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2 **ABSTRACT**

3 The purpose of the study was to investigate the acute effects of two stretching
4 interventions, proprioceptive neuromuscular facilitation (PNF) and passive static
5 stretching (PSS), and a specific warm-up (SW) on the strength and architecture of the
6 vastus lateralis and biceps femoris muscles in a subsequent performance on strength
7 training session (STS). Musculoskeletal ultrasound images were acquired from nine
8 men before and immediately after stretchings or SW, and ten minutes after STS. The
9 STS consisted of the following exercises: Leg Extension, Leg Curl, Leg Press and Hack
10 Machine Squat. The PNF resulted in lower performance for all situations. The PSS and
11 SW improved performance for the Leg Press relative to the PNF and controls (CS). For
12 the Hack Machine Squat, the SW resulted in higher performance than the stretching
13 conditions. The VL muscle fascicle length (FL) increase after STS for PNF method. The
14 BF muscle showed a higher pennation angle (PA) 10 minutes after the STS for PSS
15 method, the FL increases immediately after PSS and then decrease 10 minutes after the
16 STS for PSS method.. As our results, the SW should be performed before STS, and PNF
17 stretching should not be prescribed because this condition impairs subsequent
18 performance. These results may assist health professionals in prescribing resistance
19 training.

20 **Key words:** warm-up; passive static stretching; proprioceptive neuromuscular
21 facilitation; strength training; lower limbs; ultrasound.

22

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1

2 ***INTRODUCTION***

3 A variety of warm-up methods have been used prior to physical exercise to
4 reduce the risk of injuries and enhance performance on the subsequent main activity (5,
5 26, 27). In this respect, some authors suggest that these warm-ups can involve general
6 activities, specific warm-ups and different stretching methods (1). General warm-ups
7 consist of low-intensity aerobic activities, such as running or cycling, with the aim of
8 increasing the muscle temperature and neuromuscular function (1, 2).

9 Specific warm-ups (SW) include exercises that are similar to the main activity,
10 with a progressively increasing intensity, to enhance the neuromuscular activation
11 system. A specific warm up added to a general warm up for a 1 repetition maximum
12 (RM) test in the leg press showed an improvement of 8.4% (1). Recently, it was
13 demonstrated that a specific warm-up resulted in higher number of maximal repetitions
14 for the subsequent lower limb strength training session (STS) compared to ballistic and
15 passive static stretching methods (25).

16 Some stretching methods can also be considered as a warm-up (1). The duration
17 of the stretching stimulus performed before the main activity is directly related to the
18 performance (3). The effects of stretching methods as warm-ups previously a strength
19 performance have been studied in relation to the number of repetitions (3, 12, 13), 1RM
20 tests (2, 4), vertical jump (6) and isometric strength (21). Passive static stretching (PSS)
21 (4, 6, 21) proprioceptive neuromuscular facilitation PNF (3, 6, 15) and SW (1, 12) have
22 been manipulated according to the ACSM's recommendations (14). However,
23 controversial results can still be found as the intervals amplitudes for duration, intensity
24 and number of sets allows different protocols.

1 Overall, there is a reduction in the strength levels after stretching protocols (11,
2 15, 27, 28). This decrease has been explained by two main mechanisms. The first is
3 related to the reduction in neural activation in response to the stimulus given to the
4 Golgi tendon organ (GOT) (11). The second mechanism refers to changes in the
5 mechanical properties of the muscle-tendon unit (MTU) (16). In this case, the tendon
6 tension during stretching would alter its viscoelasticity, causing a stress relaxation
7 behavior that reduces tendon stiffness and affects the force transmitted by the muscles
8 (15).

9 Additionally, the arrangement of the muscle fibers is directly related to muscle
10 force generation (17, 19). Changes in the muscle architecture after acute stretching have
11 been reported as a decrease in the pennation angle (PA) and an increase in the fascicle
12 length (FL) (23, 24). The way that such transient architecture rearrangement could
13 influence the shortly after force production is still unclear. In fact, suggest on meta-
14 analysis (27) the inverse relationship between muscletendon complex compliancy and
15 the efficient force transmission (29).

16 Therefore, the purpose of this study was to investigate the acute effects of two
17 stretching methods, PNF and PSS, and of a specific warm-up (SW) on STS (number of
18 repetitions of four exercises). Muscle architecture of the thigh muscles will be
19 monitored before and after the stretching and specific warm up. Based on the literature,
20 the initial hypothesis is that the SW will result in performance improvements compared
21 to the stretching methods.

22 **METHODS**

23 ***Experimental Approach to the Problem***

24 This study was designed to investigate the effects of a SW and two different
25 stretching methods (PNF and PSS) on the strength performance. Muscle architecture

1 using ultrasound (US) images of the VL and BF muscles were monitored using three
2 moments of the protocol. Nine subjects visited the laboratory seven times with 48 hours
3 interval between the visits. The familiarization with the stretching methods and
4 exercises were performed on the first visit. The subjects performed the strength test and
5 were re-tested on the second and third days, respectively. The fourth to the seventh days,
6 the subjects were randomly selected to perform one of the four possible situations (PNF,
7 PSS, SW and CS). For these four visits, the subjects laid down on a stretcher for 10
8 minutes of rest before the US images were collected. Just after the US acquisition, the
9 subjects underwent the intervention with one of the proposed methods (SW, PNF or
10 PSS), and immediately afterwards, the US images were collected again. After this
11 procedure, the following four exercises were executed in this order: Leg Extension, Leg
12 Curl, Leg Press and Hack Machine Squat.

14 *Subjects*

15 Nine male volunteers (age: 24.81 ± 2.98 years, weight: 81.33 ± 11.46 kg, height:
16 1.77 ± 0.07 cm and BMI 25.81 ± 3.20 kg/m²) participated in four different, randomly
17 chosen experimental conditions. All subjects were involved in other recreational
18 physical activities such as cycling, running and swimming, but none of them was
19 practicing strength training for the last six months. Thus, these subjects were considered
20 untrained in strength training. Before data collection, the volunteers signed a free and
21 informed consent form prepared according to Resolution 196/96 of the National Health
22 Council. The subjects answered the PAR-Q questionnaire and a general questionnaire
23 designed to exclude individuals with possible cardiopulmonary diseases,
24 musculoskeletal and user ergogenic substances. The study was conducted in accordance
25 with the Declaration of Helsinki and approved by the Ethics Committee of the

1 University Hospital, Federal University of Rio de Janeiro. Subjects were informed about
2 the tests procedures with the possible risks and benefits of the study. They signed an
3 informed consent form before the tests begin. No subjects withdrew the study.

4

5 ***Procedures***

6 In the first visit the subjects were familiarized to the two stretching methods and
7 the exercises, with a sumaximal load and eight repetitions were performed. During the
8 second and third visits, subjects were initially placed in a supine position with lower
9 limbs relaxed for 10 minutes. The US images were obtained of the vastus lateralis (VL)
10 and the biceps femoris (BF) of the dominant leg muscles immediately before and after
11 the 8RM test and re-test for each exercise (Leg Extension, Leg Curl, Leg Press and
12 Hack Machine Squat), performed according to the literature (15). A 10-minute interval
13 was used among the four exercises 8RM tests that were applied with the same order.

14 In the fourth to seventh visits, the subjects initially remained in the supine
15 position on a paddle with relaxed lower limbs for 10 minutes. Afterwards, US images of
16 the VL and BF (M_{01} – time point 1) were acquired for the dominant leg. Then, one of
17 the three experimental protocols was randomly performed (PNF, PSS, and SW). A fourth
18 control condition was also included (CS) where the subjects rest at the same initial
19 supine position for the similar periods of time stretching PSS and PNF (three minutes).

20 Immediately after the experimental protocols, the subjects returned to the
21 stretcher for the STS procedure (M_{02} - time point 2). Afterwards, the participant
22 performed the STS with the following four exercises: Leg Extension, Leg Curl, Leg
23 Press and Hack Machine Squat (Rotech Equipment®, Goiania, Brazil). The four
24 exercises were performed with limited of 90° knee joint range of motion. The subjects
25 were encouraged to perform as many repetitions as possible until there was concentric

1 failure in the three sets of each exercise. The rest time between sets and between
2 exercises was 90 seconds for all protocols (8). After the training session, the US
3 procedure was repeated after the subjects rested for an additional 10 minutes with
4 relaxed legs (M_{03} – time point 3).

5 The subjects were individually tested at the same time in the morning and they
6 were instructed to maintain the nutrition habits during the experimental period.

7

8 ***Protocols for stretching and specific warm-up***

9 The PSS method involved three sets for flexors and knee extensors postures with
10 30 seconds intervals (Figure 1). The maximal joint amplitude was assisted held,
11 according to the subject limit of discomfort, for 30 seconds (14). For the PNF stretching
12 method, three series of the same knee flexors and extensors postures were performed
13 (Figure 2). Immediately after reaching the maximal amplitude, the subjects were asked
14 to isometrically contract the agonists for six seconds and then relax when further
15 amplitude was assisted reached and sustained for more 24 seconds. Both methods of
16 stretching exercises were performed with the same overall execution time.

17 For the SW, 20 repetitions of each exercise were performed, during 30 seconds
18 at a 30% 8RM load, with an interval of 30 seconds.

19

20

Insert Figure 1 and 2#

21

22 ***Ultrasound procedures***

23 The US images were acquired at four times during the fourth to seventh visits,
24 with an EUB-405 (Hitachi, Tokyo, Japan) with an 8-cm linear probe with central

1 frequency of 7.5MHz with the aid of a gel for acoustic coupling (Ultrex; Farmativa
2 Indústria e Comércio Ltda, Rio de Janeiro, RJ, Brazil).

3 The volunteers rested in the supine position on a stretcher. Longitudinal US
4 images of the vastus lateralis (VL) and biceps femoris (BF) were acquired at 50% of the
5 thigh length (9) of the dominant leg by an experienced examiner. Two images were
6 consecutively acquired. Image processing and architectural muscle parameters
7 measurements were blindly performed using a homemade routine (LabVIEW, National
8 Instruments Corporation, Austin, Texas, USA). One of the two images was considered
9 for the analysis and was selected for having the best visual definition of the fascicles.
10 Images from the second and third visits were used for reliability procedure. The PA was
11 determined as the acute angle between the deep aponeurosis and the selected muscle
12 fascicle. The fiber length (FL) was determined as the length between the superficial and
13 deep aponeurosis (Figure 2). These US architecture parameters (PA and FL) measures
14 were already validated from cadaveric measurements (10).

15
16 # **Insert Figure 3** #

18 ***STATISTICAL ANALYSES***

19 To estimate the architecture measurement reliability, the intraclass correlation
20 coefficient (ICCr), coefficient of variation (CV %) and confidence interval (CI) were
21 calculated between the FL and PA measurements of the first two-day images. The
22 reliability for the FL and PA was considered high for BF (ICCr = 0.922, CV = 4.11%,
23 CI = [0.607 to 0.997]; ICCr = 0.932, CV = 2.34% and CI = [0.676 to 0.985], for FL and
24 PA respectively) and for VL (ICCr = 0.920, CV = 8.13%, CI = [0.654 to 0.982]; ICCr =
25 0.964, CV = 2.56% and CI = [0.843 to 0.997], FL and PA respectively).

1 After confirming the normal distribution using the Shapiro-Wilk test and
2 calculating the 95% confidence level, an ANOVA with repeated measures on the
3 second factor (4x3) was applied to compare the four experimental pre-STS conditions at
4 the three US measurements moments for the muscle architecture parameters.

5 Similarly, an ANOVA was applied with two factors (4x4) to compare the
6 training volumes in each exercise. A post-hoc Fischer LSD was applied with a
7 significance level of $p < 0.05$. We performed the analysis using Statistica software
8 (Statsoft, Inc., Tulsa, OK, USA).

9

10 **RESULTS**

11 The main results of the present study are related to the influence of the type of
12 warm-up on the number of repetitions for each exercise ($F(9,144) = 3.719$, Figure 3).
13 For all exercises, the PNF stretching method reduced the number of repetitions
14 compared to the SW and PSS. The significance levels for the Leg Extension exercise
15 were $p = 0.017$ and $p = 0.031$ for SW and PSS, respectively. For the Leg Curl exercise,
16 the PNF resulted in a lower number of repetitions than the controls ($p = 0.020$, $p =$
17 0.001 , and $p = 0.006$ for PSS, SW and CS, respectively). For the Leg Press exercise, the
18 significance levels from PNF to SW and to PSS were $p = 0.031$ and $p = 0.001$,
19 respectively. In this exercise, PSS and SW resulted in a higher number of repetitions
20 than the controls ($p = 0.005$ and $p = 0.001$, respectively). For the Hack Machine Squat,
21 PSS resulted in a higher number of repetitions than the other three ($p = 0.002$ for PNF, p
22 $= 0.008$ for controls and $p < 0.001$ for SW). SW resulted in a higher number of
23 repetitions than PNF ($p < 0.001$) and controls ($p < 0.001$).

24

1 **# Insert Figure 4#**

2

3 The BF muscle showed a higher PA 10 minutes after the STS for PSS method
4 (Table 1) (M_{01} vs. M_{03} , $p = 0.008$), FL increases immediately after PSS ($p = 0.013$) than
5 decrease 10 minutes after the STS for PSS method ($p = 0.005$) (Table 2). The VL
6 muscle FL increase after STS for PNF method ($p = 0.039$) (Table 2).

7

8

Insert Table1#

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Insert Table2#

10

11 ***DISCUSSION***

12 The aim of this study was to verify the impact of PNF and PSS stretching
13 methods and the SW on a subsequent strength session for lower limbs. The main
14 findings were that the PNF stretching method caused an overall reduction in the
15 performance compared to the PSS method, SW and CS. Additionally, the PSS method
16 and SW showed an improvement in the number of repetitions for multi-joint exercises.
17 SW showed no significant effects on the muscle architecture, which means that muscle
18 fiber arrangements could not be responsible for these differences. For PSS, the FL and
19 PA of BF presented some statistical change and FL for PNF.

20 We found a significant reduction in the number of repetitions of Leg Extension
21 exercises after PNF stretching compared to control and static stretching (Figure 4). This
22 was also described by others (15) who reported a decrease of approximately two to three
23 maximal repetitions for the leg extension exercises (80% 1RM) compared to the no
24 stretching condition. A decrease in the neural activation by OTG agonist inhibition is

1 the physiological mechanisms commonly attributed to this fact (11). The OTG is an
2 autogenic inhibition reflex discharged when high tension is detected in the tendon
3 structure. With an intense stretching the traction tension can discharge this reflex
4 causing the muscle inhibition, potentiating protecting it from injuries (11). Upon PNF
5 stretching, a large tension is generated enabling the Golgi reflex discharge leading to a
6 further muscle elongation in the next stretching phase. However, it was shown that
7 although the inhibition effects are temporary, the peak passive plantarflexion torque was
8 still reduced for about one hour (11). These authors suggested that the peak torque
9 reduction lasting could be attributed to biomechanical effects. A stiffness decrease after
10 an intense stretching is reported by others for the gastrocnemius and quadriceps muscles
11 (7, 18, 20), whereas, less force is transmitted to the skeleton by a more compliant
12 structure.

13 Conversely, improvements for all exercises were recorded after PSS and SW. In
14 relation to SW, this finding is in agreement with our previous study (25) where it was
15 found an improvement in all the same exercises after a SW compared to ballistic and
16 static passive stretching. As far as we know, no other study verified the effect of SW in
17 strength training session. Although we did not measure body temperature, some authors
18 recommend 5-10 minutes of warm-up before using a strength test protocol (22). In our
19 case, SW involved 20 reps for 30 seconds at a 30% 8 RM load in each of the four
20 exercises, which approximates this recommendation. A possible explanation for the
21 improvement in performance would be an increasing muscle temperature, as suggested
22 by Sá et al. (25) after verifying the positive effects of the general warm up and the
23 specific warm up on the vertical jump performance. They suggested that muscle
24 temperature favorably affect the muscle performance by reducing viscous resistance
25 which, in turn, rises the oxidative reaction speed limits and / or increases the muscles

1 oxygen supply through a greater vasodilatation. This mechanism can explain our results
2 as the exercises use the same energy path.

3 The improvements in performance observed after passive static stretching, in all
4 exercise were less evident than for specific warm up. Commonly, there is a force
5 reduction after a stretching maneuver. For example, it is reported a decrease in the Leg
6 press performance after a passive stretching (2), contrary to ours results. This difference
7 can be related to the stretching volume where six passive stretching postures with three
8 sets each were conducted for the quadriceps and hamstrings with a total volume of 20
9 minutes. This high volume of stretching cause the same effects observed in PNF
10 stretching. For the present study, only one exercise for quadriceps and hamstrings were
11 used, with a total volume of three minutes, probably with similar benefits as the specific
12 warm up mentioned above.

13 With respect to the muscle architecture, the PSS method resulted in significant
14 changes of FL of the BF muscles, which could be linked to more complacent structures.
15 Nevertheless, the number of repetitions for the leg curl exercise did not change (Figure
16 3). Overall, the higher number of repetitions after this protocol shows that this specific
17 muscle architecture change had no negative impact on the subsequent force exertion.

18 We conclude that the SW method as well as the passive static stretching should be
19 prescribed before a strength training session and the PNF stretching should not be
20 recommended. The performance differences after the stretching methods cannot be
21 explained by changes in muscle architecture, as already discussed a (27) on meta-
22 analysis.

23

24 ***PRACTICAL APPLICATIONS***

1 Stretching exercises are commonly prescribed in training centers, gyms and
2 fitness clubs as part of a warm-up routine. The results of the present study suggest that a
3 specific warm-up (SW) and static stretching are the best warm-up options for coaches
4 and athletes to increase the performance on total repetition number for lower limb
5 resistance training session. Otherwise, we also suggest avoiding PNF stretching before a
6 lower limb training session.

7

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1

2 TABLE 1

3 Table 1. PA mean (degrees) and standard deviations for VL and BF at each measure
 4 moment (M_{01} , M_{02} and M_{03}).

	M_{01}	M_{02}	M_{03}
VL muscle			
PSS	18.80±2.17	19.12±2.72	21.13±2.30
PNF	18.60±2.34	17.84±2.28	18.78±3.19
SW	18.73±2.09	19.35±2.75	20.68±2.49
CS	19.71±2.14	19.36±3.18	20.79±3.79
BF muscle			
PSS	16.46±2.86	14.78±3.43	18.77±4.43*
PNF	14.47±1.66	16.14±3.23	16.96±3.19
SW	16.31±2.04	15.16±2.09	16.66±3.12
CS	16.14±3.16	15.92±3.64	18.26±3.65

5 *for $p < 0.05$, significant different for M_{02} .

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3 TABLE 2

4 Table 2. FL mean (cm) and standard deviations for VL and BF at each measure moment
 5 (M_{01} , M_{02} and M_{03}).

	M_{01}	M_{02}	M_{03}
VL muscle			
PSS	8.56±1.09	8.61±0.58	8.61±1.02
PNF	8.78±1.13	9.64±1.35	8.42±1.16*
SW	8.68±2.09	8.62±1.08	8.80±0.78
CS	8.27±0.62	8.96±0.91	9.36±1.59
BF muscle			
PSS	8.71±1.06*	9.91±1.52	8.19±1.31*
PNF	9.39±0.99	8.77±1.28*	8.71±1.33
SW	8.94±0.86	9.29±1.05	8.70±1.23
Control	8.99±1.24	8.78±1.07	8.28±1.41

6 *for $p < 0.05$, significant different for M_{02}

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5 Figure 1: PSS and PNF stretching of the hamstrings of the thigh muscles.

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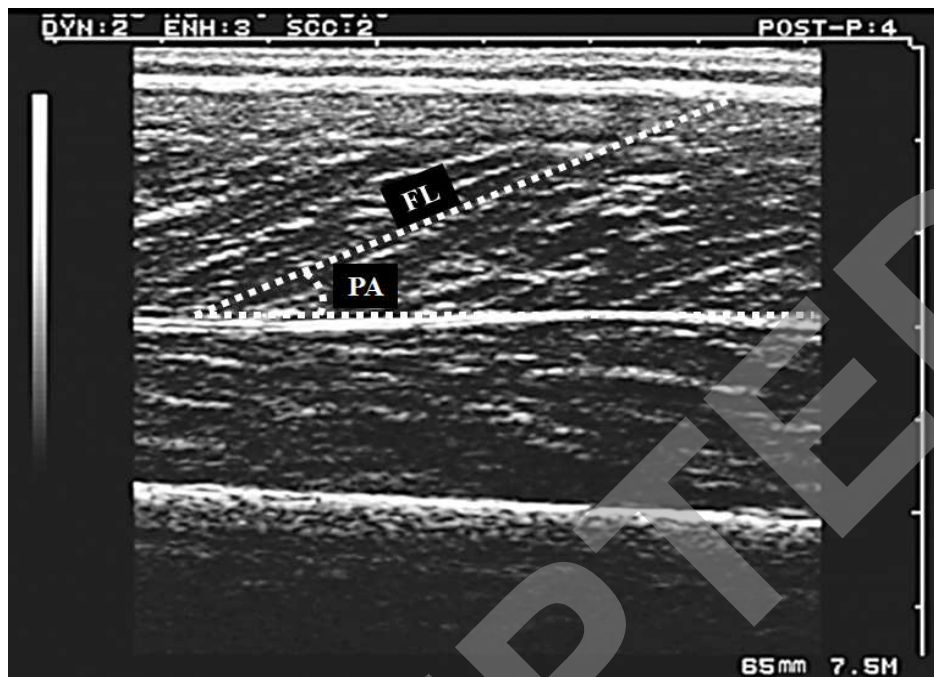


3

4 Figure 2: PSS and PNF stretching of the quadriceps of the thigh muscles.

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3

4 Figure 3: Ultrasound image of VL muscle for PA and FL measurements.

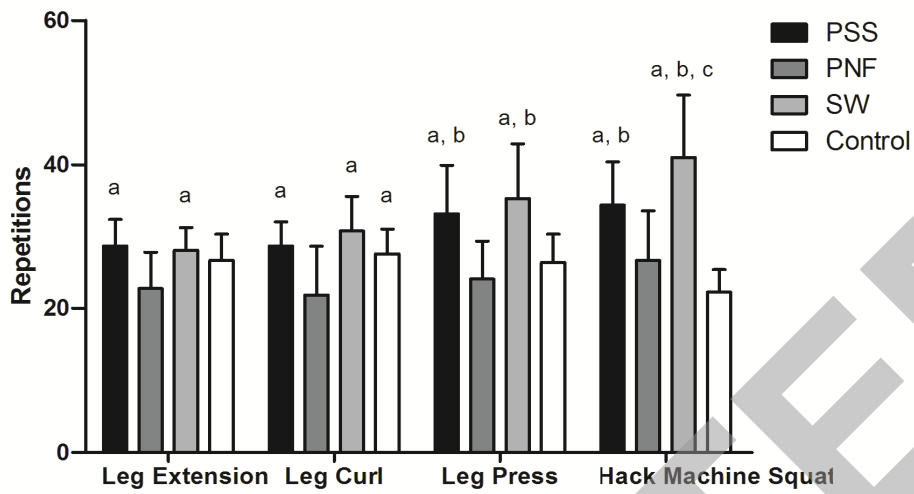


Figure 4: Representative graph of the number of repetitions for each exercise and groups. For $p < 0.05$; a - differences for the PNF; b - differences to the Control; c - difference between PSS and SW.