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A COMPARATIVE ANALYSIS OF VARIOUS PROPRIOCEPTIVE NEUROMUSCULAR FACILITATION (PNF) TECHNIQUES ON MUSCLE FLEXIBILITY AMONG EXTENDED SITTING POSTURE INDIVIDUALS

ORIGINAL ARTICLE

ABSTRACT

Purpose: The purpose of this research is to compare the effectiveness of different Proprioceptive Neuromuscular Facilitation (PNF) stretching techniques for hamstring muscle tightness, and to find out the best PNF technique for improving hamstring flexibility.

Methods: In this quasi-experimental designed study, 30 university students who were between 18-25 years-old, sitting >6 hours per day, and had a Active Knee Extension Test (AKET) >20° were recruited with convenience sampling and equally allocated into Group A, B and C, non-randomly. Hold-Relax (HR), Agonist Contraction (AC) and Contract-Relax-Antagonist-Contract (CRAC) stretching techniques were given respectively, 3 sessions/week, for three weeks.

Results: Paired t-test showed significant effect of each technique compared between pre-test value and post-test value of AKET measurements of Hold-relax (HR), AC and CRAC groups ($p<.001$). One-way ANOVA results showed significant difference between the effects of these techniques ($F(2,27)=13.069$, $p<.001$). Tukey Post-Hoc test revealed that effect was significantly greater in CRAC ($-20.033^{\circ}\pm 2.666^{\circ}$, $p<.001$) and AC groups ($-17.516^{\circ}\pm 1.658^{\circ}$, $p=.047$) than HR ($-15.100^{\circ}\pm 2.025^{\circ}$). Furthermore, CRAC ($p=.038$) was found to have significantly greater effect than AC.

Conclusion: The PNF stretching techniques used in this study are effective in improving hamstring flexibility among university students. In addition, CRAC technique was found to be the most effective one.

Keywords: Extended Sitting Posture, Flexibility, Hamstring, Proprioceptive Neuromuscular Facilitation, Stretching

UZUN SÜRELİ OTURMA POZİSYONUNA SAHİP BİREYLERDE KAS ESNEKLİĞİ ÜZERİNE ÇEŞİTLİ PROPRİOSEPTİF NÖROMÜSKÜLER FASILİTASYON (PNF) TEKNİKLERİNİN KARŞILAŞTIRMALI ANALİZİ

ARAŞTIRMA MAKALESİ

ÖZ

Amaç: Bu araştırmanın amacı, hamstring kas gerginliği için farklı Propriyoseptif Nöromusküler Fasilitasyon (PNF) germe tekniklerinin etkinliğini araştırmak ve hamstring esnekliğini geliştirmek için en iyi PNF tekniğini bulmaktır.

Yöntem: Bu yarı deneysel dizaynli çalışmada 18-25 yaşında, günde >6 saat oturan ve Aktif Diz Ekstansiyon Testi (AKET) >20° olan 30 üniversite öğrencisi elverişlilik örnekleme yöntemiyle seçilmiş ve Grup A, B ve C'ye eşit olarak ayrılmıştır. Sırasıyla tut-gevşe (HR), agonist kasılma (AC) ve kas-rahatla-antagonist kas (CRAC) germe teknikleri verilmiş ve müdahaleler deneklere haftada üç seans, üç hafta süreyle uygulanmıştır.

Sonuçlar: Paired t-testi, HR, AC ve CRAC gruplarının hepsinde ön test ve son test AKET ölçümleri arasında anlamlı farklar olduğunu gösterdi ($p<.001$). Tek yönlü ANOVA testi, bu tekniklerin etkinlikleri arasında anlamlı bir farklılık olduğunu ($F(2,27)=13,069$, $p<.001$); Tukey Post-Hoc testi, etkinin CRAC ($-20,033^{\circ}\pm 2,666^{\circ}$, $p<.001$) ve AC gruplarında ($-17,516^{\circ}\pm 1,658^{\circ}$, $p=.047$) HR'den ($-15,100^{\circ}\pm 2,025^{\circ}$) anlamlı olarak daha yüksek olduğunu ortaya koydu. Ayrıca, CRAC'ın ($p=.038$) AC'den önemli ölçüde daha fazla etkiye sahip olduğu bulundu.

Tartışma: Bu çalışmada kullanılan PNF germe teknikleri, üniversite öğrencilerinde hamstring esnekliğini geliştirmede etkili olup, en etkili olan CRAC tekniğidir.

Anahtar Kelimeler: Uzun Süreli Oturma Pozisyonu, Esneklik, Hamstring, Proprioseptif Nöromusküler Fasilitasyon, Germe

INTRODUCTION

Sitting for long periods of time, whether at a desk, behind the vehicle, or in front of a computer screen, can be detrimental. A study of 13 studies on sitting time and activity levels discovered that people who sat for more than eight hours a day with no physical exercise had a risk of dying similar to those who smoked or were obese (1). Studies have linked prolonged sitting hours with health concerns such as heart disease, cancer, depression, diabetes and obesity. Research shows that breaking up long periods of sitting with movement at least once an hour reduces those risks, while regular exercise at other times of day does not solve the purpose (2). Nowadays, people spend most time sitting because everyday life becomes more automated and computer-based. Also, extended sitting duration is required in most occupations and educational settings (3). During Covid-19 pandemic, E-learning replacing physical classes were ordered by Malaysian Education Ministry since April 2020 (4). This causes university students to stay home every day leading to increase sitting time and even increased rate of low back pain (LBP). (5-6). In sitting, hamstring muscles are not active and kept in shortened position as knees are flexed (7). With extended sitting, these cause decline blood-muscle pumping, hamstring trigger points development and shortening adaptation results in hamstring tightness (8-9)

Hamstring muscles are a collection of long, strong muscles in which both ordinary people and athletes experience a high level of flexibility inhibition. Tightness in hamstring not only causes a decrease in movement but also poses various musculoskeletal issues. If physiologically seen, the muscle relationships of length-tension act as the shock absorbing capacity of the limb which is influenced by muscle tightness. Diminished flexibility contributes in decreased range, and prompts different musculoskeletal issues (3). Therefore, majority university students had adopted prolonged sitting habit for learning and recreations which is the main cause of hamstring tightness among them (10-11). Qamar et al. (2017) also reported that high percentage of university students (82%) with extended sitting on chair (>six hours a day) had hamstring tightness.

Extended sitting habit among university students

causes high prevalence of hamstring tightness. Especially during Covid-19 pandemic, students spend more time sitting as they have E-learning classes and are restrained from going outdoors. Severe hamstring tightness can cause unnatural gait as hip, knee and ankle biomechanics were interrupted and eventually results in plantar fasciitis (12). Hamstring tightness also causes knee pathology due to the interfered distribution of load caused by muscle imbalance (9). In physical therapy and rehabilitation to deal with hamstring tightness or any muscle with tightness various physical treatment are available. One of the most popular and easy way is by stretching. Hamstring flexibility is frequently assessed using Active Knee Extension Test (AKET) in researches as it is an ideal hamstring flexibility test. Knee flexion angle $>20^\circ$ indicates hamstring tightness (13). For decades, static stretching technique has been utilised as the standard benchmark for various training programs, because it revealed that using static stretching technique used to increase flexibility in contrast to other methods of stretching (14).

In a recent study, they compared the effects of two active stretching techniques on hamstring flexibility in asymptomatic individuals; they used a modified hold-relax technique of proprioceptive neuromuscular facilitation (PNF) and neural mobilisation on male subjects to improve hamstring flexibility. Their findings showed that hold-relax and neural mobilisation are equally efficient in increasing hamstring flexibility (15). Static stretching (SS), dynamic stretching (DS), and Proprioceptive Neuromuscular Facilitation (PNF) are some of the prominent stretching treatments that can assist improve hamstring flexibility and prevent such issues (16).

In a similar study, conducted noted PNF to be superior to other stretching techniques (17). It is a stretching technique that promotes neuromuscular mechanism response through proprioceptors stimulations, that is used to increase muscle flexibility (18) It involves muscle active contraction while target muscle (TM) (muscle to be stretched) being held at its stretched position followed by relaxation and passive stretching (19). The three main PNF stretching techniques are Hold-Relax (HR), Ago-

nist Contraction (AC) and Contract-Relax-Antagonist-Contract (CRAC) (20). HR involves autogenic inhibition that increases muscles compliance to be lengthened following TM isometric contractions, whereas AC involves reciprocal inhibition following OM concentric contractions (21). While in CRAC, TM static contraction followed by OM concentric contraction has both reciprocal and autogenic inhibition involved (22).

Previous studies which compared effect of various PNF stretching techniques on hamstring flexibility of several populations showed different opinions on which technique is superior and beneficial for improving flexibility of a muscle. Therefore, in this study various PNF stretching techniques are compared and applied on subjects to identify the best technique. HR, AC and CRAC PNF stretching techniques to determine the most effective PNF technique for Malaysian university students with extended sitting to improve hamstring flexibility and prevent complications caused by hamstring tightness.

METHODS

A quantitative approach with a quasi-experimental design was applied in this study with a pre-test-posttest design to compare the effect of Hold-relax (HR), AC, and CRAC stretching techniques (independent variable) on hamstring flexibility (dependent variable) among university students with extended sitting posture. An informed consent was signed and the procedure were clearly explained to the participants. Before the first and after the last stretching session, AKET that contributed numerical data were performed to measure hamstring flexibility.

“Sampling” Subjects were recruited with a non-probability sampling method; convenience sampling is used. The sample size was calculated using the below formula with type-I error rate at 5% and type-II error rate at 20%: However, due to pandemics, there were limited available subjects and because of time limitations, only 30 subjects were recruited. After subjects were recruited based on selection criteria, they were allocated into one of the three groups purposively without randomization. Every group had 10 subjects. Group A received HR, Group B received AC and Group C received

CRAC technique. Male and female university students aged 18-25 were included in the study. Subjects who spend more than six hours sitting a day and subjects with right hamstring positive in AKET ($>20^\circ$ knee extension limitation) were included in the study. Participants who don't line up with the inclusion & exclusion criteria were not selected for the study. The data were collected in a timespan from June to August 2021.

An ethical review and ethical approval are provided by the Faculty of Health Sciences Research Review Committee, MAHSA University (FRRC) by fulfilling requirements with concern to the safety and ethics of the study. (Reference Number: FOHS/PT/21/UG61). Subjects' confidentiality and anonymity were taken care of all the time. Subjects' health status and personal information were kept safe with reference to Personal Data Protection Act 2010, and will not be revealed without the agreement of the respective subject. To ensure safety during the Covid-19 pandemic, Covid-19 Standard Operating Procedures (SOP) have been strictly adhered to throughout the study.

“Procedures” To determine the effectiveness of the techniques, hamstring flexibility was assessed using AKET, the gold standard measure, with subjects actively straightening the tested knee with hip remains 90° flexed with help of stabilizing tool and the pelvic and non- tested leg also stabilized to prevent unwanted movements. A Universal Goniometer (UG) tool was used during AKET to measure knee ROM. Target samples were approached by invitation posted in university students' online common groups, whoever was interested to participate were visited at their houses nearby university as they underwent online classes. They were briefed regarding the study objectives and procedures. Screenings were done with AKET and Participant Screening Form filled up by target samples. AKET procedure was demonstrated

before performing. Subjects' hamstring flexibility was assessed with AKET before first (pre- test) and immediately after the last stretching session (post-test). Pre-test and post-test measurements (ROM) were recorded in Data Collection Table.

“Statistical Analysis” These data were analyzed using IBM Corp. Released 2017. IBM SPSS Statistics

Table 1. Paired Samples Statistics for HR, AC and CRAC Groups

Variables		Mean	N	SD	Std. Error Mean
HR	Pre-Test	137.766°	10	4.85	1.53
	Post-Test	152.866°	10	3.91	1.23
AC	Pre-Test	138.534°	10	6.27	1.98
	Post-Test	156.050°	10	5.76	1.82
CRAC	Pre-Test	135.217°	10	4.49	1.42
	Post-Test	155.250°	10	4.98	1.57

HR- Hold Relax, AC- Agonist contraction, CRAC- Contract relax – Antagonist contract

for Windows, Version 25.0. Armonk, NY: IBM Corp with confidence interval (CI) set at 95%, significance level set at $p < .05$. To test the hypotheses, the pre-test and post-test knee ROM of each group were analyzed with a paired t-test to determine the effectiveness of each technique based on the mean difference as the measurements were taken at two separate times which were once before and once after an intervention. One-way ANOVA was used to compare the means of three distinct groups to see if there was a statistically significant difference in the effectiveness of the strategies. The post-hoc test was run, to identify the significant group by comparing each group (23). Before running these tests, I had cleaned the data, looked for missing values, and then keyed in values to the datasheet.

RESULTS

30 subjects (18 females and 12 males) were allocated into three groups. Group A (HR) had six females ($n=6$) (60%) and four males ($n=4$) (40%) aged 22.400 ± 2.412 ; Group B (AC) had seven females ($n=7$) (70%) and three males ($n=3$) (30%) aged 21.300 ± 2.451 ; Group C (CRAC) had five fe-

males ($n=5$) (50%) and five males ($n=5$) (50%) aged 20.900 ± 2.282 . To test on the hypothesis on whether there is significant effect within each technique, paired t-test was performed. For HR group, mean \pm SD of pre-test AKET measurement was $137.76 \pm 4.588^\circ$ and post-test AKET improved to $152.866 \pm 3.917^\circ$ (Table 1).

As presented in Table 2, the mean difference was $-15.100^\circ \pm 2.025^\circ$ between pre- test and post-test. Paired t-test result shows $t(9) = -23.574$, $p < .001$ indicating HR has statistically significant effect in improving hamstring flexibility. Thus, null hypothesis was rejected.

Paired t-test also performed to test hypothesis on the effectiveness of AC. According to Table 2, mean \pm SD of pre-test AKET measurement was $138.534 \pm 6.2773^\circ$ and post-test AKET improved to $156.050 \pm 5.7660^\circ$. Table 3 shows the mean difference was $-17.5160 \pm 1.6587^\circ$ between pre-test and post-test. Paired t-test result shows $t(9) = -33.394$, $p < .001$ indicating AC has statistically significant effect in improving hamstring flexibility, rejecting null hypothesis.

Table 2. Paired Samples Statistics for HR, AC and CRAC Groups

Variables		Mean Difference	SD	Std. Error Mean	95%CI of the difference		t	df	Sig.(2-tailed)
				Lower	Upper				
HR	Pre-Post	-15.100°	2.02	.64	-16.54	-13.65	-23.57	9	.000
AC	Pre-Post	-17.516°	1.65	.52	-18.70	-16.32	-33.39	9	.000
CRAC	Pre-Post	-20.033°	2.66	.84	-21.94	-18.12	-23.75	9	.000

HR- Hold Relax, AC- Agonist contraction, CRAC- Contract relax – Antagonist contra (The p value (quoted under Sig. (2-tailed)) is .000 (reported as $p < .001$) statistical significance)

Table 3. Statistical analysis using One-Way ANOVA for Comparison Between HR, AC and CRAC Groups

ROM (AKET)	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	121.68	2	60.84	13.06	.000
Within Groups	125.70	27	4.656		
Total	247.39	29	-	-	-

Paired t-test was performed to test hypothesis on the effectiveness of CRAC. Table 2 shows mean ± SD of pre-test AKET measurement was 135.217°±4.4924° and post-test AKET improved to 155.250°±4.9806°. Referring Table 2, the mean difference was - 20.033°±2.666° between pre-test and post-test. Result shows t(9)= -23.753, p<.001 indicating CRAC has statistically significant effect in improving hamstring flexibility. Therefore, null hypothesis was rejected.

To test the hypothesis of whether there is significant difference between effect of these techniques, One-way ANOVA was performed. Mean differences of AKET measurements of HR, AC and CRAC groups were compared and analyzed. Result shows statistically significant difference between effectiveness of these techniques with F (2,27) = 13.069, p<.001, rejecting null hypothesis and accepting alternate hypothesis (Table3)

To identify the significant group, multiple comparisons were done with Tukey Post-Hoc test. Results revealed improvement was statistically significantly greater in CRAC (- 20.033°±2.666°, p<.001) and AC groups (-17.516°±1.658°, p=.047) compared to HR

(-15.100°±2.025°) as presented in Table 4. Additionally, there is statistically significant difference

(p=.038) between effect of CRAC and AC, suggesting CRAC to be most effective.

The result of this study showed all three techniques had significant effect (p<.001) compared between mean of pre-test and post-test AKET measurement. One-Way ANOVA result showed significant difference (p<.001) between effect of these techniques. Tukey Post- Hoc Test revealed that CRAC (-20.033°±2.666°, p<.001) had most significant effect followed by AC (-17.516°±1.658°, p=.047), then HR (-15.100°±2.025°)

DISCUSSION

The study was conducted to determine and compare effectiveness of HR, AC and CRAC PNF stretching techniques on hamstring flexibility among university students with extended sitting posture. Result of current study showed all three techniques had significant effect (p<.001) compared between mean of pre-test and post-test AKET measurement. This study found significant effect of HR (p<.001), consistent with previous studies (24) found HR greatly improved hamstring flexibility of inactive female students with 20-session stretching with assessments done using AKET. Result of current study is also in accordance with study done by Rani and Mohanty (15) who found significant effect of HR on hamstring flexibility among asymptomat-

Table 4. Statistical analysis using Post-Hoc Test for Between Groups Multiple Comparison

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95%CI	
					Lower Bound	Upper Bound
HR	AC	-2.41*	.96	.047	-4.80	.023
	CRAC	-4.93*	.96	.000	-7.32	-2.54
AC	HR	2.41*	.96	.047	.023	4.80
	CRAC	-2.56*	.96	.038	-4.90	-.12
CRAC	HR	4.93*	.96	.000	2.54	7.32
	AC	2.51*	.96	.038	.12	4.90

HR- Hold Relax, AC- Agonist contraction, CRAC- Contract relax – Antagonist contract

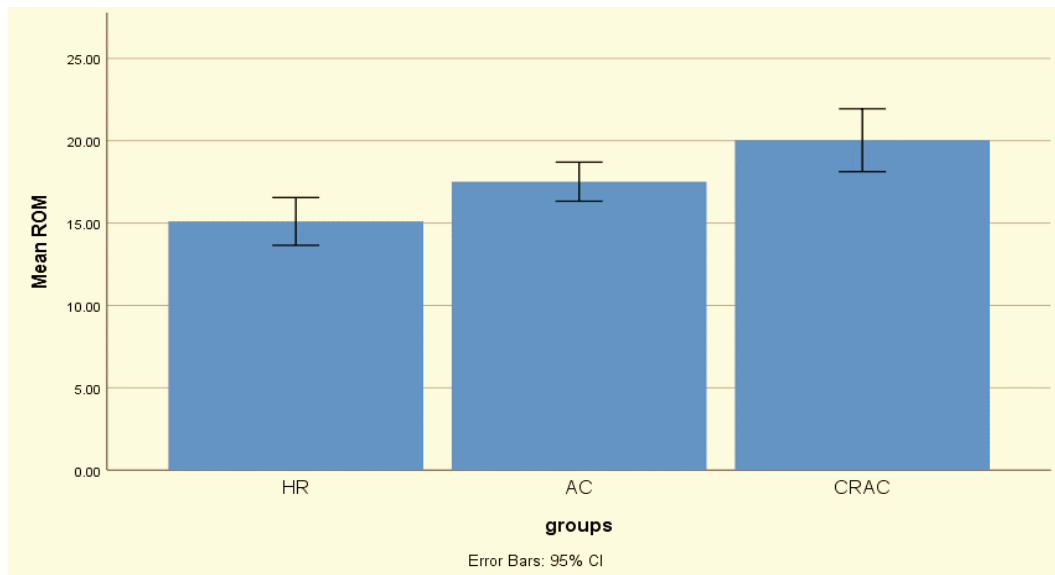


Figure 1. A Graph Representing Mean Differences of AKET of HR, AC And CRAC Groups.

ic subjects aged 20-30 in four-week intervention with $11.08^{\circ} \pm 3.08^{\circ}$ improvement as measured with AKET. Rajendran et al. (9) also found that HR significantly improved hamstring flexibility among undergraduates with 16.46° knee ROM improvement after one-session stretching but the effect was not long-lasting. Current study provided nine stretching sessions to all participants so that accumulated effect can be more obvious to be compared and perhaps the effect can last longer to be more beneficial clinically. (25,18). Such significant effect can be attributed to autogenic inhibition mechanism as TM contracted, Ib-afferent fibers in GTO activated, which it sends signals that activate inhibitory interneurons and finally the inhibitory stimulus causes TM relaxation, decreasing resistance towards stretch. (22).

Present study also showed significant effect of AC ($p < .001$) on hamstring flexibility, consistent with study done by Naga (18) who found that AC effectively improved hamstring flexibility in subjects with hamstring tightness after one-session of AC. Result of current study also demonstrated significantly greater effect of AC ($p = .047$) than HR, consistent with study done by Ferber et al. (26) who found that AC was more effective than HR and SS in improving hamstring flexibility among active elderlies as the knee ROM improvement was 29%-34% greater. Regima et al. (21) also found that AC was significantly more effective than HR on

hamstring flexibility among undergraduates. They suggested that reciprocal inhibition in AC produces greater hamstring flexibility improvement. It occurs as OM contracts which is produced by descending input and this input and Ia-afferent fiber interacted with TM Ia- inhibitory interneurons, causes TM relaxation. Moreover, OM concentric contraction moving knee joint towards maximal end range allows TM to lengthen even more as stretching force causes more neural inhibition. Also, this active stretching was suggested as another reason for AC to be more effective.

Furthermore, this study also demonstrated significant effect of CRAC ($p < .001$), in line with study done by Mani et al. (27) who found that CRAC significantly improved hamstring flexibility among male subjects with hamstring tightness after eight-week intervention with assessments done using AKET. Besides, current study found CRAC had significantly greatest effect, consistent with study conducted by Ramachandran et al. (16) and Nagarwal et al (28) who found that CRAC had significantly greater effect than HR in improving hamstring flexibility of university students after three-week stretching. Sundaram and Arun (29) also found that CRAC had most marked effect compared to HR and AC on athletes' hamstring flexibility with $25.9^{\circ} \pm 1.422^{\circ}$ knee ROM improvement although they only looked for the immediate effect. As current study applied consistent parameter for all techniques except CRAC

involved both TM and OM contraction. Thus, the superiority could be due to both neurophysiological mechanisms involvement causing more neural inhibition and muscle relaxation than AC and HR. This is supported by Etnyre and Abraham (30) who found that HR and CRAC did suppress motor pool excitability based on Hoffman Reflex responses although excitability increased after few seconds. They found greater motor pool excitability suppression and longer lasting inhibitory effect in CRAC than HR due to the addition of reciprocal inhibition. As suggested, stretching must be done immediately after contraction when muscle relaxes (31). This were done during this study and found that CRAC had greatest effect.

Effectiveness of PNF stretching is attributed to combination of several factors (32). Beside neurophysiological mechanisms, altered stretch perception could contribute to its effectiveness. Azevedo et al (33) stated that stretching itself can alter stretch perception, the addition of contraction further alters the stretch perception. Mitchell et al. (34) found stretch tolerance in subjects who received PNF stretching was greater than those receiving SS. They supported that the contraction in PNF causes stretch perception alteration. Furthermore, PNF techniques involve stretching following contraction. As musculotendinous units have viscoelastic properties, stress relaxation will occur which is the resistance of viscous material towards stress decreased during stretching. This property causes ability to endure stretching lost over time and musculotendinous units lengthen slowly. (35)

Result of present study is inconsistent with some studies. Dafda (20) found that HR was more effective than AC as assessed with AKET. However, such difference could be due to the test procedure as subjects' pelvic and contralateral leg were not stabilized and subjects' hip flexion at 90° were manually held instead of utilizing stabilizing tool. Thus, some alteration of hip and pelvic position can be suspected. Davis et al. (36) suggested that neural tension, pelvic position and stability would affect result of an outcome measure. Oh et al. (37) found no significant difference between effect of PNF stretching techniques on university students' hamstring flexibility when assessed with SLRT. There may be more pelvic rotation in SLRT especially in

persons with hamstring tightness (38). Moreover, contralateral hip flexor flexibility also affects outcome of SLRT (39). Another possibility that affects accuracy of measurement is subjects' inability to keep knee fully extended during SLRT. AKET applied in current study can overcome limitations of SLRT like pelvic movement and neurological involvements (40). Thus, such different finding of current study could be explained with the test procedure and outcome measure used.

CONCLUSION

Hamstring tightness is a common problem among university students because of extended sitting habits. It causes multiple musculoskeletal problems but is preventable with effective hamstring stretching. PNF stretching is superior to other techniques. This study compared the effectiveness of HR, AC, and CRAC PNF stretching techniques on hamstring flexibility among university students with extended sitting postures. Thirty subjects were allocated into three experimental groups. Before and after the three-week stretching, their hamstring flexibility was assessed with AKET. Based on the results, it can be concluded that all techniques are effective with CRAC to be most effective followed by AC, then HR. Thus, CRAC can bring the greatest improvement to university students' hamstring flexibility. Furthermore, the perception of pain and flexibility is also influenced by the different race of the participants (41).

This study contributes more shreds of evidence and clear confusion on which PNF technique is more effective. As this study found all techniques are effective with CRAC yielded the greatest effect, it can be applied by clinicians to effectively improve hamstring flexibility among this population who are prone to hamstring tightness. These techniques are also advisable to clinicians when treating patients with neurological disorders as these PNF techniques may help normalize tone better. The parameter applied in the current study was six seconds submaximal contraction followed by a 30-second stretch for three repetitions and found all techniques significantly improved hamstring flexibility. Thus, this parameter can serve as a reference for clinicians when applying PNF stretching. However, this study did not manage to determine

the chronic effect after cessation of PNF stretching. Furthermore, subjects were not provided with special instruction on their activity level as they were not able to be controlled during the current pandemic.

A larger sample size is recommended to ensure the representativeness of the population and for more reliable findings. Future studies should determine and compare the sustainability of the accumulated effect of these techniques. Lastly, for more accurate ROM measurement, an electro-goniometer which is more reliable is recommended for future studies.

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Author Contribution: Mr. Rahul Krishnan: Concept, Design & Supervision Ms. Ong: Concept, Design, Data Collection, analysis. Mr. Suvinlal Stalin: Critical review & writing the manuscript Mrs. Sonia Dua Dewan: Critical review & writing the manuscript Explanations: Mr. Rahul Krishnan: organizing the conduct of the research, checking on the progress Ms. Ong: Formatting the research hypothesis, evaluating & finalizing the findings. Mr. Suvinlal Stalin: reporting of the study, re-evaluating the study in a scientific sense Mrs. Sonia Dua Dewan: Critical review & writing the manuscript. Mdm. Nisha Vijayan Achary: Writing & revision of the manuscript

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