



Effect of proprioceptive neuromuscular facilitation in land and aquatic environments on adult flexibility

Efeito da facilitação neuromuscular proprioceptiva em meio terrestre e aquático na flexibilidade de adultos

Hálisson Alves Ribeiro¹, Jezrael Rossetti Dutra², Flávia Cláudia Silva², Ian Mazzetti Rodrigues Valle³, Flavio de Souza Araujo⁴, Fabíola Bertú Medeiros⁵, Rodrigo Gustavo da Silva Carvalho⁶

¹ Physiotherapy Graduate from Centro Universitário Dr. Leão Sampaio (UNILEÃO/CE). Student of the Graduate Program in Physical Education at the Federal University of Vale do São Francisco (UNIVASF), Petrolina (PE), Brazil; ² Physiotherapy Graduate from University Presidente Antônio Carlos - UNIPAC, Barbacena (MG), Brazil; ³ Graduating in Medicine at the Federal University of Vale do São Francisco (UNIVASF), Petrolina (PE), Brazil; ⁴ Master of Science from the Federal University of Vale do São Francisco (UNIVASF). Student of the Postgraduate Program in Rehabilitation and Functional Performance at the University of Pernambuco (UPE), Petrolina (PE), Brazil; ⁵ PhD in Sports Science from UFMG/MG. Permanent Professor of the Physical Education Collegiate of the Federal University of Vale do São Francisco (UNIVASF). Permanent Professor of the Graduate Program in Physical Education (PPGEF) at UNIVASF, Petrolina (PE), Brazil; ⁶ PhD in Physical Education from the State University of Londrina (UEL/PR). Permanent Professor of the Graduate Program in Physical Education (PPGEF) at UNIVASF. Permanent Professor of the Postgraduate Program in Rehabilitation and Functional Performance at the University of Pernambuco (UPE), Petrolina (PE), Brazil.

* **Corresponding author:** Flavio de Souza Araujo. E-mail: flavio.araujo@univasf.edu.br

ABSTRACT

The aim of the present study was to analyze the effect of proprioceptive neuromuscular facilitation (PNF), performed in land and aquatic environments, on the flexibility of the posterior thigh and hip extensors in healthy adults. This is a randomized clinical trial. The sample was composed of 16 adults (18 to 35 years old) of both sexes, randomized into two groups: experimental (EG, n=08), submitted to PNF stretching in the aquatic environment; and control (CG, n=08), which received PNF on land. The intervention was performed during six weeks, with two weekly sessions. Before and after the intervention, hip flexibility was assessed by range of motion (ROM) using a goniometer positioned over this joint. And to determine the flexibility of the posterior thigh muscles, the sit and reach test was used. Both interventions provided a significant increase in flexibility in the EG and CG ($p < 0.05$), however there was no significant effect on the environment ($p > 0.05$).

Keywords: Muscle stretching exercises. Hydrotherapy. Exercise.

RESUMO

O objetivo do presente estudo foi analisar o efeito da facilitação neuromuscular proprioceptiva (FNP), realizada em meio terrestre e aquático, na flexibilidade de posteriores da coxa e extensores do quadril em adultos saudáveis. Trata-se de um ensaio clínico aleatorizado. A amostra foi composta por 16 adultos (18 a 35 anos) de ambos os sexos, randomizados em dois grupos: experimental (GE, n=08), submetido ao alongamento por FNP no meio aquático; e controle (GC, n=08), que recebeu a FNP no meio terrestre. A intervenção foi realizada durante seis semanas, com duas sessões semanais. Pré e pós-intervenção a flexibilidade do quadril foi avaliada pela amplitude de movimento (ADM) utilizando um goniômetro posicionado sobre essa articulação. E para determinar a flexibilidade dos músculos posteriores da coxa foi utilizado o teste sentar e alcançar. Ambas as intervenções, propiciaram um aumento significativo na flexibilidade do GE e GC ($p < 0,05$), entretanto não houve efeito significativo de ambiente ($p > 0,05$).

Palavras-chave: Exercícios de alongamento muscular. Hidroterapia. Exercício físico.

Received in March 11, 2021
Accepted on August 28, 2021

INTRODUCTION

A sedentary lifestyle can generate a decrease in physical capabilities, including flexibility¹. In addition to sedentary lifestyle, with the aging process, this capacity is progressively reduced, which may increase the risk of injuries, pain in the lower and upper limbs, postural problems, in addition to being harmful to the performance of daily activities^{1,2,3}. Even in physically active individuals, flexibility levels can be reduced if they do not perform specific physical activities that involve the full extension of the segments, such as stretching exercises^{4,5}.

Flexibility can be defined as the range of motion (ROM) of a joint or a series of joints, which can be influenced by muscles, tendons, ligaments and bone structures⁶. Much of the resistance to movement at the extreme of its ROM is caused by the connective tissue, and more specifically by the collagen protein, and the skeletal muscle may be the greatest limitation of movement⁷. As, for example, in trunk flexion, in which the semimembranosus, semitendinosus and biceps femoris (hamstring) muscles are shortened in a resting position⁷, thus limiting this action.

The maintenance and development of flexibility levels can be achieved through stretching exercises, with a regular training program, planned and deliberate exercises

that aim to progressively increase ROM for health promotion⁸. Such exercises influence the structure and biochemical composition of connective tissues, thus reducing muscle stiffness^{8,9}.

In this sense, some methods have been used to improve flexibility, both acutely and chronically, especially proprioceptive neuromuscular facilitation (PNF)^{10,11,12}. PNF, with the contract-relax agonist-contrast (CRAC) technique, is based on neurophysiological principles such as autogenous inhibition, which refers to stimulation of the Golgi tendon organ by contraction of the muscle being stretched, and of reflex inhibition, in which the contraction of the agonist muscle induces relaxation in the muscle that is being stretched to gain ROM¹².

Another factor that can enhance flexibility improvement is the performance of exercises in aquatic environments¹³. As a form of therapeutic and rehabilitation, this environment can produce acute and chronic effects, providing ROM gains, improved quality of life, pain relief and functional aspects of the performance of its practitioners^{13,14,15,16}. However, flexibility in the water environment has not been much investigated alone^{13,14} and there is a shortage of randomized clinical trials investigating whether the water environment differs from the land environment in ROM gains using the same training protocols to improve flexibility^{15,16}.

Thus, we hypothesized that the PNF technique performed in water environment suffers a possible influence from different physical properties of this environment in improving flexibility and ROM. However, the present study aimed to analyze and compare the effect of PNF performed in land and water environments on the flexibility of healthy adults.

METHODOLOGY

STUDY DESIGN AND ETHICAL ASPECTS

This study was a randomized clinical trial, with two parallel groups, single-blind and complied with the norms of the Consolidated Standards of Reporting Trials¹⁹ (CONSORT) and was registered in the database for clinical trials, ClinicalTrials.gov (NCT03350880). The evaluators and the researcher who performed the statistical analysis were blinded as to the assignment of participants to the groups (single-blind) and the interventions lasted for six weeks.

The study was approved by the Research Ethics Committee of the Federal University of the São Francisco Valley (UNIVASF) under the following opinion number 0003/150612. All participants gave written consent and were previously informed about all assessment and intervention procedures, which only started after authorization.

SAMPLE

The sample calculation was performed using an online calculator (<https://sample-size.net/>)²⁰, based on the comparison of means of a continuous measurement of two independent groups. A type I error of 5% was considered, the power of 80%, the effect size equal to 0.8 was estimated. The total number calculated was 16, with 08 participants for each group.

Research volunteers were recruited through the dissemination of posters, leaflets and through publications on social networks. People who came into contact were checked for compliance with the following eligibility criteria (inclusion): both genders, aged between 18 and 35 years, a body mass index below 30 kg/m², sedentary, absence of neuromusculoskeletal diseases and preserved clinical and cognitive functions. These last three criteria were obtained in a self-declared form by the participants. For individuals who met the inclusive criteria, evaluation was scheduled, followed by randomization and the beginning of the flexibility training program. The exclusion criteria, in turn, were: having more than three absences, consecutive or not, during the training period, having some adverse effect, such as allergy or any dermatitis, or being unable to continue training (p. e.g. moving to another city).

Subjects were randomized into two groups: the experimental group (EG), which was subjected to PNF with the CRAC technique (PNF/CRAC) in water

(water environment); and control group (CG), which was subjected to PNF with the CRAC technique on the ground (land environment). Interventions were carried out for six weeks, with two sessions per week, totaling 12 sessions. The assignment secrecy occurred through sequential numbers kept in opaque, non-translucent and closed envelopes, with the generation of the sequence of numbers done by an independent researcher, through an online resource (<https://www.randomizer.org/>) and that it was kept confidential until the end of the study. After the inclusion of the participants, each one removed their envelope and was taken to the referred training.

EVALUATION PROCEDURES

All subjects were evaluated before and after intervention, regarding the flexibility of the coxofemoral joint (angular flexibility) considering the right and left limbs, in addition to the flexibility of the biceps femoris through the sit-and-reach test (linear flexibility).

To assess the flexibility of both outcomes, measurements were taken before and 24 hours after the end of the stretching program, performed at the same time of day and with the same level of activity before the measurement, that is, without warming up or any physical effort.

In order to assess the range of motion, in degrees, of flexion of the coxofemoral joint, a universal plastic goniometer CARCI was used, consisting of two arms of 18 centimeters each, with a 360° recording (2° scale). The measurement was taken on the lateral surface of the thigh, over the hip joint, with the knee flexed and extended. The individual was positioned in dorsal decubitus with hip abduction, adduction and 0° rotation with the axis at the level of the greater trochanter, with one of the goniometer arms pointing to the midaxillary line of the trunk and the other parallel and on the lateral surface of the thigh, towards the lateral condyle of the femur²¹. Three measurements were taken with an interval of two minutes between them, with the highest measurement being recorded (Figure 1).



Figure 1. Assessment the range of motion, in degrees, of flexion of the coxofemoral joint.

Flexibility of biceps femoris was determined in centimeters using the sit-and-reach test. For this test, a wooden box specially built for this purpose was produced, with a measuring scale between 0 and 50 cm fixed at its top, in such a way that the 23 cm value coincides with the line where the person evaluated should accommodate their feet. The distance covered by the fingers of the hand was then measured on the scale fixed on the box. Feet were placed in the box with the individual in the sitting position and with the legs extended, with the extensibility of the posterior muscles of the thigh and lumbosacral spine being the main limiting factor. Sitting on the ground, with legs extended, with the face of the bare feet resting against the bench (against the wall), with hands on the head, the subject had to move forward slowly, with both hands parallel as far as possible, keeping this position momentarily and the evaluator could lean on the individual's knees to keep them extended, however, without pressing them. For this test, three measurements were also taken with an interval of two minutes between them, and the best result of the three attempts was recorded^{22,23}.

INTERVENTION PROTOCOL

The training sessions for both groups were developed at the UNIVASF Collegiate of Physical Education complex. For the EG, the sessions took place in a heated water pool, at a mean temperature of

34 °C (standard deviation - SD: 1.3), with a dimension of 1.7 x 4 x 6 m and a water depth of 1.2 m. Water temperature was measured with a digital thermometer with a range of -10°C to +50° (Incotherm). As for the CG, the sessions took place in the weight room of the aforementioned complex.

The stretching intervention was based on PNF, with the CRAC^{24,25} technique (PNF/CRAC). The PNF/CRAC protocol was performed in both groups over a period of six weeks, with two sessions per week (totaling 12 sessions) and with a 48-hour interval between each session.

In each session, three sets of one repetition were performed on each lower limb, with a total time of 20 seconds for each repetition/set. The difference in the intervention between the groups was the way in which the PNF/CRAC was applied. The EG practiced in the water environment and the CG in the ground environment (land).

Participants in the EG were positioned orthostatically inside the pool, supporting their spine on the pool wall, while in the CG, the supine position on a stretcher was adopted (Figure 2). In both groups, the contralateral leg to the stretch was stabilized, preventing its flexion and/or preventing the movement of the lumbar.

For this technique, the limb to be stretched was taken to the maximum range of motion of hip flexion with knee extended. In this position, the contraction of the posterior muscles of the thigh

(maximum isometric contraction) was requested for five seconds, against the resistance performed by the researcher. Then, the contraction of the posterior muscles was ceased and a contraction of the

quadriceps (concentric contraction) was started for fifteen seconds, while the therapist helped in the elevation of the extended leg.

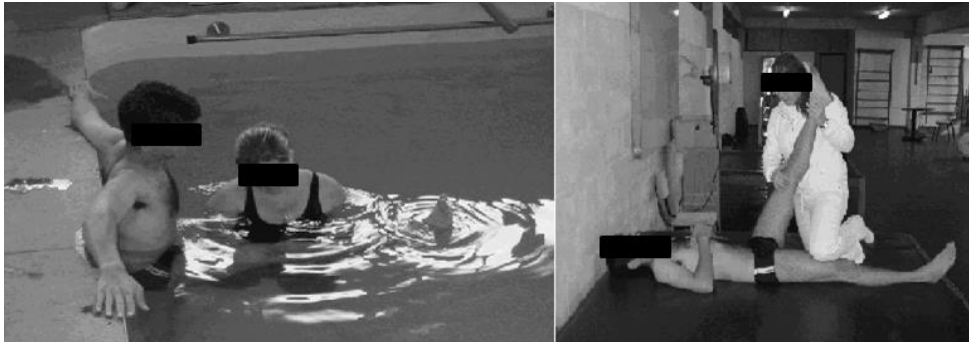


Figure 2. Proprioceptive neuromuscular facilitation technique in aquatic and land environment.

STATISTICAL ANALYSIS

A descriptive analysis with mean and standard deviation was performed. Outcome values tended to be normally distributed using the Shapiro-Wilk test. Comparisons of means between and within outcome groups were performed using Generalized Estimating Equations, with its inherent syntax, linear distribution and multiple comparisons using the Bonferroni test so that differences could be identified. Differences of means, effect size (Cohen's *d*) and 95% confidence intervals were also calculated. The statistical significance

adopted was an alpha of 5% and the analyses were performed using the Statistical Package for the Social Sciences program (SPSS 22.0, Chicago, IL, USA). Intention-to-treat analysis was considered.

RESULTS

Twenty-three subjects were evaluated for eligibility criteria. Of these, seven (30.4%) were excluded at the time of anamnesis and 16 (69.6%) participated in the study and were followed up until the end of the intervention, with an intention-to-treat analysis being performed (Figure 3).

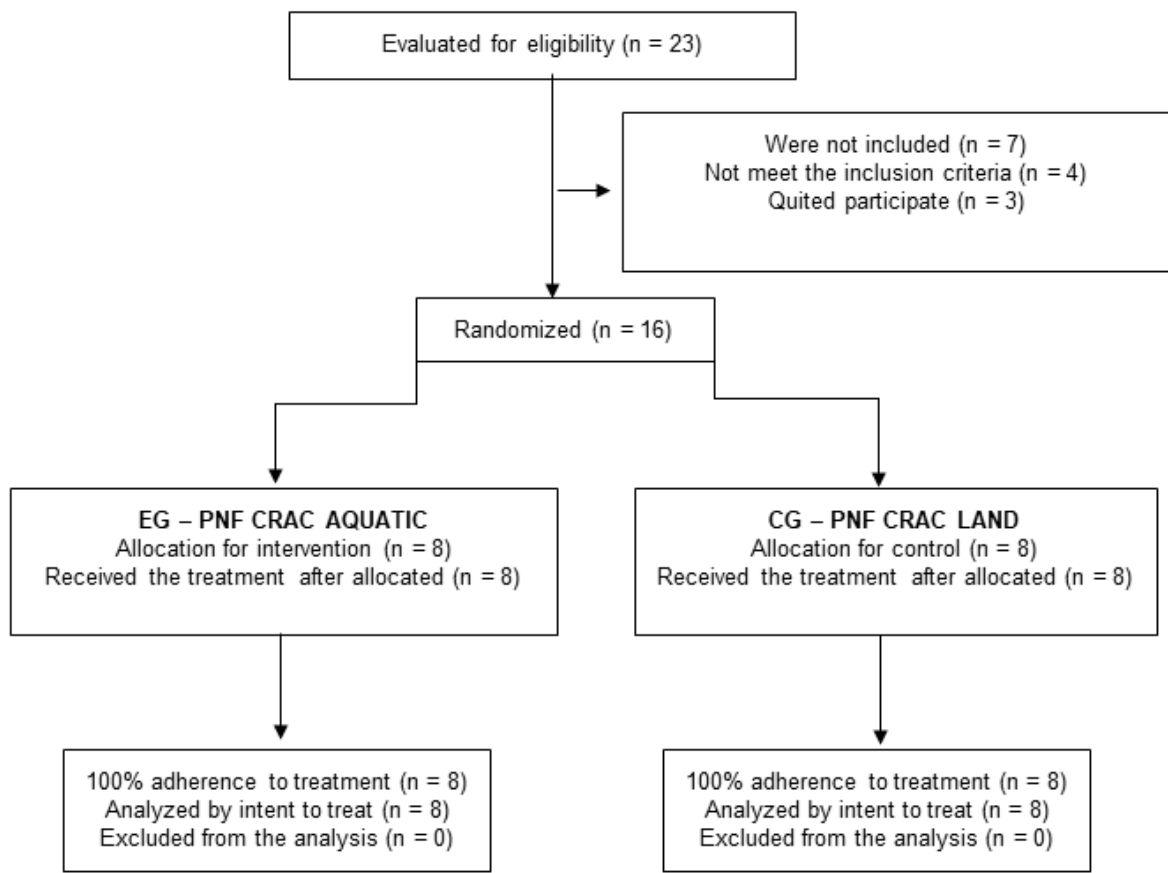


Figure 3. Sample flowchart.

In table 1, variables for sample characterization are described by groups (EG and CG), considering the variables age, body mass and height. There was no

significant difference for any of the characterization variables ($p > 0.05$), which infers that the groups have similar characteristics after randomization.

Table 1. Characteristics of the sample by groups

Variables	EG (\bar{x} (SD))	CG (\bar{x} (SD))	<i>p</i> value
Age (years)	24.2 (4.0)	26.1 (4.2)	0.381
Body mass (kg)	66.7 (16.1)	70.1 (12.6)	0.648
Height (cm)	170 (10.0)	168 (8.0)	0.759

EG = experimental group; CG = control group; \bar{x} = mean; SD = standard deviation.

Table 2 compares the outcomes between and within groups, respectively, as well as the effect size of the interventions. No significant differences were detected when comparing the groups for the

outcomes flexibility of the hip joint and biceps femoris ($p > 0.05$). There was a significant improvement ($p < 0.05$) when analyzing within the groups (before and after intervention) for the flexibility of the

hip joint (except for the CG in hip flexion with the right knee flexed) and biceps femoris (except for the CG), with a large effect size in the EG and moderate in the CG. The effect size was small for the

primary outcome and moderate for the secondary outcome for the PNF/CRAC performed in water (except for hip flexion with the left knee flexed).

Table 2. Comparison between and within groups, Pre and Post intervention, by outcome and moment

Outcomes	EG (n=08)	CG (n=08)	EG vs. CG (between groups)		Pre vs. Post intervention (within groups)	
	\bar{x} (SD)	\bar{x} (SD)	MD [CI 95%]	\bar{d} [CI 95%]	MD [CI 95%]	\bar{d} [CI 95%]
<i>Primary – Flexibility of the hip joint (degrees)</i>						
HFERK						
Pre	63.7 (6.1)	67.8 (9,8)	-4.1 [-11.6;3.4]	-0.48 [-1.5;0.5]	-16.1 [-21.4;-10.9]*	-2.6 [-4.4;-1.3]
Post	79.8 (5.2)	78.7 (8,3)	1.1 [-7.5;5.3]	0.15 [-0.8;1.1]	-10.9 [-19.2;-2.5]*	-1.1 [-2.2;-0.1]
HFELK						
Pre	64.3 (12.7)	67.7 (12.7)	-3.4 [-15.0;8.3]	-0.25 [-1.2;0.7]	-15.6 [-24.8;-6.5]*	-1.5 [-2.6;-0.4]
Post	80.0 (6.0)	78.5 (7.3)	1.5 [-4.6;7.7]	0.21 [-0.7;1.2]	-10.7 [-20.2;-1.2]*	-1.0 [-2.0;-0.1]
HFRKF						
Pre	103.6 (7.7)	107.7 (7.9)	-4.1 [-11.3;3.0]	-0.50 [-1.5;0.5]	-11.1 [-17.2;-5.0]*	-1.6 [-2.7;-0.4]
Post	114.7 (5.3)	113.6 (8.8)	1.1 [-5.6;7.8]	0.14 [-0.8;1.1]	-5.8 [-13.5;1.8]	-0.6 [-1.7;-0.3]
HFLKF						
Pre	104.4 (7.4)	106.8 (9.1)	-2.5 [-10.1;5.1]	-0.28 [-1.3;0.7]	-10.7 [-16.8;-4.6]*	-1.5 [-2.6;-0.4]
Post	115.1 (5.7)	115.6 (6.3)	-0.5 [-6.0;5.0]	0.01 [-0.9;0.9]	-8.7 [-16.0;-1.5]*	-1.0 [-2.1;-0.01]
<i>Secondary – Flexibility of the biceps femoris (Sit and Reach Test - cm)</i>						
Pre	18.7 (9.5)	16.9 (14.1)	1.8 [-9.2;12.8]	0.14 [-0.8;1.1]	-9.6 [-17.0;-2.3]*	-1.1 [-2.2;-0.1]
Post	28.3 (6.1)	23.8 (13.1)	4.5 [-4.9;13.9]	0.42 [-0.5;1.4]	-7.0 [-19.4;5.4]	-0.5 [-1.5;0.5]

* P<0.05 for Pre and Post within the group. EG = experimental group; CG = control group; HFERK = hip flexion with extended right knee; HFELK = hip flexion with extended left knee; HFRKF = hip flexion with right knee flexed; HFLKF = hip flexion with left knee flexed; \bar{x} = mean; SD = standard deviation; MD = mean difference; \bar{d} e CI 95% = confidence interval 95%.

DISCUSSION

The present study analyzed the effect of PNF/CRAC performed in land and water environments on flexibility in healthy adults. The main findings indicate that the environment (land or water) does not directly interfere with flexibility gain after stretching training with the PNF/CRAC

technique in and out of the water (Table 2). However, it was verified that the effect size (Cohen's d) was greater in the EG (water environment). Still, it is important to highlight that despite the short duration (12 sessions), it was possible to observed gains in flexibility in both groups (EG and CG).

Analyzing the literature, it is possible to point out that the improvement

in flexibility may occur due to the increased tolerance to stretching, instead of a change in the mechanical and viscoelastic properties of the muscle²⁶. It is also possible that stretching exercises have changed the viscoelastic properties of the muscle-tendon unit, reducing passive tension and stiffness of the muscle unit^{27,28}.

The time to maintain the stretch, as well as the number of sets required to produce a better result, changes according to the methodology used⁸. Different length of stretch hold do not present significant differences in ROM gains, despite having greater “clinical” effects²⁹. The specificity of training and activity has an influence on muscle flexibility in healthy individuals⁷. Stretching acutely stimulates the increase in body temperature and muscle irrigation and viscosity, which are responsible for stimulating the Golgi corpuscles that relax the muscle structure³⁰.

In this sense, when using the PNF/CRAC method, it was proposed to maintain the same characteristic for the type of stretching between the two environments (water and land). In both, the same form of stabilization of the contralateral leg allowed by a rigid structure behind the individual to be stretched was performed (what was done on the ground, the stretcher, and what was done in the water environment, the edge of the pool), it was also possible the same grip for stretching, which shows that the positioning during the training for flexibility was not responsible for the differences found.

In general, the literature indicates that the practice of exercises in water environment can provide improvements in flexibility^{13,14,15,16}. This improvement in flexibility with exercises performed in water environment may be related to the water temperature³¹. Local heating can reduce muscle spasm and facilitate joint mobility, and evidence indicates that the increase in body temperature can change the extensibility capacity of connective tissues, which would result in greater gains in flexibility¹³. As in this study the practice of stretching in water environment was performed in a heated pool (34°C), and the individuals were subjected to acclimatization in the water environment, these adaptations promoted by the increase in body temperature appear as a possible justification for a greater effect size on flexibility gains of the EG.

Another factor that may have influenced the greater effect of stretching in the EG that is related to the practice performed in warm water is the analgesic effect of heat; this effect can allow greater tolerance to hamstring stretching and tends to show a greater increase in hip flexion when compared only with stretching at usual temperature¹⁶. Skin nerve endings (temperature, touch and pressure receptors) are also affected, so the water temperature affects the pain threshold, causing it to increase and sensory extravasation as the mechanism by which pain is less perceived when the part is immersed¹⁶.

However, the ideal response dose for stretching, such as the time of the set and

session, as well as the ideal weekly frequency to produce better benefits to increase flexibility^{10,32} is not yet clarified in the literature. In addition to these variables, the determination of a more suitable environment for stretching allows, like any type of training, to obtain the greatest effect in the shortest possible time, leading to an improvement in the quantity and quality of exercise prescription.

For knowledge, there is still no evidence in the literature about the influence of the land and water environment on the flexibility response after application of the PNF method. Although this study followed the rules set forth by CONSORT¹⁹, it still has some limitations. The sample consisted of individuals of both sexes, and not stratified, which may interfere with the findings, but it was paired by age and previous characteristic (sedentary lifestyle). Another important factor to be considered is the weekly frequency (twice a week) and the total period of intervention (six weeks), which can be extended in future studies. In addition to other outcomes to be considered, such as: muscle pain, functionality and perception of improvement.

As implications for practice, stretching using PNF in both land and water environments can help improve flexibility. Thus, this type of stretching can be prescribed or included, either on land or in water, in some physical or therapeutic exercise program for people who need to improve flexibility and thus promote functionality gain.

CONCLUSION

With the results shown in this study, it is concluded that 12 sessions (twice a week) of stretching, through PNF, in land and water environments provide gains in flexibility for healthy adults. However, the environment (land or water) has no significant influence on the magnitude of these results.

ACKNOWLEDGEMENTS

The team from the Tutorial Education Program in Biomechanics at UNIVASF, Office of the Pro Dean at the Federal University of Vale do São Francisco and the Ministry of Education.

REFERENCES

1. Chaves TO, Balassiano DH, Araújo CGS. Influence of exercise habits during childhood and adolescence on flexibility of sedentary adults. *Rev Bras Med Esporte*. 2016;(22)4:256-60.
2. Mello RL, Ribeiro EK, Okuyama J. (In) atividade física e comportamento sedentário: terminologia, conceitos e riscos associados. *Caderno Intersaberes*. 2020;9(17):59-68.
3. Global Health Estimates 2016: Deaths by cause, age, sex, by country and by region, 2000–2016. Geneva: World Health Organization; 2018. http://www.who.int/healthinfo/global_burden_disease/estimates/en/index1.html.
4. Moraes AAC, Almeida CP, Ferreira TCR. Immediate and late effects of

- stretching on musculoskeletal pain, flexibility and quality of life among teachers in a municipality inland of amazon. *Rev Ciên Saúde*. 2020;5(2):28-35.
5. Rodrigues GM, Freitas FS, Rocha LSM, Bertocello D. Bodybuilding in construction workers: effects on joint flexibility and strength muscle. *ConScientiae Saúde*. 2018; 17(2):179-86.
 6. Lima TR, Martins PC, Moraes MS, Silva DAS. Association of flexibility with sociodemographic factors, physical activity, muscle strength, and aerobic fitness in adolescents from southern Brazil. *Rev Paul Pediatr*. 2019; 37(2):202-8.
 7. Cayco CS, Labro AV, Gorgon EJR. Hold-relax and contract-relax stretching for hamstrings flexibility: A systematic review with meta-analysis. *Phys Ther Sport*. 2019; 35(1):42-55.
 8. Thomas E, Bianco A, Paoli A, Palma A. The Relation Between Stretching Typology and Stretching Duration: The Effects on Range of Motion. *Int J Sports Med*. 2018; 39(4):243-54.
 9. Iwata M, Yamamoto A, Matsuo S, Hatano G, Miyazaki M, Fukaya T, et al. Dynamic Stretching Has Sustained Effects on Range of Motion and Passive Stiffness of the Hamstring Muscles. *J Sports Sci Med*. 2019; 18(1):13-20.
 10. Navega MT, Paleari B, Morcelli MH. Assessment and comparison of the effects of two techniques on hamstring flexibility. *Fisioter Mov*. 2014; 27(4):583-9.
 11. Santos D, Dias GP, Schwabe H, Klosiensi TB, Moreira, NB. Acute effect of different stretching techniques of hamstring flexibility. *Fisioter Bras*. 2017; 18(6):708-18.
 12. Moesch J, Mallmann JS, Tomé F, Vieira L, Ciqueleiro RT, Bertolini GRF. Effects of three protocols of hamstring muscle stretching and paravertebral lumbar. *Fisioter. mov*. 2014; 27(1):85-92.
 13. Cunha MG, Carvalho EV, Caromano FA. Effects of single session of watsu. *Cad. Pós-Grad. Distúrb. Desenvolv*. 2010; 10(1):103-9.
 14. Candeloro JM, Caromano FA. Effects of a hydrotherapy program on flexibility and muscular strength in elderly women. *Rev. bras. Fisioter*. 2007; 11(4):267-72.
 15. Silva LA, Tortelli L, Motta J, Menguer L, Mariano S, Tasca G, et al. Effects of aquatic exercise on mental health, functional autonomy and oxidative stress in depressed elderly individuals: a randomized clinical trial. *Clinics*, 2019; 74:e322.
 16. Britto A, Rodrigues V, Santos AM, Rizzini M, Britto P, Britto L, Gracia JBS. Effects of water- and land-based exercise on quality of life and physical aspects in women with fibromyalgia: A randomized clinical trial. *Musculoskeletal Care*. 2020; 18:459-66.
 17. Ansari S, Elmieh A, Alipour A. The effect of aquatic exercise on functional disability, flexibility and function of trunk muscles in postmenopausal women with chronic non-specific low back pain: randomized controlled trial. *Science & Sports*. 2021; 36(3):e103-e110.
 18. Antunes JM, Daher DV, Giaretta VMA, Ferrari MFM, Posso MBS. Hydrotherapy and crenotherapy in the

- treatment of pain: integrative review. *BrJP*. 2019; 2(2):187-98.
19. Boutron I, Altman DG, Moher D, Schulz KF, Ravaud P. CONSORT Statement for Randomized Trials of Nonpharmacologic Treatments: A 2017 Update and a CONSORT Extension for Nonpharmacologic Trial Abstracts. *Ann Intern Med*. 2017; 167(1):40-7.
20. Brito CJ, Grigoletto MES, Nóbrega OT, Córdova C. Dimensionamento de amostras e o mito dos números mágicos: ponto de vista. *Rev Andal Med Deporte*. 2016; 9(1):29-31.
21. Paula AR, Paula SC, Polese JC. Descomplicando a Goniometria: Um Guia Para a Prática Clínica. 2019; 1(1):1-58.
22. Camilo IB. O teste de sentar e alcançar como avaliação de flexibilidade em escolares do ensino fundamental da rede pública de um município da região central de Rondônia. *ACTA Brasileira do Movimento Humano*. 2016; 6(1):64-75.
23. Bezerra ES, Martins SL, Leite TB, Paladino KDV, Rossato M, Simão R. Influence of the modified Sit-and-Reach Test in flexibility of different age groups. *Motriz*. 2015; 11(3):3-10.
24. Gunn LJ, Stewart JC, Morgan B, Metts ST, Magnuson JM, Iglowski NJ, et al. Instrument-assisted soft tissue mobilization and proprioceptive neuromuscular facilitation techniques improve hamstring flexibility better than static stretching alone: a randomized clinical trial. *J Man Manip Ther*. 2019; 27(1):15-23.
25. Yıldırım MS, S Ozyurek, Tosun OÇ, Uzer S, Gelecek N. Comparison of effects of static, proprioceptive neuromuscular facilitation and Mulligan stretching on hip flexion range of motion: a randomized controlled trial. *Biol Sport*. 2016; 33(1):89-94.
26. Opplert J, Babault, N. Acute Effects of Dynamic Stretching on Muscle Flexibility and Performance: An Analysis of the Current Literature. *Sports Med*. 2018; 48(2):299-325.
27. Lempke L, Wilkinson R, Murray C, Stanek J. The Effectiveness of PNF Versus Static Stretching on Increasing Hip-Flexion Range of Motion. *J Sport Rehabil*. 2018; 27(3):289-94.
28. Moraes MA, Spinoso DH, Navega MT. Effectiveness of performing hamstring stretches under physiotherapeutic supervision. *ConScientiae Saúde*. 2015; 14(2):298-305.
29. Behm DG, Alizadeh S, Anvar SH, Drury B, Granacher U, Moran J. Non-local acute passive stretching effects on range of motion in healthy adults: a systematic review with meta-analysis. *Sports Med*. 2021; 51(5):945-59.
30. Lyle MA, Nichols TR. Evaluating intermuscular Golgi tendon organ feedback with twitch contractions. *J Physiol*. 2019; 597(17):4627-42.
31. Cubas SRO, Ribas DIR. Positive effect of isostretching in aquatic environment on muscle flexibility in elderly people. *Geriatr Gerontol Aging*. 2017; 11(1):37-41.
32. Zotz TGG, Loureiro APC, Valderramas SR, Gomes ARS. Stretching - an important strategy to prevent musculoskeletal aging: a systematic review and meta-analysis. *Top Geriatr Rehabil*. 2014; 30(4):246-55.