

The effect of adding Proprioceptive Neuromuscular Facilitation (PNF) training on quadriceps and hamstrings muscle strength in cerebral palsy athletes

Efecto del entrenamiento con Facilitación Neuromuscular Propioceptiva (FNP) sobre la fuerza muscular de cuádriceps e isquiotibiales en atletas *con parálisis cerebral*

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Abstract

Introduction: Athletes with CP were more prone to limb injuries. Stretching had long been considered beneficial for improving muscle performance and reducing the risk of injury. Proprioceptive Neuromuscular Facilitation was a technique used to enhance muscle elasticity and increase peak torque and muscle strength.

Objective: To analyze the effect of adding Proprioceptive Neuromuscular Facilitation exercises on quadriceps and hamstring muscle strength in cerebral palsy athletes.

Methodology: The study was conducted at the NPC Indonesia Training Center. The subjects consisted of 24 athletes diagnosed with cerebral palsy, aged 18-40 years, who underwent intervention three times per week for four weeks on both limbs. The subjects were divided into two groups: 12 individuals in the treatment group received a resistance training program with additional Proprioceptive Neuromuscular Facilitation exercises, and 12 in the control group underwent resistance training only.

Results: There was a significant increase in muscle strength in the treatment group for the dominant quadriceps (p=0.043), non-dominant quadriceps (p=0.001), dominant hamstring (p=0.002), and non-dominant hamstring (p=0.008). In the control group, significant increases were observed in the dominant hamstring (p=0.024) and non-dominant hamstring (p=0.013), but not in the dominant quadriceps (p=0.530) or non-dominant quadriceps (p=0.420). Compared to the control group, a significant difference was found in non-dominant quadriceps strength (p=0.020), but no differences were observed in dominant quadriceps strength (p=0.396) or hamstring strength (p=0.271, p=0.204). Conclusions: The addition of Proprioceptive Neuromuscular Facilitation exercises significantly increased quadriceps and hamstring strength in cerebral palsy athletes compared to those without it.

Keywords

Cerebral palsy athletes; hamstrings; muscle strength; proprioceptive neuromuscular facilitation; quadriceps.

Resumen

Introducción: Los atletas con parálisis cerebral son más propensos a las lesiones. Los estiramientos se utilizan desde hace mucho tiempo para mejorar el rendimiento muscular y prevenir lesiones. La facilitación neuromuscular propioceptiva (FNP) se utiliza para mejorar la elasticidad muscular, el par máximo y la fuerza.

Objetivo: Analizar el efecto de agregar entrenamiento PNF sobre la fuerza de los músculos cuádriceps e isquiotibiales en atletas con parálisis cerebral.

Metodología: En el estudio, realizado en el Centro de Entrenamiento NPC Indonesia, participaron 24 atletas con Parálisis Cerebral de entre 18 y 40 años. La intervención se aplicó tres veces por semana durante cuatro semanas en ambas extremidades. Doce atletas pertenecían al grupo de tratamiento que realizó entrenamiento de resistencia con FNP, y 12 atletas al grupo de control que sólo realizó entrenamiento de resistencia.

Resultados: En el grupo de tratamiento hubo un aumento en la fuerza de los músculos cuádriceps dominante (p=0,043) y no dominante (p=0,001), isquiotibiales dominante (p=0,002) y no dominante (p=0,008). En el grupo control, se observaron mejoras en los isquiotibiales dominantes (p=0,024) y no dominantes (p=0,013), pero no en los cuádriceps dominantes y no dominantes (p=0,530 y p=0,420). Entre grupos se encontraron diferencias significativas en la fuerza de los músculos cuádriceps no dominantes (p=0,020), pero no en los músculos cuádriceps dominantes (p=0,271, p=0,204). Conclusiones: El entrenamiento PNF mejoró significativamente la fuerza de cuádriceps e isquiotibiales en atletas con Parálisis Cerebral en comparación con aquellos sin parálisis cerebral.

Palabras clave

Atletas con parálisis cerebral; isquiotibiales; fuerza muscular; facilitación neuromuscular propioceptiva; cuádriceps.





Introduction

The incidence of cerebral palsy (CP) is 2.5 per 1000 live births and is increasing (Gunawan et al., 2023). Sports participation for individuals with CP progresses in rehabilitation to professional sports, including the Paralympics. The development of CP athletes is similar to that of non-disabled athletes, but more specialized attention is required due to limitations that affect preparation, participation, and increase the risk of injury, such as musculoskeletal injuries (Runciman & Derman, 2018).

Athletes with cerebral palsy (CP) are at higher risk of overuse syndrome, muscle strain, chronic knee pain, patellofemoral problems, and chondromalacia, with injury patterns similar to non-disabled athletes (Pinheiro et al., 2021). CP athletes are prone to leg injuries due to spasticity which causes muscle imbalance, increasing muscle tone but limiting joint range of motion (LGS) (Choi et al., 2014). Weakness in eccentric hamstring strength can also increase the risk of injury due to overuse in athletes (Isna et al., 2024). Stretching has long been seen as beneficial for improving muscle performance and reducing the risk of injury during exercise, as well as improving ROM and function after injury (McCarthy et al., 1997). Dynamic stretching is a kind of warming up to optimize performance and reduce the risk of injury (Salsabila Zahroh et al., 2024). Static stretching is effective in improving explosive power, particularly in movements that utilize the elastic bonds of the muscle to store and release energy in the form of velocity during a rapid stretch-shortening cycle (Jean-Berchmans et al., 2024).Many athletes stretch before training either statically or dynamically for injury prevention, decreased muscle soreness and improved performance (Hough et al., 2009; Pearce et al., 2009).

Static and dynamic stretching methods have evolved considerably, and many sports experts now recommend the use of Proprioceptive Neuromuscular Facilitation (PNF) techniques. PNF techniques are effective for improving muscle elasticity, active (AROM) and passive (PROM) range of motion of joints, and increasing peak torque and muscle strength. Clinical evidence suggests PNF is beneficial in restoring ROM, increasing strength, and reducing spasticity in cerebral palsy patients. Several studies mentioned that PNF was also shown to increase ROM in both trained and untrained individuals and showed similar effectiveness to weight training in increasing muscle strength (Hindle et al., 2012; Nelson et al., 2005). PNF stretching before exercise can decrease the maximal effort of muscle contraction required such as during sprinting, jumping, plyometrics, weightlifting and other high-intensity exercises (Bradley et al., 2007; Hindle et al., 2012; Mikolajec et al., 2012). This can occur due to stretching will improve the proper balance between the agonist-antagonist muscles of the thigh which is important for joint stabilization during dynamic movements to reduce the incidence of injury (Gerdijan et al., 2021). PNF stretching is also thought to have an effect in reducing muscle spasticity in cerebral palsy athletes, thus reducing the risk of injury during competition and can improve muscle strength and performance during competition (Sharma, 2021). Combination resistance training with agonist-antagonist paired set method can be an alternative to increase muscle mass within a relatively short training time (Pringga et al., 2021).

Further research on PNF exercises in athletes with cerebral palsy has never been done, therefore the authors are interested in conducting research on the effects of adding Proprioceptive Neuromuscular Facilitation (PNF) exercises on quadricep and hamstrings muscle strength in cerebral palsy athletes.

Method

Study design

This was a clinical experimental study with a randomized pre-test and post-test group experimental design. The sampling technique used total sampling, with a total of 24 research subjects divided into two groups: the treatment group (P), which was given static and dynamic stretching exercises with Proprioceptive Neuromuscular Facilitation (PNF) exercises, and the control group (K), which only received static and dynamic stretching exercises. Both groups received regular training of 8 exercises with a frequency of 3 times a week for a total of 4 weeks of training.





Participants

The study population was all athletes with cerebral palsy who were at the Training Center of the National Paralympic Committee Indonesia (NPCI) Surakarta City, Indonesia, who agreed to participate in the study and met the inclusion and exclusion criteria. Inclusion criteria included (1) athletes diagnosed with Cerebral Palsy class CP5, CP6, CP7, and CP8 according to the Cerebral Palsy-International Sports and Recreation Association (CP-ISRA) classification aged 18-40 years, (2) BMI 18.5-24.9 kg/m², (3) no alterations in cognitive function, and (4) could do stretching exercises with the PNF method for 15-30 minutes. Exclusion criteria included (1) musculoskeletal injury in the past 1 month, (2) CP class CP4 or more severe based on the Cerebral Palsy-International Sports and Recreation (3) pregnancy, (4) having pain in the lower limbs with WBFS > 4, and (5) had done PNF training before. Of the 36 total athletes at NPCI, there were 24 athletes who met the criteria, and they were randomly assigned to groups using a computer-generated randomization process to be divided into 2 research groups, namely the treatment group (P) and the control group (K). Each subject's group assignment was determined automatically by the software, ensuring equal opportunity for placement in any group.

Experimental design/exercise

Cerebral Palsy (CP) athletes aged 18-40 years at Pelatnas NPC Indonesia were given verbal and written explanations about the study procedures and asked to sign a consent or refusal form. Subject data (name, age), subjective, physical examination, and other data for inclusion-exclusion were recorded. Anthropometric measurements were taken to determine body mass index. CP classification was determined based on CP-ISRA. PNF stretching exercises were performed 3 times per week for 4 weeks. Quadriceps and hamstring muscle strength evaluation was done before and after 12 PNF exercise sessions for 4 weeks using the DIERS Myoline machine, followed by data processing. All training processes and evaluation of research results were carried out at the training center of the National Paralympic Committee Indonesia (NPCI) Surakarta City in May - June 2023.

Exercise protocol

The control group received an exercise program that included three types of exercises. First, static and dynamic stretching was performed three times a week for 15 minutes to improve muscle flexibility. Second, strength exercises such as squats, leg extensions, leg curls, and calf exercises were performed twice a week at an intensity of 60-80% of 1 repetition maximum (1RM). Each session consisted of 4-6 sets of 8-12 reps. Third, circuit training involving movements such as step-ups, vertical jumps, kangaroo jumps, hurdle jumps, running back and forth, and burpees was performed once a week, with 4-6 sets in each session. The intervention group received the same treatment as the control group, resistive strengthening exercises 2 times per week. The intervention group also received an additional exercise program with the PNF method. This movement was done alternately for both legs, each leg for 4 sets of PNF with a total time of 30 minutes, done three times a week. Figure 1 illustrates the PNF exercise protocol.

Figure 1. Proprioceptive Neuromuscular Facilitation (PNF) protocol. (A) Stretching the hamstring, (B) hamstring isometric contraction 20-70% MVC (C) quadriceps concentric contraction 20-70% MVC







Ethical considerations

Ethical approval was obtained from the Health Research Ethics Committee of RSOP Prof. Soeharso Surakarta with Number: IR.03.01/D.XXV.3/3997/2023. Each subject had signed a written informed consent after receiving verbal and written explanations regarding the purpose, objectives, procedures, and benefits of the study.

Outcome assessment

Outcome measurements were taken at the training center of the National Paralympic Committee Indonesia (NPCI) Surakarta City one day after the last intervention was given to prevent fatigue during assessment. Muscle strength measurement was assessed using the DIERS Myoline machine. This tool has been widely used to assess muscle strength objectively. Muscle strength measurement is done by the subject sitting upright and positioning the feet properly on the DIERS Myoline machine, then pushing both legs forward to assess the strength of the quadriceps muscles. Hamstring muscle assessment is done in the same way, but both legs are pushed backwards.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, version 27.0 (IBM Corp., Aronk, N.Y., USA). Descriptive analysis for the characteristics of the research subjects was performed to retrieve mean \pm standard deviation (SD) or frequency (percentage). In addition, bivariate analysis was performed using paired t-test or Wilcoxon Signed Rank test to compare muscle strength before and after PNF training for 4 weeks in each group (P and K), and unpaired t-test or Mann Whitney test to compare muscle strength between groups P and K. The p-value was considered significant if p < 0.05.

Results

Characteristic of research subjects

This study was an experimental study with a randomized controlled trial design and pre-post test conducted at the NPC Indonesia Training Center in Solo from April to June 2023. A total of 24 subjects were divided into two groups: 12 subjects in the treatment group who received routine stretching and strengthening exercises plus PNF method three times a week for 4 weeks, and 12 subjects in the control group who only underwent routine stretching and strengthening exercises with the same frequency. Prior to statistical analysis, the normality test showed normal data distribution, and the homogeneity test showed that the baseline characteristics between the two groups were not significantly different.

Table 1 shows that each group consisted of 12 subjects, with 11 males and 1 female per group. Five subjects (41.67%) were right leg dominant and seven subjects (58.33) were left leg dominant. All subjects met the CP ISRA criteria of 5-8. The mean age of the treatment group was 27.41 ± 4.37 years, body weight was 60.16 ± 9.63 kg, height was 1.68 ± 6.84 cm, and body mass index was 21.03 ± 2.07 kg/m². The mean age of the control group was 27.50 ± 5.91 years, weight 60.33 ± 7.01 kg, height 1.65 ± 6.24 cm, and body mass index 21.83 ± 2.65 kg/m². All data were normally distributed and there were no significant differences between the two groups.

		Interventions	Control		
Variable		(n = 12)	(n = 12)	p-value	
		Means ± SD	Means ± SD	-	
Age (years)		27.41 ± 4.37	27.50 ± 5.91	0.969	
Weight (kg)		60.16 ± 9.63	60.33 ± 7.01	0.962	
Height (m)		1.68 ± 6.84	1.65 ± 6.24	0.204	
BMI (kg/m2)		21.03 ± 2.07	21.83 ± 2.65	0.318	
Dominant foot	Right	5 (41.67%)	5 (41.67%)	NS	
	Left	7 (58.33%)	7 (58.33%)	NS	
Quad pre (N)	Dom	205.91 ± 46.51	200.50 ± 35.91	0.752	
	NDom	132.25 ± 45.99	134.75 ± 46.17	0.895	
	Dom	75.83 ± 19.59	73.66 ± 17.89	0.780	
Hams pre (N)	NDom	49.91 ± 15.00	48.91 ± 14.61	0.870	

Table 1. Basic characteristics of the study subjects

Note: figures for age, BW, TB, BMI, and muscle strength difference are mean ± deviation, p-value (normality) < 0.05 indicates abnormal, p-value (homogeneity) < 0.05 indicates abnormal.





Muscle strength values before intervention in the treatment and control groups are shown in table 1. The Saphiro Wilk normality test (table 1) showed that the data were normally distributed, so the parametric independent sample t-test was used. Table 1 shows there was no significant difference in muscle strength before the intervention, indicating the jumping ability of both groups was similar.

Comparison of muscle strength before and after intervention

In the treatment group, Table 2 shows that after the addition of PNF exercises for 4 weeks, there was an increase in the strength of both muscles (quadriceps and hamstring) on both the dominant and nondominant side of the leg. The paired t-test showed significant improvements in dominant quadriceps (p = 0.043), non-dominant quadriceps (p = 0.001), dominant hamstrings (p = 0.002), and non-dominant hamstrings (p = 0.008). Large effect size was obtained in non-dominant quadriceps (d = 1.353), dominant hamstrings (d = 1.141), and non-dominant hamstrings (d = 0.941), while medium effect size was obtained in dominant quadriceps (d = 0.659).

In the control group, Table 2 shows an increase in muscle strength (quadriceps and hamstrings) on both the dominant and non-dominant side of the leg. The paired t-test showed significant improvement in dominant hamstrings (p = 0.024) and non-dominant hamstrings (p = 0.013), but not significant in dominant quadriceps (p = 0.530) and non-dominant quadriceps (p = 0.420). Large effect size was found in non-dominant hamstrings (d = 0.857) and medium effect size in dominant hamstrings (d = 0.756), while small effect size in dominant (d = 0.187) and non-dominant quadriceps (d = 0.242).

Group	Muscles		Pre-training (N)	Post-training (N)	p-value	Cohen's d
Interventions	Quadriceps	Dom	205.91 ± 46.51	236.00 ± 54.99	0.043	0.659
	Quadriceps	NDom	132.25 ± 45.99	187.08 ± 45.29	0.001	1.353
	II	Dom	75.83 ± 19.59	92.58 ± 22.87	0.002	1.141
	Hamstrings	NDom	49.91 ± 15.00	65.33 ± 19.19	0.008	0.941
Control —	Owederland	Dom	200.50 ± 35.91	213.16 ± 73.06	0.530	0.187
	Quadriceps	NDom	134.75 ± 46.17	145.91 ± 34.23	0.420	0.242
	II and a tailor and	Dom	73.66 ± 17.89	82.83 ± 19.29	0.024	0.756
	Hamstrings	NDom	48.91 ± 14.61	55.50 ± 17.52	0.013	0.857

Description: Numbers are mean standard deviation (SD).

*P value is significant (p<0.05).

Comparison of muscle strength between groups

Table 3 shows there was no significant difference after the intervention, except for the non-dominant quadriceps muscle (p = 0.020).

Table 3. Comparison of Muscle Strength in Treatment and Control After Intervention
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Muscles		Interventions (N)	Control (N)	p-value	Cohen's d
Quadriaana	Dominant	236.00 ± 54.99	213.16 ± 73.06	0.396	0.353
Quadriceps No	Non-Dominant	187.08 ± 45.29	145.91 ± 34.23	0.020	1.025
Hamstrings	Dominant	92.58 ± 22.87	82.83 ± 19.29	0.271	0.461
	Non-Dominant	65.33 ± 19.19	55.50 ± 17.52	0.204	0.535

Description: Numbers are mean standard deviation (SD). *P value is significant (p<0.05).

Figure 2 (A-B) shows an increase in dominant quadriceps muscle strength in the treatment group from 205.91 ± 46.51 to 236.00 ± 54.99, and non-dominant from 132.25 ± 45.99 to 187.08 ± 45.29. The control group increased from 200.50 \pm 35.91 to 213.16 \pm 73.06 (dominant) and 134.75 \pm 46.17 to 145.91 \pm 34.23 (non-dominant). Figure 2 (C-D) shows an increase in dominant hamstrings muscle in the treatment group from 75.83 ± 19.59 to 92.58 ± 22.87 , and non-dominant from 49.91 ± 15.00 to 65.33 ± 10.59 to 92.58 ± 22.87 , and non-dominant from 49.91 ± 15.00 to 65.33 ± 10.59 to 92.58 ± 22.87 , and non-dominant from 49.91 ± 15.00 to 65.33 ± 10.59 to 92.58 ± 22.87 , and non-dominant from 49.91 ± 15.00 to 65.33 ± 10.59 to 92.58 ± 22.87 , and non-dominant from 49.91 ± 15.00 to 65.33 ± 10.59 to 92.58 ± 22.87 , and non-dominant from 49.91 ± 15.00 to 65.33 ± 10.59 to 92.58 ± 10.59 to 92.19.19. The control group increased from 73.66 ± 17.89 to 82.83 ± 19.29 (dominant) and 48.91 ± 14.61 to 55.50 ± 17.52 (non-dominant).





Figure 2. Muscle Comparison Diagram. (A) Quadriceps Dominant, (B) Quadriceps Non-dominant, (C) Hamstring Dominant, (D) Hamstring Non-dominant.

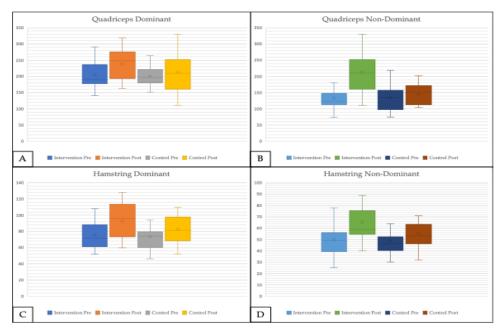


Table 4. shows significant differences (p < 0.05) in quadriceps (p = 0.009) and hamstrings (p = 0.031) on the non-dominant side, while the improvement on the dominant side was not significant (p = 0.110) and (p = 0.136). Large effect size (d > 0.8) was found in quadriceps (d = 1.176) and non-dominant hamstrings (d = 0.940), and medium effect size ($0.5 \le d < 0.8$) in quadriceps (d = 0.679) and dominant hamstrings (d = 0.632) after 4 weeks PNF training.

Table 4. Comparison	of Changes in Muscle Stre	ength in Treatments and Controls

Muscles		Interventions (N)	Control (N)	p-value	Cohen's d
A Que duierre	Dominan	34.25 ± 26.63	13.50 ± 34.02	0.110	0.679
Δ Quadriceps	NonDominan	50.67 ± 36.85	11.16 ± 29.95	0.009	1.176
Δ Hamstrings	Dominan	16.75 ± 14.67	9.50 ± 6.88	0.136	0.632
Δ Hallisti liigs	NonDominan	15.41 ± 10.84	6.58 ± 7.68	0.031	0.940

Keterangan: Angka adalah rerata (mean) standar deviasi (SD)

*Nilai p bermakna (p<0,05)

Discussion

This study was conducted at the National Paralympic Committee (NPC) Indonesia in April-June 2023, involving 24 cerebral palsy athletes aged 18-40 years. They were divided into two groups: 12 athletes in the treatment group who underwent Proprioceptive Neuromuscular Facilitation (PNF) stretching exercises for 4 weeks, and 12 athletes in the control group who performed conventional stretching. Each group consisted of 11 males and 1 female, with gender homogenization not possible due to the limited number of subjects. Several studies have reported that strength per unit of cross-sectional area or lean body mass, does not differ much between genders the same strength output normalized to lower limb muscle mass in males and females. So in this study measuring lower limb muscle strength, it is assumed that there is no difference between genders (Bartolomei et al., 2021).

Subject characteristics between the two groups (age, weight, height, body mass index) were not significantly different, with a mean age of 27 years, indicating that the average participant is still in the productive age range that allows muscle strengthening training with a relatively smaller risk of injury and has good awareness (Schoenfeld, 2010). The mean BMI is in the normoweight category based on Asia Pacific criteria (WHO, 2000). This indicates no significant difference in the anthropometric profiles of the two groups.

The mean muscle strength of the treatment group was higher than the control, especially in the dominant and non-dominant limb muscles. This is consistent with the study of Latouf et al. (2023) who





found PNF effective in increasing hamstrings muscle strength, and in line with the increase in quadriceps and hamstrings strength in the treatment group in this study.

In this study, measurement of lower limb muscle strength was carried out with the DIERS Myoline tool to measure strength isometrically (Marco, 2019). The results showed a significant increase in muscle strength after a 4-week PNF intervention in quadriceps and hamsting muscles in both dominant and non-dominant legs. Large effect sizes were also found in non-dominant quadriceps, dominant hamstrings, and non-dominant hamstrings, while moderate effect sizes were found in dominant quadriceps. These findings are in line with the study of Latouf et al. (2023), which showed a significant increase in hamstring strength after 8 weeks of PNF training. Other studies also support these findings, showing that PNF is effective in increasing strength and neuromuscular activity in lower limb muscles. Funk et al. (2003) indicated that PNF can increase strength by training the neuromuscular system to coordinate muscle fibers during movement. Nelson et al. (2005) also reported improved muscle strength and athletic performance after 8 weeks of PNF training. Caplan et al. (2009) found an increase in stride speed and stride length after a five-week PNF protocol. In addition, Zaidi et al. (2023) showed increased EMG activity during isometric contractions after four weeks of PNF intervention, and Miyahara et al. (2013) reported increased hamstring strength in young university students. Borges et al. (2018) concluded that PNF is effective in improving hamstring strength and activation in athletes.

PNF exercises cause repetitive pulling and contraction of the muscle, which causes damage to the muscle myofiber. This damage increases proinflammatory factors such as $\text{TNF}\alpha$, $\text{TGF}\beta$, and IL6, which in turn increase satellite cell activity. The increased satellite cells will stimulate HGF and IGF factors, which increase muscle cell proliferation and repair, as well as reduce myostatin and increase TGFb. The result is an increase in muscle strength and viscoelasticity. The decrease in myostatin also prevents the degradation of the sarcomere molecule titin, which is important for muscle strength in individuals with cerebral palsy (Hotta & Muller-Delp, 2022). In addition, increased muscle strength can also occur from resistance training performed 2 times a week. This exercise causes physiological adaptations such as muscle hypertrophy and hyperplasia at moderate to high intensity (Kisner et al., 2017).

In the control group, there was a significant increase in the strength of dominant and non-dominant hamstrings after 4 weeks of conventional stretching exercises, with a large effect size in non-dominant hamstrings and a medium effect size in dominant hamstrings. Although there was no significant improvement in dominant and non-dominant quadriceps, resistance training 2 times a week may have contributed to the increase in muscle strength, in line with previous studies (Dominguez-Navarro et al., 2023; Stricker et al., 2020).

There were no significant differences between the treatment and control groups in muscle strength before and after the intervention, but there were significant differences in muscle strength on the non-dominant side in both the quadriceps and hamstring muscles, with large effect sizes in these two non-dominant muscles. The muscle strength of the treatment group increased more than the control group, possibly due to the PNF technique that combines active contraction with stretching (Arazi et al., 2012; Kisner et al., 2017). This finding supports the results of Arazi et al. (2012) who showed significant improvements in strength, flexibility and muscle volume in the resistance training + PNF group compared to resistance training only

Conclusions

There was an increase in quadriceps and hamstrings muscle strength for both dominant and nondominant muscles in the control and intervention groups. More significant increases in muscle strength occurred in cerebral palsy athletes who received additional training with PNF than those who did not.

Limitation of study

The number of samples in this study is relatively small and comes from the same training center environment so that the results may differ in each place and other broad environments.





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