fiber splitting and showed significant increased fiber splitting of up to 45% after 1 week of stretching compared with the no stretched control muscle. Mean fiber area increased from 0.90 ± 0.26 µm in the control muscle to 1.22 ± 0.43 µm in the intervened muscle. The authors discussed fiber splitting after reaching a critical size of the muscle fiber. It is hypothesized that splitting starts at the Z-disk and disruptions could be filled with developing elements of the sarcotubular system. In this study, authors stated an increase of 50% in muscle cross sectional area as well as myofibril splitting of 45% after one week. Growth of skeletal muscle originates from increased level of protein synthesis and protein catabolism with an elevated protein RNA level. Authors also refered to protein catabolism as possible explanation for fiber splitting and Ca2+ activated neutral protease could play a role in initiation of fiber splitting. Furthermore, it is shown that passive stretch disrupted the normal alignment of myofibrils resulting in wavy Z-lines, what can lead to an increased liberation of Ca^{2+} from the sarcoplasmatic reticulum, and therefore, possibly to an increased Ca^{2+} activated protease.

14.1.4 Effects of Passive Stretch on Growth and Regression of Muscle from Chickens of Various Ages

C. R. Brown, W.K. Palmer, P.J. Bechtel, (1986) [63]

The PAT of the left wing of 6-week-old, 10-month-old and 28-month-old chicken was stretched for about two weeks. Furthermore, regression of adaptations was investigated after an intervention period for up to 22 days. In six-week-old chicken, there was an increase of 60% over control level, but returned to control weight within 13 days. In 10-month-old chicken there was an increase of 40% due to 11 days of stretch, which decreased fast after removing the stretch. In 28-month-old chicken, there was a decrease in the stretched muscle. There were also increases in muscle fiber cross-sectional area and DNA level as well as protein concentration. Authors showed an influence of age on responses of the muscle on stretching stimulus. While total protein increased during hypertrophy process, the protein concentration was constant and decreased to baseline due to atrophy. Study pointed out reduced responsibility to stretch interventions in older chicken. The muscle showed a very decreased reaction to a stress situation compared to younger animals.

14.1.5 Involvement of PI3K/Akt/TOR Pathway in Stretch-Induced Hypertrophy of Myotubes

N. Sasai, N. Agata, M. Inoue-Miyazuk, K. Kawakami, K.Kobayashi, M. Sokabe, K. Hayakawa, (2010)[261]

There are many signaling pathways involved in hypertrophy process of the muscle. IGF-1, PI3K/Akt/TOR pathways as well as mitogen activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK) pathway seem to regulate muscle mass by proliferation, differentiation and survival of muscle cells. There are indications from striated muscle from the myocard indicating that MAPK pathway may play an important role in hypertrophy-related gene expression, resulting in hypertrophy. Cell cultures of muscle cells from the breast muscle of chicken embryos were dissected and stretched by using a stretching apparatus. To examine the influence of PI3K/Akt/TOR pathways, muscle cells were treated with inhibitors (wortmannin) leading to decreased hypertrophy of stretched myotubes. To examine the influence of the MEK Pathways, muscle cells were treated with the inhibitor U0126 showing involvement of MEK/ERK pathway in the basal downregulation of myotube thickness. Authors concluded that there seems to be acceleration of protein synthesis as well as suppression of protein degradation of PI3K/Akt/TOR pathway, which could be stimulated by mechanical stretch or IGF-1 treatment to induce hypertrophy. Hypertrophy effects are decreased, when PI3K/Akt/TOR pathway is inhibited. S6K (S6 Kinase) is known as downstream effector of PI3K/Akt/TOR pathway and is activated as response to acute stretch of myotubes. However, there are more factors influencing the muscle growth since mTOR/p70s6K can be regulated by mechanical stresses independently from PI3K/Akt.

14.1.6 Hypertrophy and Hyperplasia of Adult Chicken Anterior Latissimus Dorsi Muscles Following Stretch With and Without Denervation

O.M. Sola, D.L. Christensen, A.W. Martin, (1973) [282]

Authors of previous studies reported increase of DNA content in hypertrophied chicken anterior latissimus dorsi [72]. From this, the authors conclude evidence of nuclear proliferation which has been noted in other hypertrophying or regenerating muscles. Furthermore, the authors described indications for hyperplasia effects pointing out small round darkly stained fibers (after myosin ATPase-staining preparation) adjacent to or interdigitated with intermediate fibers suggesting those as formation of new fibers. Therefore, even with more evidence for hypertrophy, they suggested that it *"does not mean that there may not be a gradual replacement of fibers in normal life, nor that there may not be hyperplasia if the stimulus is adequate"* [282, p.78]. The left wing of chicken was stretched by using a weight of 100g and 200g and in two groups, the innervating nerve of the stretched muscle was removed. The anterior latissimus dorsi, posterior lastissimus dorsi and teres minor were assessed. Authors showed that there was an influence of the attached weight (intensity). Highest hypertrophy could be determined in the group with innervated muscle and 200g weight to induce the stretch (up to 169% increased). Also, for fiber number, there was influence of the weight: higher weight produced more rapid increase in fiber number in denervated and innervated muscle. *"It is stretch that causes the hypertrophy of muscle"* [282, p.93] that was shown in studies performed before 1950 [201, 284]. Before studies included denervation, there was no possibility to separate effects of motor innervation and stretch, however, in this study it was shown that also denervated muscle showed significant hypertrophy due to stretch. Nevertheless, there were also studies investigating the influence of dissection of the synergists (Tenotomy) on the target muscle in combination with denervation showing atrophy [115, 130, 150, 289]. Muscle growth was only reported when stretch was applied to the denervated muscle for example by bone elongation (stretch of the corresponding muscles). However, highest effects were reported in innervated muscles that were stretched with high weights. Therefore, authors state *"If stretch can be maintained, there appears to be little limit to extent and duration of the hypertrophy."*[282, p.95]. However, the study confirmed responses to stretch dependent on the muscle fiber distribution of the stretched muscles [131, 137, 283]. *"From the studies of many previous workers and the results of this investigation, we have come to the opinion that hyperplasia follows adequate stretch"* [282, p.97]. Authors suggested that hyperplasia is the result of longitudinal muscle fiber splitting or fiber differentiation occurring in clusters around mature fibers. Afterwards those so-called daughter fibers could establish themselves as independent new fibers [218]. This fiber formation could be attributed to satellite cells. Therefore, fiber counting became necessary. Authors described the formation of new fibers as follows *"In our examination of longitudinal sections of the anterior latissimus dorsi muscle we have seen that some new fibers originate in bundles of vesicular nuclei surrounding a large fiber and that these smaller fibers are separated from the larger fiber by this nuclear mass. In other instances, two or three smaller caliber fibers will appear to be emanating from a segment of paced nuclear and cellular material located at the abrupt end of one large fiber."* Furthermore: *"It is suggested that new fibers are separated from mature fibers by this region of myogenic activity and arise de novo confirming in the growth pattern to that of embryonic tissue [. . .], expression ATPase activity that differs completely from the mature fibers but confirming to that of newly developing*

muscle fibers [129]. New fibers appeared at the 2-week time in severely stretched muscles and, by 6th week, many of the darkly staining fibers were as large as normal." [282, p.98].

14.1.7 Regression of Skeletal Muscle of Chicken Wing after Stretch-Induced Hypertrophy

M.P. Sparrow, (1982)[286]

60 cockerels were divided into 10 groups of six birds each and the left wing was stretched with an attached weight of 160g. After the first week, weight was removed from 30 birds and six birds were decapitated immediately. Remaining birds were killed and dissected after further three, seven, 13 and 29 days. After 28 days, weight was removed from the other birds and birds were killed immediately, after five, 13, 25 and 35 days and the muscles of the intervened wing and the control wing were examined. Muscle weight increased by 86.31% (from 0.913 $\pm$ 0.02g to 1.701 $\pm$ 0.073g) after seven days of stretch and 99.4% from 0.928 $\pm$ 0.026g to 1.850 $\pm$ 0.07g) after 28 days of stretch. Protein content increased after seven days of stretching by 59% and after 28 days by 99% compared to the control condition. After removing the weight, regression of the muscle was finished to a large extent after 13 days, even when weight was removed after 28 days. Protein concentration was the same in controls as during the muscle regression (except for the group with seven days of hypertrophy, in which it was lower). RNA concentration increased very fast after inducing stretch (peak was seen at day three of stretch) but was only 16% bigger than control after 28 days of stretch. While regression, total RNA decreased fast and reached the level of the control group after day 29. Changes in muscle mass were attributed to changes in protein synthesis due to stretching stimulus induced hypertrophy. Muscle RNA could be seen as a predictor of the following progression of the DNA content, and therefore the protein content. There were also changes in collagen content. Author suggested that hypertrophy (and atrophy) of the muscle is related to the tension per cross sectional area of the muscle fiber but referred to missing knowledge of corresponding intracellular events of protein degradation.

14.1.8 Stretch-Induced Growth in Chicken Wing Muscles: Role of Soluble Growth-Promoting Factors

P.J. Summers, C.R. Ashmore, Y.B. Lee, S. Ellis, (1985)

Barnett et al. (1980) [35] and Holly et al. (1980) [139] showed rapid growth of muscle cross sectional area and muscle length of PAT in the wing of chicken due to stretching. Weight increased by 65% and muscle cross-sectional area by 55% after one week of stretching. This study investigated the possible increase of growth promoting factors when hypertrophy occurs by static stretching. Therefore, an increase in DNA, RNA, protein and metabolic enzymatic activity is hypothesized. Myoblast and fibroblast cell cultures were examined and effects of stretch on cell proliferation, cell differentiation, creatine kinasis were observed. Stretching was induced for 5 days as described previously. An increase of the 1.5 times in muscle wet weight was observed after 5 days of stretching compared to the control muscle. Cell proliferation was stimulated and creatine kinasis activity was three times higher than in control. Authors resume

that growth promoting factors seem to be highest when muscle growth is existing. As soon as growth stopped, a decreased activity of growth promoting factors could be observed. Matsuda et al. (1984) [202] showed that more growth factors were present in the ST-fiber-dominant anterior latissimus dorsi compared to FT-dominant pectoralis major. The present study found increased transferring activity in the intermedial spaces (perhaps because of higher vascularity of the anterior latissimus dorsi compared to pectoralis major). Passive stretch of white fibers of the patagialis muscle resulted in increased oxidative enzyme activity and capillary/fiber ratio [35, 139]. Strong increase of DNA and increased number of muscle fiber nuclei in the basement membrane was pointed out as well [35].

14.1.9 Mechano-Biology of Skeletal Muscle Hypertrophy and Regeneration: Possible Mechanism of Stretch-induced Activation of Resident Myogenic Stem Cells

R. Tatsumi (2010), [301]

Satellite cells are stated as resident myogenic stem cells in postnatal muscle in the basal lamina that surround the muscle and in the sarcolemma of the fiber and are mostly in quiescent state in adult muscles. When muscle fibers are damaged (e.g. after mechanical overuse from stretching) satellite cells get activated and migrate to the injured place to replicate DNA, divide and fuse to injured place or form new fibers [53, 205, 349]. Therefore, there is an accumulation of numbers of myonuclei in the fibers with an increase in protein synthesis leading to hypertrophy and hyperplasia. Satelite cell activation is therefore hypothesized as a part of muscle grow and regeneration after training. Authors write: *"By learning how satellite cell activation is controlled, we will be able to design new procedures to enhance muscle growth and repair, contributing the meat-animal production, humans sports and health sciences aimed for physical performance enhancement in athletes and medical therapies on muscular dystrophy and age-related atrophy (sarcopenia)"*. Furthermore, the activation of satellite cells, by hepatocyte growth factor (HGF) and nitric oxide (NO) is described in the paper. Research by Bischoff [52] showed that damaged muscle tissue extracts *"phosphate-buffered saline (PBS) which stimulates quiescent satellite cells to proliferate"*. The so-called crushed muscle extract was examined and it was shown that it is released from the extracellular matrix when a muscle fiber is damaged and is called hepatocyte growth factor (HGF). HGF is known as a heterodimer and was related to regeneration process of the liver which stimulates hepatocyte proliferation. In vivo, HGF seems to be the only growth factor which activates quiescent satellite cells to enter in cell cycle in vivo. Earlier experiments demonstrated that known growth factors as insulin-like growth factor 1 (IGF-1) and fibroblast growth factors (FGFs), platelet-derived growth factor beta (PDGF-BB), transforming growth factor beta (TGF-beta1 and 2) and epidermal growth factor (EGF) were not included in activation of satellite cells. HGF was localized in undamaged muscle fibers in the extracellular domain and from there, it fastly "associates" with satellite cells after injury of the muscle. Further, Nitric oxide radicals (NO) seem to be another factor, which is located in sarcolemma of the muscle fibers [61]. NO radical production is very low in quiescent satellite cell status, however, it is upregulated by muscle damage and mechanical stretch. Therefore, NO radical is released due to mechanical stimulation and initiates molecular pathways which are involved in muscle growth and

regeneration [13, 14]. Satellite cells in quiescent and activated status express c-met receptor as mediator for signalling pathway of HGF [6, 302] when HGF associates with satellite cells after damaging, which is also the case after mechanical stretch. Furthermore, results showed that calcium-calmodulin is included in mechanotransduction and mediates the HGF release which is upstream of NO radical synthesis. Mechanical stretch seemed to activate quiescent satellite cells to enter in cell cycle, which is induced by an intracellular signaling cascade mediated by calcium-calmodulin, with a responsibility for NO radical synthesis resulting in a release of HGF. The authors hypothesized that *"it is a molecular cascade of events including calcium-calmodulin formation, NO radical production, MMP activation, liberation of HGF with associated extracellular segment of proteoglycans and the subsequent presentation to the receptor c-met to generate a signal for satellite cell activation"* [301, p.17] and *"in conclusion therefore, a mechanism exists to translate mechanical changes in muscle tissue into chemical signals that can activate sateliite cells [. . .]"*. [301, p.17].

14.1.10 Fiber Number, Area, and Composition of Mouse Soleus Muscle Following Enlargement

B. F. Timson, B.K. Bowlin, G.A. Dudenhoeffer, J.B. George, (1985) [310]

Ablation of the gastrocnemius led to increase of the soleus muscle in mice of up to 39.1%, however, no higher fiber number could be obtained with an increase in the fiber composition of type 1 fibers. The authors questioned histological determination of fiber number in muscle with parallel fiber arrangement. The authors *"provide strong evidence against hyperplasia as a mechanism for muscle enlargement in the mouse soleus muscle"*.

14.1.11 The Importance of Stretch and Contractile Activity in the Prevention of Connective Tissue Accumulation in Muscle

P.E. Williams, T. Catanese, E.G. Lucey, G. Goldspink, (1988) [342]

Immobilisation in a shortened position resulted in a loss of sarcomeres and reduced muscle compliance [295] with an increase in collagen, possibly explaining increased stiffness of the muscle. Proportion of collagen increased after only few days of immobilization while loss of serial sarcomeres needs more time. However, it seems that immobilization is not the main explanation for increased connective tissue, as this appears only when muscle is immobilized in shortened position. Furthermore, fiber length decreased after immobilization in shortened position as well as if the muscle only works over reduced range of motion. It was hypothesized that a reduction of serial sarcomeres would lead to the ability of the muscle to produce more tension in short fiber length. It was explained by the adaptation of the fibers to the functional length of the muscle to the shortened position. In addition, if the muscle had to work at reduced range of motion, there was also an increase in connective tissue, as it was observed in shortened immobilized position. To investigate, 40 rabbits were divided into five groups: 1. control group, 2. Soleus was immobilized in a shortened position (foot was held in plantar flexion), 3. immobilized in a shortened position in combination with stimulation of the muscle, 4. Immobilized in a stretched position, 5. only stimulation with a intervention duration

of one week. Afterwards, animals were killed and the soleus was removed and examined for the parameters fiber length, and serial sarcomere number, and connective tissue. There was a decrease in serial sarcomere number and an increase in connective tissue when muscle was immobilized in a shortened position. Immobilization and stimulation in a shortened position resulted in a larger loss of serial sarcomeres, but there was no change in connective tissue, as in the group with sole stimulation of the muscle. Immobilization of the muscle in a stretched position resulted in no loss of sarcomeres and no change in connective tissue concentration. The absence of increase of connective tissue in stretched position is attributed to the mechanical effects of stretch by the authors. *"However, it has been shown for the immobilized soleus muscles of the rat that, whereas in the shortened position EMG activity falls rapidly, in the stretched position resting EMG activity remains at normal levels"* [138]. Authors hypothesized that it may be possible that neural activation of the muscle prevents increase in connective tissue. Stimulation only led to decrease in serial sarcomere number, but no change in connective tissue, which led authors to the assumption, that contractile activity may be important for normal connective tissue.

14.1.12 Physiological and Structural Changes in the Cat's Soleus Muscle due to Immobilization at Different Length by Plaster Casts

J.C. Tabary, C. Tabary, C. Tardieu, G. Tardieu, G. Goldspink, (1972) [295]

The adaptability of striated muscle in length is stated to be first demonstrated by Marey [198] due to change the position of the distal end of the triceps surae muscle farther down the calcaneum. Authors showed a lengthening of the muscle within a few weeks. Immobilization of a muscle in a shortened position showed a reduced muscle length, a change in the length-tension curve and a reduced ROM [5], while immobilization of the muscle in a lengthened position resulted in developing new contractile tissue [300]. 27 cats were divided into 5 groups. The hindlimbs of one group were immobilized in a dorsiflexed (stretched) position, the hindlimbs of another group were immobilized in a plantarflexed (shortened) position. In those two groups, cats were dissected after 4 weeks, and the muscles were investigated. In another group, hindlimbs were immobilized in a shortened position, but after 4 weeks of immobilization, the plaster cast was removed to reach 4 weeks of recovery. Furthermore, another group with 4 weeks of immobilization in a shortened position, followed from a 4-week immobilization in an intermediate position. Finally, one control group was included. Immobilization of the muscle in a stretched position induced an increase in fiber length (total number of sarcomeres in series) of 19% but a reduction of sarcomere length of 5%. Immobilization of the muscle in a shortened position led to a decrease of 40% in sarcomeres in series. 4 weeks of recovery with normal activity after immobilization led to no difference compared with the control group. However, the immobilization in an intermediate position after immobilization in a shortened position led also to a reduction in sarcomere number.

14.1.13 Contractile Properties of Aged Avian Muscle after Stretch-Overload

S.E. Alway, (1994a) [8]

The ALD of one wing of young quails (10 weeks old) and old quails (90 weeks old) was stretched with 12% of the own bodyweight of the birds for 30 days showing that older birds had significant higher contraction time than younger birds (149 ± 9ms vs 174 ± 16ms). Long-lasting static stretching led to an increase in contraction time to 162±7ms and 215±14ms in young and old quails, respectively. Muscle mass increased in young birds from 26.7 ± 1.2mg to 71.6 ± 3.0mg and from 28.5±1.5mg to 67.4 ± 4.4mg in old animals. Maximal force increased in young animals from 58.3±2.8mN to 115.4 ± 5.9mN and from 57.4±3.1mN to 112.1±6.1mN in old animals, while relative force (strength per muscle mass) did not change. In older animals' muscle adapted to mechanical overload with hypertrophy as well, but ageing was associated with slowing of skeletal muscle because of reduced contraction velocity. Slowing of contraction time could be explained by a shift of myosin heavy chains to slower myosin heavy chains. Stretching also resulted in slowing down of contraction velocity. The myosin heavy chain expression may have been altered by stretch overload; a shift toward slower isoforms (slow myosin 1 to slow myoin 2) was shown in another study [7] but it was not clear whether this shift results in altered contraction velocities. It was discussed that aging can lead to slowing down of the muscle fiber and therefore increasing the contraction time as well.

14.1.14 Force and Contractile Characteristics after Stretch Overload in Quail Anterior Latissimus Dorsi Muscle

S.E. Alway, (1994b) [8]

ALD of the left wing of 12 adult quails was stretched with 12% of the birds bodyweight for 30 days. Muscle mass increased from 25.7 ± 0.9mg to 66.8 ± 0.8mg, muscle length increased from 15.3±0.3mm to 19.4 ± 0.4mm and absolute maximal strength increased (relative maximal strength did not change). Fiber hyperplasia was measured with 1189±78 fibers in the control and 1766 ± 99 fibers in the stretched muscle. Furthermore, contraction velocity decreased in response to stretch although there was no change in percentage FT fiber contribution; the total FT fiber number increased. ATPase activity decreased in both, slow myosin 1 and slow myosin 2 isoform after stretch overload. The author hypothesized that due to hyperplasia new fibers are involved in force production, because of an assumed strength increase of only 60% due to hypertrophy, but a determined increase of about 95%. From this, the author suggests that new fibers are functional.

14.1.15 Muscle Fiber Formation and Fiber Hypertrophy during the Onset of Stretch Overload

S.E. Alway, W.J. Gonyea, M.E. Davis (1990) [11]

There are previous hypotheses that hyperplasia might be the result of fiber splitting or branching due to stretching, while no increase in fiber number was observed after ablation of the synergists. Fibers would grow to a point where they become mechanically or metabolically inefficient resulting in splitting into two or more *"daughter fibers"*. The anterior latissimus

dorsi of adult quails was stretched unilaterally for $1 - 7$ days with 10% of their own body weight. Authors showed increases in muscle mass of up to 64±8.4% with 44.6 ± 3.2mg in the control muscle and 72.3±5.1mg in the intervened muscle. Furthermore, there was an increase of up to 40.2 ± 2.7% in fiber length, 28.9 ± 12.3% of mean fiber area and 28.6±7.0% of fiber number compared to the control muscle. While fiber length increased after 24 hours, however, fiber size and number needed more time (one week). The authors hypothesized that the increase muscle mass of the first three days can be attributed to the increase of non-muscular tissue to a large extent. The decrease of muscle cross sectional area in the first days was attributed to mechanical stretch applied to elastic components of the muscle (like stretching a rubber band). Therefore it was speculated that hyperplasia did not occur after reaching critical fiber size due to hypertrophy. However, authors stated that their methods were not sensitive enough to examine new small fibers (<500µm) with nitric acid digestion and mechanical tweezing. To assess smaller fibers, it would be essential to use other methods as fiber autoradiographic techniques [348]. Alternative hypothesis is that new fibers are formed by activating of quiescent satellite cells, because new fibers seemed to be an embryonic type of myosin rather than an adult from of Myosin from *"parent fiber"*. Winchester et al [348] also provided data for satellite cells entering the cell cycle after the first week of stretch. Maybe hypertrophy and hyperplasia are independent of each other, especially since hyperplasia was not present after ablation or tenotomy of a synergist. Data from this study do not support the hypothesis that hyperplasia is a result of finished hypertrophy.

14.1.16 Varying Amounts of Stretch Stimulus Regulate Stretch-Induced Muscle Hypertrophy in the Chicken

D.K. DeVol, J. Novakofski, R. Fernando, P.J. Bechtel, (1991) [89]

Muscle adaptations on increased demands can be attributed to many biological adaptations, such as increases in the release of growth homone (GH) and insuline like growth factor 1 (IGF1) [90, 249]. Previous research showed that passive stretching of the patagialis, anterior latissimus dorsi as well as the biceps brachii results in large hypertrophy [23, 35, 139, 174]. Some studies investigated the biochemisty which could be attributed to the muscle growth of the patagialis [64, 174] hypothesizing that mechanical tension (e.g. via stretch) seems to be of crucial importance for muscle hypertrophy [115, 131]. Sparrow [286] showed, that removing the mechanical tension via stretch resulted in rapid atrophy of the stretched muscle. The authors described that the stretch induced hypertrophy could be described as a unique-model for examining muscle hypertrophy and atrophy. The muscle of one wing was stretched using a cardboard sleeve as it was done in previous research for a) 24h/day and b) 4h/day for 5,10,15,20 and 25 days. Increasing stretching duration as well as intervention period led to higher increases in muscle mass. The regression of muscle mass after removing the continuous stretch stimulus could be inhibited by inducing intermittent stretch in the regression phase. However, there was a decrease in hypertrophy by prolonged intervention period, which were explained by the authors with different theories. Firstly, the PAT reached maximal growth potential at 50% above the control level. Secondly, the induced mechanical tension per muscle fiber decreased because of increased muscle length due to serial sarcomere accumulation, therefore, the mechanical tension stimulus per sarcomere decreased.

14.1.17 Changes in RNA, DNA and Protein Content and the Rates of Protein Synthesis and Degradation during Hypertrophy of the Anterior Latissimus Dorsi Muscle of the Adult Fowl (Gallus Domesticus)

G.J. Laurent, M.P. Sparrow, (1977) [174]

Attaching a weight to one wing of the quail showed that increases in muscle mass were induced. Authors showed that after 6 days of stretch wet weight of the muscle increased by 74%, protein content by 44%, RNA by 203% and DNA by 83% compared with the non-stretched contralateral control muscle. Rates of protein synthesis and degradation were measured over the full period of 14 days showing an increase in protein synthesis without a change in degradation.

14.1.18 Stretch Induced Non-Uniform Isomyosin Expression in the Quail Anterior Latissimus Dorsi Muscle

S.E. Alway, (1993) [7]

There was high degree of hypertrophy in ALD of quail with 50% enhancement after 7 and 150% enhancement after 30 days of stretch, which was often attributed to fiber hypertrophy and new fiber formation. It seemed that new fiber formation occured as a result of the activation of satellite cells. In contrast to other studies, some authors suggested that stretch-overload induced remodelling results from increased expression of fast myosin isoforms relative to total myosin ([203]. Other authors showed increases of slow myosin isoform 2 after stretch. Stretching was performed for the ALD for 30 days with 12% of the own body weight. Myosinisoform determination was performed via electrophorese and Myosinheavy chain determination was examined by immunocytochemistry. Muscle length of the stretched muscle was 25.4±4.6% higher compared to the intraindividual control (16.2±0.4 vs. 13.7±0.4). In both, the intervened and the control muscle two slow myosin isoforms (SM2 and SM1, slow myosin-2 and 1) and two fast isoforms (fast myosin 3 and 2) were found. Long-lasting stretching led to increased expression of slow myosin 2 and decreased expression of slow myosin 1. In addition, total fiber number was increased in the stretched muscle with 46.2±3.3% and fiber area increased by an average of 62% in ST-fibers, while there was no increase in fiber area of FT fibers. Those fibers were even 26-35% smaller in the distal region compared to other regions of the muscle. Author discusses changes in relative myosin expression due and in percentage fiber type distribution due to static stretching. To explain fiber formation and hypertrophy, the author referred to studies showing inclusion of satellite cell activation in hypertrophy and hyperplasia [349].

14.1.19 Muscle Fiber Splitting in Stretch-Enlarged Avian Muscle

J. Antonio, & W.J. Gonyea, (1994) [19]

If a muscle has been stretched for long durations, fiber hypertrophy and hyperplasia occured [12, 121, 123, 282]. There are two prominent hypotheses to explain hyperplasia: Satellite cell proliferation and longitudinal fiber splitting. *"It has been speculated that longitudinal fiber splitting occurs only after fibers hypertrophy to a point where they become either metabolically or mechanically compromised, subsequently, these large fibers could split into two or more smaller fibers that are more efficient than the large fiber"* [19, p.973]. Branched fibers after resistance training were stated as extremely low with <0.20% after 150 week of resistance training in cats [211], and in weightlifting rats with 0.5% [299]. The ALD of 18 quails was stretched unilaterally with progressive increasing weight of 10-35% for 16 and 28 days (see Figure 20 in the Discussion). After 16 days and 28 days, muscle mass of the stretched muscle increased of 188.1% and 294.3% respectively compared to the control muscle. Fiber number increased 29.7% after 28 days of stretch, while after 16 days, no differences could be observed. The authors discussed the following theory to explain hyperplasia: some studies showed fiber hyperplasia due to stretch and speculate that fibers would split after reaching this size, fibers would split into two or more *"daughter fibers"* [122, 205, 282]. Inducing intermitted stretch for 16 days, there were less than 0.3% fibers showing a splitted fiber profile, but after continuous stretch in the second half of the intervention period, there were 5.25% fibers with splitted profiles. Authors suggest that the resting phases in the first half of intervention period reduces the injury or gave the fiber the opportunity to repair damaged fibers and the muscle could adapt to the stretch related injuries with hypertrophy. In the second half of the investigation period, there was little hypertrophy compared to the first half, however, chronic stretch with no change in intensity led to higher hyperplasia. Authors assumed that the absence of rest intervals prevented the adaptation due to hypertrophy. Consequently, fiber splitting could be the consequence of a chronic stretch overload. This study showed separation of hypertrophy and hyperplasia in quails.

14.1.20 Progressive Stretch Overload of Skeletal Muscle Results in Hypertrophy before Hyperplasia

J. Antonio, & W.J. Gonyea, (1993) [18]

Muscle hypertrophy and hyperplasia lead to muscle enlargement. Sola et al. [282] showed muscle growth due to chronic stretch in the ALD, which can be attributed to hypertrophy and hyperplasia. Previous research showed that new formed small fibers containing an embryonic type of myosin and were at the outside of the other muscle fibers, which indicates that they were not part of injuried fibers. Wichester & Gonyea [350] pointed out a correlation between fiber hyperplasia and morphological indicators of fiber injury. Including rest intervals in stretching procedure seems to minimize injury and led to different responses in ALD (Hypertrophy instead of hyperplasia). ALD of 26 quails was stretched unilaterally for 28 days with progressive stretching protocol. Birds were killed after 12, 16, 20, 24 and 28 days. Muscle mass increased up to 318.6±31.5%, muscle length with about 50%, mean fiber area with a peak of 141.6±32.6% and fiber number of 82.2±17.1% compared with non-interverend control

muscle. ST fiber area increased with 152.5±38.6% at day 16, however, there was a decrease in hypertrophy at day 28 (41.0±5.4%). Fiber cross-sectional area of the fast fiber was significantly increased at day 12, 16 and 20 days, with a peak increase at day 16 with 123.5±33.3%. In the ALD, there was an average of 86% of ST fibers. Muscle length seemed to contribute to a high extent to increased muscle mass but did not increase with longer stretching duration. In the first half of the intervention, fiber cross sectional area increased to a large extent and declined by chronic stretch in the second half of the intervention, while hyperplasia was only found in the second half of the study. Hyperplasia was attributed to fiber splitting because of changed metabolism of enlarged fibers; to reach normal metabolic processes of the muscle, fibers could split into daughter fibers. The second option could be seen in the activation of satellite cells due to injury and degeneration of muscle fibers via stretch. The activation of satellite cells could lead to fusion and therefore to new fibers. Authors prefered the hypothesis of fiber splitting because they speculated about reduced muscle cross sectional area, while hyperplasia is at its peak. If new fibers were formed, muscle cross sectional area would be assumed to increase. For the last hypothesis, then authors suggested injury of the muscle fiber led to degenerative processes in each fiber resulting in satellite cell activation and a replacement of the old, damaged fiber. Winchester et al. [349] showed a satellite cell activation in the first three weeks of chronic stretch and a temporal correlation between fiber injury and hyperplasia effect. *"The decrease in fiber cross-sectional area coupled with an increase in muscle mass and fiber number suggests that these muscle fibers hypertrophied to a critical size. [. . .] this response may be simply the result of chronic stretch of sufficient duration"* (p.1270).

14.1.21 Role of Muscle Fiber Hypertrophy and Hyperplasia in Intermittently Stretch Avian Muscle

J. Antonio, & W.J. Gonyea, (1993) [18]

There are studies using animal model to investigate an overload related muscle enlargement via surgical ablation [113, 122, 113, 122] and stretching, discussing the influence of hypertrophy and hyperplasia to increases in muscle mass. Increases due to long-lasting stretching was greater than reported increases from surgical ablation models or exercise [121, 123], and there were differences regarding hyperplasia. It seemed that rest intervals between stretching sessions seem to induce difference adaptive responses. Authors speculated that intermitted stretch allows the muscle tissue to recover while chronic stretch leads to stronger injury of muscle fibers and therefore, it was hypothesized that hyperplasia may be attributed to large injury of the muscle. The ALD of one wing was stretched with 10% of the bird's bodyweight for 24 hours with a 48–72-hour rest interval in between for 15 days. After 5 days of intermittent stretch there was an increase in muscle mass of 53.1±9.0% compared with the non-stretched control. ST fiber area increased with 28.6±5.7% and fast fiber area with 18.5±8.4%, however, no difference in fiber number between the intervened muscle and the control muscle could be determined. In accordance with previous listed studies, muscle mass increase due to intermitted stretching protocol seemed to be related to hypertrophy, not to hyperplasia. The authors discussed hyperplasia due to chronic stretch and hypothesized that chronic stretch might lead to segmental necrosis, abnormal shapes, vacuolation and phagocytosis and that fiber hyperplasia may replace destroyed and degenerated muscle fibers. However, increase in present study could be attributed to increased fiber cross sectional area and fiber length.

14.1.22 Stretch-induced Growth in Chicken Wing Muscles: Effects on Hereditary Muscular Dystrophy

C.R. Ashmore, (1982) [23]

Passive stretch led to increased weight of 60% over unstretched control, which can be attributed to increases in fiber length and fiber cross sectional area. The authors did not find new fiber formation. Muscle fiber DNA was also increased in response to stretch induced enlargement. Furthermore, it an increase of oxidative enzymes and capillary to muscle fiber ratio doubles as well, while glycolytic enzyme activities decreased. Adaptations of the muscle to passive stretching were comparable with active strength training or endurance training. However, authors stated that EMG showed no signal in response to stretch (Holly et al., 1980). Dystrophic and normal chickens were included in the study and stretched for one and six weeks. In both stretching groups there were increases in muscle weight of 63% after one week vs. 200% after 6 weeks of stretch. *"Passive stretch of the normal PAT muscle at 6 weeks of age has previously been shown to be a powerful inducer of muscle growth"* (p. C178). It is stated that not all fibers are equally involved into stretch and therefore didn't react identically. Dystrophic chicken reacted with hypertrophy, if stretch was induced at the age of 1 week. Previous research showed that stretching led to significant hypertrophy in innervated and denervated muscle. *"The common factor present in all cases of muscle growth is that tension on the myofibrils is present. The tension may be actively or passively conveyed to the contractile proteins. It seems likely that rate of muscle growth is proportional to the time that tension is applied to the muscle fiber."* (p. C183). It was hypothesized that chronic stretch leads to very high potential for growth, as mechanical tension can be induced without metabolically produced high-energy metabolites. Active tension was intermittent and limited by high-energy phosphate synthesis.

14.1.23 Stretch-Induced Growth in Chicken Wing Muscles: A new Model of Stretch Hypertrophy

R.G. Holly, H.G. Barnett, C.R. Ashmore, R.G. Taylor, P.A. Molé, (1980)

Stretch induced growth was determined in smooth muscle in cardiac muscle [243] and skeletal muscle [282]. In Vitro, passive stretch showed increased amino acid incorporation into myosin heavy chains with a reduced protein degradation. The right wing of Chicken was stretched by a *"spring-loaded device"* with a force of 550g. Stretch was performed with progressively changing intensity for 5 weeks. The anterior latissimus dorsi and the patagialis muscle were stretched and showed a growth in muscle mass of 81% and 63% respectively. The authors explained the increased muscle length with serial sarcomere accumulation. A response to stretch was higher in ST-fiber dominant anterior latissimus dorsi compared to the FT-fiber dominant patagialis. However, no increase in fiber number was observed. This could be explained by different locations for fiber number measurement. The authors suggested that neuronal activity is not the stimulus for stretch-induced muscle growth since EMG did not show improved signaling.

14.1.24 Myosin Isozyme Expression in Response to Stretch-Induced Hypertrophy in the Japanese Quail

W. Matthews, R.R. Jenkins, W.J. Gonyea, (1990) [203]

"Stretch has been widely demonstrated, both in vivo [35, 95, 282] and in vitro [318] to be an extremely effective stimulus for increasing muscle mass. Muscle stretch has been shown to increase greatly the protein turnover, with synthesis exceeding degradation." [175] Increased muscle mass was attributed hypertrophy and hyperplasia. Kennedy et al.[154] demonstrated that stretch led to increased isozyme expression of slow myosin 2 instead of slow myosin 1. The right anterior latissimus dorsi and biceps brachii of 10 quails was stretched with 10% of the bird's bodyweight for 33 days resulting in 247±91% increased muscle mass and higher fiber cross sectional area in the stretched muscle (985±291µm vs 520±96 µm). In the ALD there were slow myosin 1 and slow myosin 2, while a very low percentage of fast fibers could be detected. Authors suggested that the accumulation of slow myosin could be explained by a more favorable energetic state to handle chronic muscle overload. Expression of fast myosin could possibly be attributed to new fiber formation, because usually syntheses of muscle and myosin expression follows steps as embryonic → neonatal → fast → slow myosin isoform. The transition of muscle fiber seemed to depend on received innervation. J. M. Kennedy et al. [154] demonstrated the expression of embryonic myosin in response to stretch, which was disappeared after 28 days of stretch.

14.1.25 Myosin Expression in Hypertrophied Fast Twitch and Slow Tonic Muscles of Normal and Dystrophic Chickens

J.M. Kennedy, R. Zak, L. Gao, (1991) [155]

Myosin consists of one pair of myosin heavy chains and two pairs of myosin light chains in each isomyosin. In chicken muscle there were 3 fast isomyosins (FM3,2,1) and 2 slow isomyosins (SM2,1). Furthermore, authors reported different light chains with LC1f, LC2f, LC3f. E.g., a decrease in proportion of MLC LC1f with a parallel increase of MLC LC3f led to an accumulation of FM1- isomyosin. Stretching led to a shift from SM1 to slower MHC SM2, which could have influence on the rate of growth in ALD muscle. Stretch was induced to anterior latissimus dorsi and the patagialis muscle by 10% of the birds bodyweight of the chicken for five weeks in normal and dystrophic chicken. The response to overload was similar in both: SM1 isomyosin was reduced, while there was an increase in embryonic myosin heavy chain phenotype in extrafascicular spaces.

14.1.26 Regional Injury and the Terminal Differentiation of Satellite Cells in Stretch Avian Slow Tonic Muscle

P.K. Winchester, W.J. Gonyea, (1992) [350]

Stretching produced significant hyperplasia and hypertrophy in previous research [156, 282]. Satellite cells were stated to play an important role during postnatal muscle growth as it was suggestes that they would fuse with growing myofibers to increase the number of true myonuclei [219] resulting in in a response to chronic stretch. Activation and Proliferation of

satellite cells could be associated with damaged myofibers and were suggested to be involved in the replacement of necrotic myofibers in muscle regeneration. Kennedy et al. [156] showed myofibril and Z-Band disruptions in stretched ALD, which did not lead to muscle cell apoptosis but could play an important role in activation and proliferation of satellite cells. Ashmore et al. [24] demonstrated necrosis in stretched PAT due to stretching. One wing of quails was stretched with 10% of the birds body weight for 1,2,3,5,7,10,14,21 or 30 days (see Figure 1 in Winchester & Gonyea, 1992 [350]).
After five days there was a difference in mass of stretch ALD of 33.6±7.1% while 30 days of stretching showed an increase of 115.3±8.0% compared to the contralateral control muscle. After 5 days there was a significant increase of muscle injury in the distal region of the muscle of about 49.0±24.8%, after seven days of 30.4±14.2% and after ten days of 26.9±13.6%. Myofiber degeneration was only observed in the middle and distal region of the muscle. At day 1 of stretch, activated satellite cells were detected between the basal lamina and the plasmalemma of the muscle fibers, which was often seen in stretch related injury of muscle fibers. Furthermore, the authors stated the presence of small myofibers in the interstitial spaces in stretched muscle with new myotubes containing many central nuclei, which are surrounded by contractile protein. *"In order to evaluate the role of the satellite cell in an enlarging muscle, it was first important to determine whether chronic stretch resulted in fiber injury because satellite cell activation has been associated with muscle fiber degeneration and regeneration in the adult animal [264, 280, 281]."* Changes after stretching included Z Band and myofilament disruption and are mainly present at the first ten days of stretch. Khan [157] also described Z-Band disruptions due to stretch in the teres major in animal model. Kennedy et al. [156] showed indications of muscle fiber necrosis and degeneration due to chronic stretch in the anterior latissimus dorsi. It was speculated that satellite cell activation may play an important role in initial phase of injury for regeneration and in the second phase it might play a role for hypertrophy.

14.1.27 Adaptations of Myonuclei to Hypertrophy in Patagialis Muscle Fibers From Aged Quail

J. Lee, S.E. Alway, (1996) [176]

ST fibers and small fibers have smaller DNA units than FT fibers and large fibers (nuclei with controlling cytoplasmic domain); consequently, muscle hypertrophy would lead to an increase in myonuclei number. Rosenblatt et al. [253] showed that hypertrophy depends on the ability of satellite cell activation in the rat muscle. Satellite cells are present in a quiescent status between the basement membrane and the plasmalemma of the muscle fiber. Investigating stretch-mediated hypertrophy, quails were often used to investigate stretch- because they were stated mature at the age of six weeks and no growing could be observed after six weeks. Previous studies showed that hyperplasia and hypertrophy seem to be present in the anterior latissimus dorsi after stretch in young and old quails. Both adaptations were stronger in young birds, but satellite cell activation seemed not to decrease with age. The authors hypothesized that satellite cell activation could not be the critical factor to induce hypertrophy. The patagialis of young (12-week-old), of adult animals (52 weeks old) and old (90 weeks old) quail was stretched with 12% of the bird's bodyweight for up to 30 days. There was a

significant increase of muscle weight in young birds at all time points with a peak at day 30 with 44% (131.5±1.7mg vs. 189.4±6.4mg) but also in adult animals with 35% (128.6±1.7mg vs 174.8±4.4mg) and in old animals with 25% (126.7±1.3mg vs 158.5±4.8mg). The average of fiber number per muscle did not change dependent on age. The authors discussed different adaptations because of fiber distribution and specific physiological and innervating conditions.

14.1.28 Modulation of IGF mRNA Abundance during Stretch-Induced Skeletal Muscle Hypertrophy and Regression

S.M. Czerwinski, J.M. Martin, P.J. Bechtel, (1994) [85]

Adaptations of the muscle can be attributed to a series of biochemical and physical adaptations. Hypertrophy due to stretching can be seen as a response to increased demands. *"The physiological and biochemical changes that occur during stretch-induced growth of the PAT muscle have been investigated [...] and it has been suggested that stretch or increased tension on a muscle is a major component contributing to muscle mass increases[...].* Insulin like growth factors (IGF) seem to be included of regulation of the protein synthesis. Previous studies provided information about an increase of IGF-1 mRNA increases during muscle hypertrophy [315]. The left wing of 57 chicken was stretched for 11 days; a part of the group got no food at day 0 of stretching. Muscle weight of the PAT increased with 13% and 44% at day 2 and 11, respectively. After removing the stretch stimulus, muscle mass decreased. There was a response of IGF1 mRNA to stretch at time points 11, 13, 18 and 25. After removing the stretch stimulus, IGf-1 mRNA decreased, but stayed elevated over a period of regression. From this, it was stated that muscle hypertrophy and growth is accompanied by increasing of IGF-1 mRNA.

14.1.29 Stretch-Induced Growth in Chicken Wing Muscles: Biochemical and Morphological Charaterization

J.G. Barnett, R.G. Holly, C.R. Ashmore, (1980) [35]

Inducing chronic stretch to one wing of a chicken or quail to stretch the slow tonic anterior latissimus dorsi muscle resulting in 80% increase in muscle weight after one week and 180% after five weeks [282]. Laurent and Sparrow [174] and Laurent et al. [175] pointed out elevated protein, DNA and RNA accompanying stretch induced muscle hypertrophy. Holly et al. [139] stretched the wing by using a stretching apparatus showing significant growth in muscle cross-sectional area, length and muscle weight. EMG showed no significant neuromuscular activity in the stretched muscle compared with control. The patagialis muscle and biceps muscle were stretched for ten days. Stretch led to an increase in muscle weight of the PAT of 67%. The biceps muscle showed similar responses with reduced magnitude. Hypertrophy was accompanied by an increase of DNA concentration with a peak at day 7 and an increase in RNA concentration with a peak of 122% at day 5. There were two phases of growing. In the first phase there was large hypertrophy and large increases of DNA and RNA, in the second phase there was slower hypertrophy with a decline in DNA and RNA concentration. Stretching for seven days led to increased fiber sizes in the middle region of the muscle; no hyperplasia effect could be obtained. Hyperplasia in the anterior latissimus dorsi was explained by the fact

of different fiber distribution and therefore, different innervation of the muscle and different responses. However, destruction of Z-line and A-I junctions were determined. The authors discussed muscle growth with a) regenerative response to injury of the transverse tubules or sarcoplasmatic reticulum or b) damaging the sarcoplasmatic reticulum could result in elevation of Ca^{2+} level in the sarcoplasm, which has previously shown to stimulate the RNA protein synthesis.

14.1.30 Stretch Overload-Induced Satellite Cell Activation in Slow Tonic Muscle from Adult and Aged Japanese Quail

J.A.Carson, S.E. Alway, (1996) [67]

There are many stimuli responsible for entering satellite cells in the cell cycle as IGFs, fibroblast growth factors and muscle damage [52, 51, 271]. Fusion of satellite cells resulting in an addition of myonuclei and therefore a constant DNA-to-cytoplasma ratio during growing or regenerating muscle. Stretching led to a large stretch-induced muscle enlargement of the ALD including fusion of satellite cells to new myotubes, *"which evolve into fully innervated muscle fibers [...]"* (p.C578). In aged birds reduced adaptations could be observed and a reduced responsibility for this alterations was discussed in the study. One wing of 15 12-week-old quails and 15 90-week-old quails was stretched for seven and 14 days. There was an increase of 141.6±9.5%in muscle mass and 32.7±3.8% in fiber number after 14 days of stretch in the adult animals and of 106.9±11% in muscle mass and 18.9±3.4% in fiber number in the old animals. The authors discussed the influence of age on hypertrophy adaptations in the muscle of the anterior latissimus dorsi due to stretching. There were different pathways included to satellite cell activation and proliferation with identical mechanical load on the muscle.

14.1.31 Time Course of Hypertrophic Adaptations of the Anterior Latissimus Dorsi Muscle to Stretch Overload in Aged Japanese Quail

J.A. Carson, S.E. Alway, M. Yamaguchi, (1995) [68]

Aged muscle showed diminished ability to respond on stretching stimulus via hypertrophy and hyperplasia. There seemed to be no linear relationship, since there were 50% of the increase in fiber number of 30 days was reached after 7 days of stretch. 94 quails (45 young and 49 old animals) were divided into two groups and one wing was stretched with 10% of the bird's bodyweight for seven and 14 days. Muscle mass increased dependent on age with 134.7±5.8% in adult animals and with 102.4±6.2% in old animals, while the fiber number also increase with 31.6±2.1% and 19.2±2.2%, respectively. Fiber cross sectional area increased by 51.6±7.0% in adult and 39.6±8.5% in old animals. Ageing had influence on adaptations of the muscle on stretching stimulus. Satellite cell activation seemed to be responsible for hyperplasia and fiber formation potential seems to be diminished with age.

14.1.32 Hypertrophy and Proliferation of Skeletal Muscle Fibers from Aged Quail

J.A. Carson, M. Yamaguchi, & S.E. Alway, (1995) [69]

There seem to be specific adaptations of muscle to specific stimulus: if muscles repeatedly contract against resistance (Goldberg et al., 1975) or if it undergoes mechanical tension [11], muscle seem to increase. Stretching led to an increase in muscle mass in the anterior latissimus dorsi of quails and chicken due to hypertrophy and hyperplasia [12, 282]. Furthermore, there was a shift in muscle fiber distribution in myosin isoforms from slow myosin 1 to slow myosin 2. One wing of 32 quails (16 adult and 16 old animals) was stretched for 30 days. Results showed an increase in muscle mass of 178.7±7.1% with an increase in fiber number of 59.6±8.0% in adult animals and of 142.8±7.9% in muscle mass with 47.2±8.1% in fiber number in the old animals. An increase in fiber cross-sectional area in young adults was higher with 63.8±7.8% in adult animals compared to 49.1±5.4% in old animals. The results showed an influence of age on adaptations of the muscle due to stretching stimulus. An increase in slow myosin 2 compared to slow myosin 1 was obtained. There was no significant increase in fast twitch fibers in aged animals, consequently the authors explained this with decreased capacity to innervate FT fibers in old muscles.

14.1.33 Effects of Graded Duration of Stretch on Normal and Dystrophic Skeletal Muscle

J.R. Frankeny, R.G. Holly, C.R. Ashmore, (1983) [105]

Static stretching showed hypertrophy and reduced atrophy in skeletal muscle, also in denervated muscle. *"Continuous stretch of dystrophic muscle not only stimulated hypertrophy, but also had a dramatic protective effect against the cytological pathology associated with the disease"* (p.269). The patagialis muscle of 47 chicken was stretched with different durations between 2x15 min per day to 24 hours chronic stretch for six weeks. There was a significant increase of 121% in muscle mass by inducing chronic stretch for 6 weeks, while 30 min per day led to an increase of about 70%. Increase in mass was greater than in fiber cross-sectional area, consequently, it was hypothesized that there was also longitudinal hypertrophy. The authors suggested to confirm muscle adaptations due to stretching in humans. *"We conclude that passive stretch applied for as little as 30 min/day to as long as 24 hr/day is a powerful inducer of growth of both normal and dystrophic skeletal muscle"* (p.276).

14.1.34 Effect of the Position of Immobilizaion Upon the Tensile Properties of the Rat Gastrocnemius Muscle

M.J. Jiirvinen, S.A. Einola, E.O. Virtanen, (1992) [149]

Atrophy due to immobilization is a common problem in clinical practice. Injuries of the gastrocnemius muscle tendon unit are the most common muscle-tendon strains seen in clinical sports medicine. Immobilization of the muscle in a lengthened position seemed to be beneficial compared with an immobilization in a shortend position, as this led to a decrease extensibility. To investigate the adaptations of different positions of immobilization, 52 rats were divided

into two groups. 26 hindlimbs of the rats were immobilized in shortened position and 26 in lengthened position for 7,14, and 21 days. There was a continuous decrease in muscle weight of the muscle immobilized hindlimbs in shortened position of 15% at day 7 to 36% at day 21. In both groups immobilization led to a decrease in maximal strength, however, there was less decrease in the group with immobilization in lengthened position. Elastic stiffness also increased in both groups, however, to a significant higher degree in the shortened position group (69% vs 46%). The authors highlighted that the degree of atrophy of the rat gastrocnemius was dependent on its position during immobilization.

14.1.35 Stretch-Induced Transformations in Myosin Expression of Quail Anterior Latissimus Dorsi Muscle

W.J. Roman, S.E. Alway, (1995) [251]

Stretching led to a slowing down in contraction velocity, however, it is not clarified if the adaptations can be attributed to changes in myosin heavy chain, myosin light chain or both. Stretching model as described previously was used and the anterior latissimus dorsi of one wing was stretched unilaterally with 10% of the bird's bodyweight for 7, 14 and 21 days. There was a decrease of 53.6%, 67% and 70.2% after 7, 14 and 21 days. Furthermore, stretch resulted in an increased expression of slow myosin 2 isoform from 43.1±1.7% in the control muscle to 55±1.2% in the stretched muscle and a decrease of slow myosin 1 (faster isoform) from 34.1±1.7% to 24.6±1.2% in the stretched muscle. In addition, there was a small increase in fast myosin isoform, which was not statistically significant at any point in time. In accordance with isomyosin, the myosin heavy chain distribution shifted from SHC2 to SHC1 without any change in fast myosin heavy chains. No change in the myosin light chain distribution could be observed. The authors suggest that slowing of contraction velocity may be attributed to shift in myosin heavy chain contribution to SHC2 and, therefore to slow myosin 2 Isomyosin.

14.1.36 Changes in Muscle Mass and Phenotype and the Expression of Autocrine and Systemic Growth Factors by Muscle in Response to Stretch and Overload

G. Goldspink, (1999) [117]

Mechanical stimuli can be transduced into chemical signals regulating gene expression. Gene expressions in osteocytes, fibroblasts and muscle cells are strongly influenced by mechanical factors, thus, these cell types are named mechanocytes [118]. Function and size of an organ seem to depend on metabolic activity and mechanical loading. Mechanical tension therefore induces hypertrophy and DNA synthesis e.g. in bone and reorganization of collagen or extracellular matrix proteins [151]. Therefore, via cellular biology it seems to be possible to *"regulate the regulators"* by influencing gene expression to induce structural changes. There are some factors known to be responsible for muscle growth as myogenin and MyoD [65]. *"Stretch has been shown to be a powerful stimulant of muscle protein synthesis and muscle growth"* (p. 325).
Mature muscle adapted to load factors with functional length by removing or adding new sarcomeres in series [295]. If new tissue is developed, muscle protein synthesis seems to be of

high relevance. Force activation and stretch are major factors in activating protein synthesis and a combination of these stimuli seems to have an additive effect.
To increase muscle size, an increase in RNA has been shown due to stretching. Since rRNA was mainly enhanced, it could be hypothesized that muscle hypertrophy is regulated by translation in ribosomes. From this, muscle size and phenotype could be regulated by the level of gene translation.
Skeletal muscles show different types of fiber compositions. Type 1 or ST fibers have long cycle time with slow contraction. FT fibers show more rapid myosin cross bridge cycling rates and are mainly recruited in powerful movements or maximal strength production. It wass hypothesized that all muscle fibers stayed as fast phenotype, until they were activated via stretch or neuronal innervation. Immobilization seemed to express fast myosin again. Studies also showed that muscle phenotype is strongly determined by gene trainscription [207] which is influenced by expressed growth factors due to physical activity.

14.1.37 Use of Intermittend Stretch in the Prevention of Serial Sarcomere Loss in Immobilized Muscle

P.E. Williams, (1990) [341]

Immobilization of the muscle in shortened position seems to result in a loss in fiber length and number of serial sarcomeres [295]. Animal studies showed a large reduction of the ROM when a muscle is immobilized in a shortened position, however, it seemed that short stretching periods of 15 min seem to be sufficient to prevent the reduction of serial sarcomeres. Reduction of serial sarcomeres could be attributed to a change in functional length of the muscle, because in shortened position, there is no optimal length in sarcomeres leading to an appropriate overlap of actin and myosin. A reduction of serial sarcomeres would lead to enabling the muscle to develop higher tension from a shorter position. Accordingly, when a muscle was immobilized in a stretched position, there was a serial accumulation of sarcomeres and therefore, the length/tension curve can be assumed to be shifted to the right. There were six groups with six mice each included in the two-week study. The soleus was immobilized in one group for two weeks, while in the other groups, immobilization was removed for 15 min, 30 min, one hour or 2 hours daily. In the immobilization group, there was a loss in sarcomeres and ROM of about 19%. Stretching for 30 min per day or more prevented reduction of serial sarcomeres and loss of ROM. Two hours of daily stretched led to an increase in serial number of sarcomeres of 10%.

14.1.38 Intermittent Stretch Training of Rabbit Plantarflexor Muscles Increases Soleus Mass and Serial Sarcomere Number

D. de Jaeger, V. Joumaa, W. Herzog , (2015) [86]

Stretching is commonly used to increase ROM in humans. However, in animal studies stretching of 40 min 3 days per week for 4 weeks led to increased length, serial sarcomere number and enhanced cross sectional area in the soleus of rats and reduced the passive dorsiflexion torque angle curve. Plantar flexors of seven rabbits were stretched with intermittent stretching protocol three times per week for four weeks. Passive torque and stiffness were reduced

after training in five of seven animals. Compared to the control muscles, muscle mass and serial sarcomere number of the gastrocnemius and plantaris was significant increased in the stretched muscle. Differences in muscles could possibly be attributed to different fiber types.

14.1.39 The Relationship between Duration of Stimulus per Day and the Extent of Hypertrophy of Slow-Tonic Skeletal Muscle in the Fowl, Gallus Gallus

G.P. Bates, (1993) [36]

Muscle hypertrophy was induced by using long-term stretching interventions in animals. stretch was applied on the wing of a muscle for 5, 10, 20 and 30 days. The results showed an increase of 96%, 116% and 105% after 10, 20 and 30 days of stretch. Furthermore, stretching was induced to another group for 5 weeks with 0.5,1,2,4 and 8 hours per day showing significant increases. 30 min stretching per day resulted in 57% increase in muscle mass, one hour in 59.7%, two hours in 67% and four hours in 72%. However, there was a high increase in muscle weight of about 150% due to eight hours stretching training on muscle mass. Authors suggested that because of lack in EMG signaling while stretching, increases in muscle mass could be attributed to mechanical tension induced by stretching. A possible threshold for increased muscle mass due to stretching was discussed to be less than 30 minutes per day in animal experiment.

14.2 Short Study-Summeries of Human Research

14.2.1 Cross Education Training Effects are Evident with Twice Daily, Self-Administered Band Stretch Training

S.L. Caldwell, R.L.S. Bilodeau, M.J. Cox, D.G. Behm, (2019) [66]

Flexibility training is well known to be associated with enhancements in flexibility [39, 40, 15] in rehabilitation process [15]. Furthermore, static stretching is often included to training programs in rehabilitation and injury prevention [87]. Mahieu et al. [197, 196] demonstrated increases in ROM of 11.5-20.8% in plantarflexors due to a daily stretching program for 6 weeks. In this study, the authors investigated the effect of daily versus twice per day stretching for quadriceps and hamstrings on knee extension and knee flexion MVC and flexibility as well as the cross over effect. 30 participants were included to the study. Stretching was performed 3x30 sec with 15 sec recovery between sets. Main findings of the study were improvements in quadriceps MVC of 7.1%, d = 0.8 in the stretched and 6.6%, d = 0.45 in the non-stretched leg as well as flexibility improvements in the stretched leg with up to 12.8%, d = 2.82. Surprisingly, passive static ROM was not increased due to the intervention. Lack of improvements can possibly be attributed to test specificity (other testing than stretching). The authors attributed the increases in MVC to increases in ROM and therefore, the possibility to place more tensile stress on the muscle over a higher ROM. Furthermore, another explanation would be the contraction of the muscle versus the stretching bands. The increases in MVC in the contralateral leg could be possibly attributed to neuronal changes.

14.2.2 A 10-Week Stretching Program Increases Strength in the Contralateral Muscle

A.G. Nelson, J. Kokkonen, J.B. Winchester, W. Kalani, K. Peterson, M.S. Kenly, D.A. Arnall, (2012) [229]

Strength training can improve maximum strength in the trained and the non-trained contralateral limb, known as cross education effect, which could be possibly attributed to neuromuscular adaptations. Zhou [362] suggested that EMS would lead to cross training effects because there are afferences present. The authors were led to the hypothesis that long-term static stretching protocols could lead to cross training effects as well as to increases in muscle strength [160, 161, 357]. To investigate the effects, the authors performed a 10-week stretching training protocol for the plantar flexors for 4x30 sec for one leg. 25 participants were randomly assigned to an intervention group (n=13) and a control group (n=12). To induce stretching, participants had to stand with the right leg on a beam which was 30 cm above the floor, while the left foot stabilized the body. Participants were instructed to let the heel of the right foot hang unsupported over the edge of the beam and stretching was induced by pushing the heel down to get in maximal dorsiflexion. The authors showed increased flexibility of 8% (d = 0.58) in the right calf muscle, but in no changes in the left muscle and in the control group. Maximal strength increased with 29% (d = 1.24) in the stretched calf and 11% (d = 0.46) in the unstretched calf, while no increase could be stated in the control group. The authors discussed that stretching could increase maximum strength capacity without activating the motor neurons but could activate afferences, cross educational effects could be expected by long-term stretching programs. There are also studies showing no significant contralateral effect after stretching the plantar flexors 10 minutes per day, 5 days per week for 6 weeks [128]. The authors stated that increases in maximum strength may be attributed to stabilization of the body while stretching. Moreover, the authors referred to the possibility to use stretching training to increase maximum strength, if there is no possibility to perform strength training (e.g. when traveling).

14.2.3 Plantar-Flexor Static Stretch Training Effect on Eccentric and Concentric Peak Torque – A comparative Study of Trained versus Untrained Subjects

A.A. Abdel-Aziem, W.S. Mohammad, (2012) [1]

It is the goal of many exercise programs to increase strength and flexibility. Strength capacity is mostly improved by resistance training but may diminished by stretching. Most studies investigating the acute effects of stretching training with conflicting results showing decrease performance after stretching [28, 41, 228, 241] ranging from 4.5% to 28% independent on testing procedure (isometric, isotonic, isokinetic) [254], but there are only few studies investigating the long-term effects of stretching programs on maximal strength. Some studies investigated PNF [357] showing significant increases in flexibility (6.3%) and increases in knee extensor and flexor eccentric peak torque of 23.0% and 18.2% and isometric peak torque increases of 11.3% and 9.4% [132]. 57 participants were included to this study and divided into three groups of untrained participants, trained participants, and a control group. Participants were instructed to stretch the plantar flexors by facing a wall with their right foot and moving the

foot backward while staying with the left foot forward. Stretching was performed 5x30 sec twice per day on 5 days per week for six weeks. There were significant increases in concentric peak torque in the trained and untrained group of up to with 14.6%, d = 0.98 and in eccentric peak torque of 12.5% with d = 1.09. Dorsiflexion ROM increased in the untrained group with 71.1% and d = 2.48 and in the trained group with 10.0% and d = 0.42. The authors stated that there are also studies showing no increase in ROM due to stretching [56], also showing inconsistency in results regarding flexibility gains. The authors referred to structural changes presented in animal studies, but stated that many authors who performed stretching in humans showed no significant changes in stiffness and electromyographic activity. Therefore, the authors suggested that increases in ROM may be attributed to central adaptations rather than to peripheral adaptations and that higher ROM may be attributed to increased pain tolerance [194]. The authors attributed the increases in peak torque to increased storage of potential energy during eccentric loading, which can be used in the concentric phase of the movement. The increases in eccentric torque could be possibly attributed to increased compliance of the elastic components in series, which could lead to greater ability to store potential energy [26, 346]. The authors concluded that chronic static stretching leads to increased muscle strength in concentric and eccentric movements and suggested including static stretching to regular training routines instead of using it immediately before physical activity.

14.2.4 Chronic Effects of Static and Dynamic Stretching on Hamstrings Eccentric Strength and Functional Performance: A Randomized Controlled Trial

G.M. Barbosa, G.S. Trajano, G.A.F. Dantas, B.R. Silva, W.H.B. Vieira, (2020) [34]

The implementation of regular stretching programs led to significant increases in flexibility, but the chronic effects of static stretching on performance were not finally clarified. Shrier [274] reviewed the available literature suggesting that the regular usage of stretching programs could lead to an improvement of muscle performance, but authors did not differentiate the type of stretching because of a limited number of included studies. There are conflicting results of using static stretching over a period of few weeks on muscle performance. 45 participants were divided into three groups and performed then sessions of 3x30 sec stretch for the hamstrings with 30 second rest in between. The results showed a significant decrease in eccentric peak torque (-15.4 $\pm$ 10.4%, d = 1.03) and a distance of triple hop (-3.7 $\pm$ 4.1%, d = 0.29). No significant change in 20 m sprint-test was found. Static stretching affected the performance parameters, while dynamic stretching did not seem to influence strength and/or jumping and sprinting performance. Eccentric torque is of high importance for rehabilitation and performance in sports. The authors suggested including a full warm up routine to diminish negative effects of static stretching to promote greater muscle activation. Decreases performance in jumping performance could be possibly attributed to muscle tendon stiffness and therefore reduced capacity to use stretch-shortening cycle.

14.2.5 Chronic Static Stretching Improves Exercise Performance

J. Kokkonen, A.G. Nelson, C. Eldredge, J.B. Winchester, (2007) [160]

Flexibility is important for physical fitness to enhance performance level and to prevent injuries [278]. Even though it is often integrated in warm-up routines, stretching showed negative effects on maximum strength production [228] and performance parameters as jumping performance and running speed. The question arises about regarding adaptations on maximum strength and performance capacity if stretching is performed over a longer period of many weeks several times per week. Shrier [274] reviewed 9 studies and pointed out beneficial effects in seven of those studies. To investigate long-term stretching effects 40 participants were divided into an intervention group and a control group. The intervention group performed 15 exercises to stretch the lower extremities with 3x15 sec and 15 sec rest in between on three days per week and 10 weeks. The results showed significant increases in flexibility (17.7%, d = 1.15), standing long jump (2.2%, d = 0.11), vertical jump (3.9%, d = 0.14), 20m sprint (1.3%, d = 0.1), knee extension (28.5%, d = 0.72), knee flexion (14.1%, d = 0.44), maximum strength and endurance with 28.1%, d = 1.3 for knee extension and 29.7%, d = 1.24 for knee flexion. The authors therefore recommend including stretching routines to regular training activities. However, the authors also stated that static stretch could be related to muscle hypertrophy in animal studies [82, 287]. Another hypothesis was that increased maximum strength could be attributed to increased muscle length, as Lieber [181] showed that stretching and increased bone length could be responsible for muscle lengthening. In animal studies, Coutinho et al.[82] and Williams et al. [342] showed that stretching for 30 and 40 min respectively resulted in an increased number of sarcomeres after 3 weeks.

14.2.6 Combined Effects of Static Stretching and Electrical Stimulation on Joint Range of Motion and Muscle Strength

T. Mizuno, (2019) [212]

Several studies showed that static stretching can increase ROM and muscle strength [107, 228], can induce changes in muscle architecture or increase MTh [107, 277]. The author suggested that increased ROM could be attributed to reductions in passive resistive torque and an increased stretching tolerance [197, 224], which are influenced by stretching duration and intensity [107]. No previous studies reported an increase in muscle strength and muscle hypertrophy in human subjects. The author hypothesized that this combination induced more tension than stretching alone. 35 participants were divided into three groups, a stretching group, a stretching and electrical stimulation group as well as a control group. The calf muscles were stretched for 8 weeks, 4x30 sec with 30 sec rest in between. Stretching was induced with a stretching board by the highest intensity participants could withstand without pain. While stretching in the corresponding group electrical stimulation using 80Hz were induced additionally. Static stretching led to increases in ROM of 12.7 ± 24.6%, d = 1.0, static stretching and electrical stimulation led to increased ROM of 14.3 ± 6.6%, d = 0.97. Maximum strength increased in the stretching group with 20.2± 28.7%, d = 0.72, stretching and electrical stimulation led to an increase of 22.4± 27.6%, d = 0.46, while in the control group, there was an increase of 6.4± 18.3%, d = 0.13. There was no statistically significant difference between

both intervention groups. The results showed significant increases in muscle thickness in both intervention groups, however, no original datasets are available. The author found no correlation between increases in maximum strength and muscle thickness. He hypothesized that neuronal adaptations could be responsible for increases in maximum strength and ROM, while increases in muscle thickness were attributed to the induced stretching tension.

14.2.7 Effects of Flexibility Training on Eccentric Ecercise-Induced Muscle Damage

C.H. Chen, K. Nosaka, H.L. Chen, M.J. Lin, K.W. Tseng, T.C. Chen, (2011) [73]

Eccentric exercise induces muscle damage with typical symptoms of muscle weakness or DOMS [75, 74, 242]. Pre-exercise static stretching had negative impact on following power performance and strength [257] and was not sufficient to reduce DOMS through muscle damage due to eccentric contractions [250]. However, McHugh et al. [206] stated that compliant muscles are less vulnerable to symptoms of muscle damage after eccentric exercise, consequently it is hypothesized that stretching over a period of some weeks could help to reduce DOMS via eccentric exercise. 30 participants were divided into three groups, a static stretching group, a PNF group and a control group. Static stretching was performed by sitting on the floor and trying to touch the toes of the foot, the investigator pushed the subject forward until a mild discomfort was felt. Stretch was performed for 30 seconds and repeated 30 times with a 30 sec rest in between three times per week for eight weeks. PNF was also performed for the hamstring (further description in the original paper). The results showed significant increases in ROM in the stretching group of 24 $\pm$ 3° 25.0%, d = 6.57 and for PNF of 28 $\pm$4° (28.9%, d = 7.99). Furthermore, there were increases in concentric maximum strength in the knee flexors of 8.7%, d = 2.03 in the stretching group and 15.5%, d = 2.89 in the PNF group, while the knee extensor maximum strength increased in the stretching group with 3.0%, d = 0.47 and in the PNF group with 6.1%, d = 1.09. Furthermore, a shift the optimal angle of force production to a longer muscle length and reduced the muscle damage after eccentric exercise independent of used training protocol. Flexible muscles were less susceptible to eccentric exercise-induced damaging. The authors concluded that stretching had protective effects for muscle damage through eccentric exercise and could possibly be attributed to the other changes measured in the study: higher maximum strength and increased ROM. The authors suggested that the higher the ROM and longer the optimum muscle length, the less muscle damage may be developed.

14.2.8 Effects of 6-Week Static Stretching of Knee Extensors on Flexibility, Muscle Strength, Jump Performance, and Muscle Endurance

N. Ikeda, T. Ryushi, (2021) [145]

Maximum strength, power, endurance and flexibility are of high importance for athletes. Recent studies showed that long-term stretching programs induce sufficient stimulus to improve flexibility [128] and maximum strength [274], however, other authors showed no improvements [37, 54]. The authors referred to high heterogeneity in study design as explanation for differences in results, as there were high differences in training period with 3-10 weeks, training

frequency with 3-14 times per week [37, 160, 229] and duration of training with 1.5 – 40 minutes [37, 143, 160, 229, 357]. There are recommendations of the American sports medicine society to stretch the muscle 2-3 times per week for 60 seconds if the aim is to improve flexibility. Furthermore, the authors pointed out increased protein synthesis responses resulting in structural changes in collagen and muscle hypertrophy by stretching. 25 participants were divided into a stretching group and a control group. Participants performed a 6x30 sec stretching program on 3 days per week for six weeks. The results showed that the stretching routine led to increases in flexibility with 9.7%, d = 0.29 and maximum strength in leg extension with 10.16%, d = 0.43. The authors discussed that hypertrophy is one of the major factors influencing maximum strength and requires long stretching times.

14.2.9 The Effects of 12 Weeks of Static Stretch Training on the Functional, Mechanical, and Architectural Characteristics of the Triceps Surae Muscle-Tendon Complex

S. Longo, E. Cè, A.V. Bisconti, S. Rampichini, C. Doria, M. Borrelli, E. Limonta, G. Coratella, F. Esposito, (2021) [185]

Stretching has a wide application in sport and rehabilitation to enhance flexibility and muscle performance. Stretching also showed negative acute impact on maximum strength and rate of force development [241], which seemed to be accompanied by a decrease in the amplitude of surface EMG [40, 83]. From this, it was hypothesized that acute negative effects can be possibly attributed to neuromuscular (reduced activation) and mechanical (changed elastic properties) mechanisms [195, 217]. Long-term effects were discussed controversially. There is evidence for increases in maximum strength [229], while others did not find any changes [4]. The authors referred to studies from animal experiments showing large increases in fiber- and muscle size [139, 282] via increased protein synthesis due to stretching stimulus [120]. Thirty (30) participants were included to this study and were randomly divided into an intervention and a control group. The participants of the intervention group performed a stretching program for 12 weeks with 5 sessions per day with a stretching 5x45 sec with a 15 sec rest in between. Two exercises were performed to stretch the plantar flexors. The stretching routine was not able to induce maximum strength increases, alterations in muscle architecture or increases in muscle thickness. However, an increase in ROM of 21.5%, d = 1.59 and a decrease in muscle stiffness was found. From this, the authors hypothesized that enhanced flexibility may be attributed to reduced stiffness, however, because of a lack of correlation between changes in stiffness and ROM, it may not be the main factor. The authors had no explanation for the discrepancy between their results and results from previous studies but discussed the possibility of lower muscle overload in the presented study or differences in measuring devices.

14.2.10 Comparison Between High- and Low-Intensity Static Stretching Training Program on Active and Passive Properties of Plantar Flexors

M. Nakamura, R. Yoshida, S. Sato, K. Yahata, Y. Murakami, K. Kasahara, T. Fukaya, K. Takeuchi, J.P. Nunes, A. Konrad, (2021) [225]

Stretching is used to improve many parameters of movement functions as ROM and stretch tolerance in many settings of fitness and health. Stretching intensity seems to have a high impact for muscle adaptations [21]. However, there are some studies showing no difference regarding adaptations in ROM by using different stretching intensities [110, 296, 297]. The authors hypothesized that high intensity stretching training would induce more intensive stimulus on the muscle and would cause higher magnitude of adaptations in muscle strength and architecture. 40 participants were randomly divided into three groups, a high intensity stretching group, a low intensity stretching group and a control group. Participants were instructed to stretch their calf muscle by using a stretching board for four weeks with 3x60 seconds and a 30 second rest in between on three days per week. Intensity was determined via a verbal numerical scale. The results showed higher decreases in muscle stiffness of the high intensity group compared to the low intensity group, whereas both showed significant attenuations. The authors were not able to point out increases in maximum strength or muscle thickness due to stretching, regardless of the intervention group.

14.2.11 Chronic Effects of a Static Stretching Program on Hamstring Strength

S. Nakao, T. Ikezoe, M. Nakamura, H. Umegaki, K. Fujita, J. Umehara, T. Kobayashi, S. Ibuki, N. Ichihashi, (2019) [226]

Stretching is commonly used to improve flexibility and decrease muscle tendon unit stiffness and muscle hardness [199]. There are conflicting results regarding chronic stretching intervention and their long-term effects on maximum strength. The authors pointed out studies showing increases and studies without an influence on maximum strength. Thirty (30) subjects were included to the study and divided into an intervention group and a control group. Stretching for the hamstrings was induced for 5 minutes, three times per week for four weeks. A reduction of muscle tendon unit stiffness in the hamstrings, but no significant changes in isometric and isokinetic peak torque was observed. The authors hypothesized an increase in fiber length by increase sarcomere number, because the optimal angle for maximal peak torque shifted to longer muscle length.

14.2.12 Chronic Stretching and Voluntary Muscle Force

D.O. LaRoche, M.V. Luissier, S. J. Roy, (2008) [171]

Stretching is used to improve flexibility, to reduce risk of injury and to improve performance, but literature shows conflicting results. The authors referred to Shrier [274] showing that long-term effects showing significant increases, while acute effects were of detrimental character regarding maximum strength/muscle performance [28, 101, 128] from this, the authors recommended avoiding stretching prior to exercise.

Twenty-nine (29) participants were included and randomly divided into three groups: a static stretching group, a ballistic stretching group and a control group. Stretching was performed 10x30 sec stretching, three times per week for four weeks, showing small effects (7.5%, d = 0.34) on maximum strength in the hamstrings, however, there were also increases in the control group (3.8%, d = 0.17). Those effects could possibly be explained by habituation.

14.2.13 A Randomized Controlled Trial for the Effect of Passive Stretching on Measures of Hamstring Extensibility, Passive Stiffness, Strength, and Stretch Tolerance

P.W.M. Marshall, A. Cashman, B.S. Cheema, (2011) [199]

Hamstring extensibility is often measured in pre-season in soccer and therefore, there is high interest to improve flexibility, but there is also a high relevance in therapies of low back pain [200]. Twenty-two (22) participants were divided into two groups. The intervention included four stretching exercises, which were performed 3x30 seconds on five days per week for four weeks. The authors did not find increases in hamstring strength due to stretching program, while extensibility increased due to intervention.

14.2.14 Muscle Architectural and Functional Adaptations Following 12-Weeks of Stretching in Adolscent Female Athletes

I. Panidi, G.C. Bogdanis, G- Terzis, A. Donti, A. Konrad, V. Gaspari, O. Donti, (2021) [236]

Muscle tissue responds to mechanical loading due to molecular and structural changes and therefore can modify physiological and contractile properties ([96, 102]. There seem to be a few options of mechanical loading by concentric and eccentric muscle contraction, but also via stretching, which can provide sufficient stimulus to induce architectural muscle changes [104] and increase fascicle length [277]. Adaptations of the muscle can be seen as a response to changed functional demands of the muscle in daily life [338]. Cross sectional studies indicated that due to growing bones, muscle cross-sectional area and fascicle length also increase [49]. Both, mechanical load, and growth seemed to influence muscle architecture. The authors suggested that improvements in jumping height and contractile function [160] may be attributed to addition of sarcomeres in series, which could possibly enables higher contraction velocity over a higher ROM [182]. *"Interestingly, from infancy to adulthood, ankle joint ROM decreases about 1.5% per year [49], and during adolescence levels of flexibility tend to plateau or decrease at the time of the adolescent spurt [...]"*. The authors referred to the absence of studies investigating high volume stretching training on muscle properties. Twenty-six (26) volleyball players were recruited. Stretching was performed with one leg, the other leg served as control leg. A 12-week static stretching training for the plantar flexors was performed with stretching duration of up to 900 seconds (15 minutes) per session on 5 days per week for 12 weeks. The stretching duration was progressively increased all three weeks. Six different stretching exercises were included to the stretching protocol. The results showed significant increases in ROM and fascicle length. Furthermore, an increase in muscle cross-sectional area of $23 \pm 14\%$ vs $13 \pm 14\%$ in the intervened and the control leg as well as increases in unilateral

jumping height of 27 ± 30% in the intervened and 17 ± 23% in the control leg were presented. Changes in muscle cross-sectional area could be possibly attributed to stretching, maturation of the youth volleyball players as well as the accompanying volleyball training.

14.2.15 The Effects of Static Stretching Programs on Muscle Strength and Muscle Architecture of the Medial Gastrocnemius

S. Sato, K. Hiraizumi, R. Kiyono, T. Fukaya, S. Nishishita, J.P. Nunes, M. Nakamura, (2020) [262]

Stretching is used to improve flexibility or decrease muscle stiffness [32, 224, 223]. Acute stretching showed acute decrease of maximum strength, which is called stretching induced force deficit. However, performing stretching over a period of weeks, there might be different adaptations. Animal studies showed that stretching may provide sufficient stimulus to induce hypertrophy by triggering myogenic growth factors, stretch activated channels, AKT/mTOR pathway and protein synthesis [215, 248, 301].
As previous studies showed that muscle size has a strong influence on maximum strength [160, 212, 229], it could be hypothesized that stretching could also lead to hypertrophy and maximum strength gains in humans, but literature showed inconsistency in results. 24 participants were divided into a stretching group stretching the plantar flexors for 3 times per week 1x120 seconds, and a second stretching group with a one-time per week stretching training for 360 seconds using a stretching board. No significant effects could be induced in maximum strength or muscle thickness. The authors referred to many aspects influencing the results as intensity, volume and nutrition.

14.2.16 Effect of a 5-Week Static Stretching Program on Hardness of the Gastrocnemius Muscle

R. Akagi, H. Takahashi, (2013) [4]

Static stretching is a useful activity to reduce muscle hardness. The authors suggested that, when stretching is performed for a comparatively long duration (5-6 minutes) ROM increases due to decreased muscle tendon unit stiffness. Authors' issue was to clarify long term effects of static stretching over a period of some weeks instead of acute effects. For this, 19 participants were included to the study. The non-stretched leg served as contralateral control leg. Participants performed 3x2 min of static stretching on six days per week for 5 weeks. The results showed significant effects on passive ROM in dorsiflexion and MTU stiffness, but no effects for muscle thickness and joint torque.

14.2.17 Stretch Training Induced Unequal Adaptation in Muscle Fascicles and Thickness in Medial and Lateral Gastrocnemii

C.L. Simpson, B.D.H. Kim, M.R. Bourcet, G.R. Jones, J.M.Jakobi, (2017) [277]

The authors hypothesized that stretching could induce similar stress on the muscle as it can be observed in strength training. The authors referred to animal studies showing that chronic stretch overload was sufficient to induce increases in muscle fascicles, muscle size and weight, if stretching is performed with high intensity and duration. Using commonly used stretch training in humans might not be able to induce comparable effects assuming that stretching intensity and volume could be low. 21 participants were divided into a stretching and a control group performing a training routine which was performed on 5 consecutive days per week with two days of rest for six weeks. Stretching was performed for 3 minutes using a leg press machine pushing the foot in dorsiflexed position with progressively increased weight. The results showed an increase in Muscle thickness of 5.6%, however, no increase in maximum strength could be obtained. The authors discussed results based on results from animal studies, suggesting that muscle thickness may increase by stimulation of the protein synthesis based on muscle damage via tension induced microtraumatization.

14.2.18 Effects of a High-Volume Static Stretching Programme on Plantar-Flexor Muscle Strength and Architecture

K. Yahata, A. Konrad, S. Sato, R. Kiyono, R. Yoshida, T. Fukaya, J.P. Nunes, M. Nakamura, (2021) [359]

Stretching is mostly used to increase ROM, decrease muscle stiffness [224, 223] or induce enhancements in muscle thickness [277]. Dynamic stretching, PNF and static stretching can be performed to improve ROM, however, only static stretching showed reductions in muscle stiffness [164, 165]. Stretching led to acute reduction in maximum strength and speed strength performance, which is called the stretching induced force deficit [276], however, there were several studies showing increases in maximum strength and jumping after performing static stretching for a period of weeks [73, 229]. Furthermore, animal studies showed significant hypertrophy after chronic static stretching, however, in humans, the highest weekly volume performed in humans was 36 min stretching per week [4] which was hypothesized not to be a sufficient volume to induce comparable changes in the muscle. Sixteen (16) participants were included to the study, the dominant leg served as intervention leg, the non-stretched leg as the control leg. Stretching was performed for 6x5 minutes with 60 seconds rest in between on two days per week for five weeks. The results showed an increase in maximum strength in some strength measurements of up to 6.9% with d = 0.35, muscle architecture and muscle thickness did not show any changes due to the stretching routine. The authors referred to Fowles et al. [100] showing no significant increase of muscle protein synthesis after one bout of 27-minute static stretching and concluded that low intensity stretching seems not to provide sufficient stimulus to induce hypertrophy and that high volume may not substitute low intensities, referring to Nunes [232].

14.2.19 Sprint and Vertical Jump Performances Are Not Affected by Six Weeks of Static Hamstring Stretching

D.M. Bazett-Jones, M.H. Gibson, J.M. McBride, (2008) [37]

Long-term stretching interventions are often used to decrease injury [230, 255] and to enhance performance [290]. There are conflicting results of stretching on maximum strength and performance parameters. The study focussed the effects of a long-term stretching training on performance parameter as sprinting and jumping. Twenty-one (21) participants were divided into a stretching group and a control group. The stretching group performed 4x45 sec stretching with a 45-60 second rest in between on four consecutive days per week for 6 weeks. Stretching was performed without any stretching device. The authors were not able to find any change in 55m sprint and vertical jump performance due to stretching, however, there was an increase in ROM.

14.2.20 Effect of Hamstring Stretching on Hamstring Muscle Performance

T.W. Worrel, T.L. Smith, J. Winegardner, (1994) [357]

Performance enhancement is the main goal of coaches, athletes and therapists. There are only few studies investigating the relationship between flexibility and force production. The authors hypothesized that increasing the ROM, more force could be absorbed in the eccentric and therefore more force could be generated in the concentric. 19 participants were included to the study. Different stretching methods were performed on the legs, no control group was included. The authors stated an increase in flexibility of 8° by static stretching and 9.5° by PNF. Peak torque increase with about 13.5%. The authors ruled out learning effects because they stated familiarity of the measurement device and ICC and SEM showing no learning process.

14.2.21 Effect of Static Stretch Training on Neural and Mechanical Properties of the Human Plantar-Flexor Muscles

N. Guissard, J. Duchateau, (2004) [128]

Stretching is commonly used to improve flexibility and is therefore used in rehabilitation and sport related activities. However, only few studies investigated the effects of long-term stretching programs. Twelve (12) participants were included to stretch plantar flexors of the right leg, while the contralateral leg served as control leg. Stretching was performed for a total of 10 minutes per session, five times per week for six weeks. Therefore, five stretching exercises were performed for 5x30 sec with a 30s rest in between. There was an increase in ROM of 30.8%, with the highest increase after the first ten days. MVC increased after 30 sessions with 5.4%, $d = 0.71$, after 30 days rest after the intervention period, there was no decrease in MVC. Furthermore, stiffness decreased in the stretched muscle. The authors referred to increases in compliance of the muscle tendon unit as long-term adaptations through stretching. However, a control group is missing in the study design.