

# Research Paper: Hip Abductor Muscles Strengthening's Effect on Lower Extremity's Function of Runners With Iliotibial Syndrome



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## ABSTRACT

**Introduction:** Iliotibial Band Syndrome (ITBS) is among the most common injuries in distance runners. Thus, the present study investigated the effect of a special strengthening exercise program for abductor's muscles according to NSCA protocol, on pain reduction and lower extremity function improvement among elite distance runners with ITBS.

**Materials and Methods:** The subjects of the present study were 32 elite distance runners who were randomly divided into two groups of control and experimental (n=16/group). The experimental group performed the exercise program for 8 weeks; however, the control group followed their routine program. The pain was assessed by the Visual Analogue Scale of pain (VAS) and the lower extremity function was assessed by the Lower Extremity Functional Scale (LEFS). In the statistical procedure, the repeated-measures Analysis of Variance (ANOVA) and Bonferroni test was applied to determine the within-group and between-groups differences of the study variables, and for assessing the stability level of protocols' effect on the experimental group and comparing the stages, respectively. The significance level was considered 0.05, test power as 95%, and effect size as 0.7; SPSS was used for all data analyses.

**Results:** The present study data indicated that the strengthening exercise program on hip abductor muscles caused a significant decrease in pain (P=0.0001). The lower extremity function manifested a significant improvement in the experimental group after 8 weeks of strengthening exercise protocol (P=0.0001); it was durable even 3 months after the end of the exercise program.

**Conclusion:** Hip abductor muscle strengthening, based on NSCA protocol, can be an effective approach in reducing pain and improving lower extremity function in elite distance runners with ITBS.

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## Introduction

Iliotibial Band Syndrome (ITBS) is among the most common injuries among runners [1]. ITBS occurs in the outer part of the knee joint, between the joint line and the hip's external epicondyle, with pain or tenderness to palpation [2]. ITBS is non-impact damage, often associated with major weakness of the hip's abductor and external rotator muscles [2, 3]. In a patient with ITBS, the pain initiates when the knee is flexed about 30° or slightly less; it occurs at the beginning of the stance phase of running. During this phase of the running cycle, the eccentric contraction of tensor fascia lata and Gluteus Maximus (GM) muscles cause the legs to be slow and create tension in the iliotibial band [4]. Previous studies reported the prevalence of ITBS to approximately be 62% and 38% in women and men runners, respectively, indicating a high frequency of this injury in runners [1].

Different factors cause ITBS, i.e., referred to as intrinsic and extrinsic in numerous studies. The anatomical risk factors are thought as intrinsic; they include decreased flexibility of the iliotibial band, leg length discrepancy, and increased prominence of the lateral femoral epicondyle. For example, the leg length discrepancy caused some changes in the position of the pelvic and hip that insert more tension to ITB [5-8].

Some kinetic and kinematic characteristics may also cause ITBS in runners [9]. Moreover, because of ITB's source and its insertion, these biomechanical abnormalities are mostly visible in the hip and knee [10, 11]. Thus, in a runner with ITBS, the extents of knee internal rotation and peak hip adduction are increased [12]. The incorrect position of the knee can enhance the ITB's tension and strain, resulting in ITB friction or its compression versus lateral femoral epicondyle [13].

Other biomechanical risk factors can be expressed as the peak knee flexion, rearfoot eversion, and abnormality in the foot and tibia [14]. The abnormality of the foot and tibia may happen because of anatomical connection and the interrelationship of the foot and tibia [15].

The deficits in the strength of Gluteal muscles, especially Gluteus Medius can be also proposed as a marker of ITBS outbreak in runners; it can happen due to the predominant occurrence of running in the sagittal plane [16]. The goal of Gluteus Medius is to improve hip abduction. Researchers supported its presence in runners with ITBS. They reported further extents of tension on

ITB, induced by greater hip abduction; they found that a program for hip abductor strengthening is useful for reducing the symptoms of ITBS [17]. Thus, the weakness or fatigue of GM muscle can be mentioned as the major internal risk factor in ITBS [18]. Since GM muscle causes the external rotation of the hip, its inefficiency may generate the internal rotation of the hip and hardness of ITB on the hip's external condyle; this stiffness increases the band abrasion, resulting in ITBS [18].

The treatment of ITBS can be performed by invasive methods, like surgeries or non-invasive approaches. The surgical treatment of ITBS involves removing or unwrapping the damaged lower part of ITB, releasing the affected part, or extent the bands' length, or removing the underneath bursa [19]. These interventions are used in the resistant cases of ITBS or when non-invasive methods are ineffective; the preferred view of athletes and coaches for managing ITBS is non-surgical methods and their emphasis is on non-invasive manners. Some of the non-invasive methods can be expressed as a combination of rest, exercise patterns correction, pain management, stretching, and the strengthening of muscles and non-contractile tissues. The application of Non-Steroid Anti-Inflammatory Drugs (NSAID), corticosteroids, deep frictional massage, ultrasound, and ice therapy packs can also be pointed as other conservative treatment methods in ITBS. Some studies reported the positive effects of various conservative techniques on reducing ITBS-induced pain; however, the extent of the effect and the exact function of these methods in the recovery of pain and motor function remains unclear [20, 21].

Rest is the first step, i.e., recommended to runners with ITBS for pain recovery. Moreover, it is offered for preventing the recurrence of symptoms by avoiding pain stimulating activities, gradually returning to the usual lifestyle [5]. Some studies considered the modalities of electrotherapy alone or in combination with exercise; however, few of them have used corrective exercises or exercise therapy as a complete method for ITBS management. Furthermore, traditional exercises including ITB stretching are partly effective in curing ITBS [5]. The exercise therapy program in such a study was designed to increase lower extremity balance, stretch in ITB, and gradual returning of the patient to sports activities. The researchers of these studies stated that these exercises were partly useful in curing ITBS. They suggested the evaluation of exercises' effects with an emphasis on hip abductor muscles for treating and preventing ITBS, along with traditional stretching exercises, in future investigations [21].

McKay et al. conducted a study on female runners, in two groups of control and general stretching exercises [5]. Their results revealed no significant difference between the study groups [5]. Fredericson et al. also examined hip muscles strength in distance runners with ITBS. In this survey, the hip abductor strength of the injured knee was compared with the non-injured knee of runners, and the control group. The relevant results indicated that the hip abductor muscles of control subjects and the non-injured knee of runners with ITBS were stronger than those of the injured knee of runners with ITBS [21].

van der Worp et al. studied the biomechanics of the lower extremity and trunk in running activity and the strength of hip abductor muscles in patients with ITBS [22]. They argued that the strength of the abductor's muscles in the lower extremity of the knee with ITBS was less than that in the healthy knee. The difference in the evaluation of strength in 6 groups of hip muscles on the involved leg of runners with ITBS, compared to their non-involved leg, also control subjects of healthy runners demonstrated an imbalance of strength in the hip abductor muscles of the injured knee, compared to the healthy knee of distance runners [23].

Stastny et al. stated that the weakness of hip abductor muscles is a muscle disability that causes movement disorders. According to them, this disability leads to undesirable changes, such as the possibility of injury and pain increase, the kinematic shifts, and the performance decline of athletes, especially in the lower extremity. The researchers also documented that the athletes with hip abductor muscles' deficit encounter further injuries than the athletes with higher abducting strength of the hip joint; therefore, some recommendations were presented to strengthen the hip abductor muscles, i.e., based on the anatomical function of these muscles [24]. However, the specific concepts of exercises on hip abductor muscles during training programs remain unaddressed [25, 26].

In general, most studies of this field examined the effects of non-invasive methods on ITBS. In a few of them, the effect of traditional stretching exercises has also been considered; however, evidence about the application of strengthening exercises on the hip abductors in lower extremity injuries, especially ITBS in men is scarce [5]. Thus, the current study aimed to investigate the effects of strengthening hip abductor muscles, based on National Strength and Conditioning Association (NSCA) protocol on pain and lower extremity function of elite male distance runners with ITBS. Furthermore, the important

case with less attention in previous studies was the lack of evaluation on the durability of applied exercise's effect in Iliotibial complication of elite distance runners. For this reason, the present study discussed the persistence effect of applied exercise therapy protocol on the experimental group 3 months after the end of a treatment program, to understand the durability of its effect.

## Materials and Methods

This was a quasi-experimental research with a pre-test-posttest and a control group design. The statistical population of the study was elite male distance runners in the age range of 20-30 years, who encountered unilateral ITBS for at least 3 months before the data collection, according to specialists' confirmation.

For determining the minimum number of samples in the present study, the G \* POWER sample volume estimation software was used; its test power, effect size, and significance level were considered 0.95, 0.80, and 0.05, respectively. This software determined 13 subjects as the minimum samples size required for each group in the present study. However, according to samples availability, the researcher chose 32 distance runners as the subjects of study, i.e., more than the necessary minimum sample size [27].

These study subjects had a participation background in Iran's track and field national team or one of its authentic teams at different age ranges; they hold valid rankings in national level or international competitions, and; they had an average of 8 years of background for regular training in distance running sport. The researcher recruited the study subjects by confirming the medical background by a physician and all of them participated voluntarily in this study. Furthermore, all experiments on these subjects were performed with their sufficient understanding and providing written informed consent forms.

The runners with a record of disorder in the knee areas, such as patella hip pain, joint inflammation, meniscus injury, or a history of any previous ITBS-related treatment were excluded from the study. The presence of ITBS in the research subjects was confirmed by checking their medical records and physical exam by an orthopedic specialist. In the clinical examination of the subjects, the presence of lateral knee pain was considered as the primary cause of diagnosis; it is a prominent symptom in all injured runners and is exacerbated by running, especially on a sloping surface. The other factors for diagnosis were also the presence of local sensitivity on external epicondyle, the existence

**Table 1.** Outline of strengthening exercise protocol for the experimental group based on NSCA design

Exercise Therapy Protocols		Exercise Dose: Time (sec), Repeat (No.), and Set
First Exercise	Progression Modified side plank	Week 1: 30×2*
		Week 2: 40×* (each side)
	Side plank with clamshell	Week 3: 10 repeat×2 light elastic traband
		Week 4: 15 repeat×3 light elastic traband
	Side plank	Week 4-6: 15 seconds×2* and 20 seconds×3 (each side)
		Week 6-8: 10 repeats×2 and 10 repeats×3
Second Exercise	Side plank with hip abduction	Week 1: 30×2*
		Week 2: 40×* (each side)
	Progression Side-Lying Hip Abduction	Week 1: 10×2
		Week 2: 10×3
	Lateral Monster Walk	Week 3: 15×2 light elastic traband**
		Week 4: 15×3 light elastic traband**
	Monster walking with external shoulder rotation	Week 5: 15×2 light elastic traband**
		Week 6: 15×3 medium elastic traband**
Monster movement along with walking	Week 7: 15×2 light elastic traband**	
	Week 8: 15×3 medium elastic traband**	
Third Exercise	Progression Hip Hikes	Week 1: 10×3***
		Week 2: 20×3***
	Scott motion on one leg	Week 3: 10×2***
		Week 4: 12×3***
	Abduction of hip and end of the knee with elastic band	Week 5: 12×2 light elastic traband**
		Week 6: 12×3 light elastic traband**
Fourth Exercise	Skater-running man	Week 7: 15×2***
		Week 8: 15×3***
	Plank motion with elastic traband	Week 1: 10×3 medium elastic traband
		Week 2: 15×3 medium elastic traband**
	Pelvic lift at full angle	Week 3: 10×2***
		Week 4: 12×3***
	Plank motion with one bent leg	Week 5: 10×2***
		Week 6: 12×2***
Raising both hips with the waist in lying down position	Week 7: 12×2	
	Week 8: 15×3	

\*Each side, \*\*Each direction, \*\*\*Each leg

of repeatable pain with Noble's compression test and the occurrence of pain during bending, and stretching of the knee while applying pressure on the hip's external condyle with maximum pain at almost 30° of knee flexion. After clinical evaluation and diagnostic confirmation, the study samples were randomly divided into two groups of control and experimental (n=16/group). The control group received no regular sports and therapeutic exercises and they had daily activities. In return, the subjects of the experimental group went under an eight-week of exercise therapy program, three times per week and with the researcher's supervision.

The exercise therapy protocol of this study was designed by modeling the proposed protocol of NSCA [5]. The training protocol was conducted in 8 weeks, according to the principle of overload and gradual progress, in the experimental group. This protocol had the necessary complexity and predicted progress during the period. Moreover, it was performed by considering the improvement to multi-joint motions, in lying down on the side, sitting, and standing positions; while it was shifting from symmetrical and bilateral exercises to asymmetric and unilateral exercises and adding some upper extremity movements, balance exercises and finally incorporating with functional exercises. It was performed based on the progress level and individual characteristics of the study subjects (Table 1).

The pain in the Iliotibial area of the present study subjects was assessed by the Visual Analogue Scale (VAS), a day before the first training session and a day after its last session. This scale includes a horizontal line, i.e.,

scored from 0 to 10; number 0 indicates the absolute pain lack and number 10 reflects unbearable pain [28].

To evaluate the lower extremity function of the study subjects, the Lower Extremity Functional Scale (LEFS) was also used a day before starting the exercise program and a day after the program's completion. The LEFS is a valid result-oriented scale of an individual's performance, i.e., used to measure the performance and injuries of the lower extremity. This scale was first used by Binkley et al. in patients with lower extremity musculoskeletal disorders [29-32]. The LEFS contains 20 questions, with 5 choices for each question, i.e., graded as 0-4; the respondent must choose an option for each question which indicates the lower extremity's physical. In this test, 0 indicates severe difficulty in performing the activity or lack of activity performance and 4 reflects no restriction or difficulty in the lower extremity for performing the intended activity. The lower score of a subject in this test addresses the poorer function of the lower extremity. Notably, the internal reliability for LEFS was measured to be excellent ( $\alpha=0.96$ ); its test-retest reliability was evaluated as 95%; therefore, the LEFS is a valid tool, in comparison to the 36-Item Short Form Health Survey (SF-36). This questionnaire has been used in numerous studies [5, 29-32].

In this study, the Shapiro-Wilk test was used to check the assumption of data normality. According to the normality of collected data, i.e., established by this test, the parametric method of repeated-measures Analysis of Variance (ANOVA) was applied for defining the within-group and between-groups differences of two variables

Table 2. Descriptive information of the study subjects

Variables	Mean±SD	
	Control Group	Experimental Group
Age (y)	24.33±3.9	24.5±4.8
Weight (Kg)	62.9±5.8	63.6±9.2
Height (cm)	171.0±5.2	173.0±6.4
BMI (kg/cm <sup>2</sup> )	21.4±5.6	21.3±5.2
Sport's background (y)	8.2±2.1	8.33±3.4
Pain history (mon)	4.21±1.4	4.61±1.2
Training sessions per week	7.46±5.3	7.11±6.6
Training duration of each session (min)	132.77±40.35	126.56±35.87



**Table 3.** Mauchly's test of Sphericity data

Tests	Mauchly's W	Degree of Freedom	P
Pain	0.021	2	0.0001
LEFS	0.217	2	0.0001

LEFS: Lower Extremity Functional Scale.

**Table 4.** Repeated-measures ANOVA data in the study groups on 3 stages of study

Variables	Groups	Mean±SD			ANOVA (Group×Time), P	ANOVA (Time), P
		Pretest	Posttest	3 Months Later		
Pain	Experimental	4.5013±1.35007	1.619±0.98415	1.6025±0.9085	F=15.263 P=0.0001	F=30.506 P=0.0001
	Control	4.7425±1.83074	4.2325±1.79706	4.2456±1.801		
LEFS	Experimental	44.8125±3.83351	70.875±3.44238	70.50±3.2249	F=247.349 P=0.0001	F=291.88 P=0.0001
	Control	44.750±1.73205	46.00±2.3094	45.8125±2.5356		

LEFS: Lower Extremity Functional Scale.

**Table 5.** Two-by-two comparison of the study variables in 3 stages with Bonferroni test

Variables	Test Stage	Pretest	Posttest	3 Months Later
Pain	Pretest	-	0.0001*	0.0001*
	Posttest	0.0001*	-	0.342
	3 months Later	0.0001*	0.948	-
LEFS	Pretest	-	0.0001*	0.0001*
	Posttest	0.0001*	-	0.103
	3 months Later	0.0001*	0.210	-

LEFS: Lower Extremity Functional Scale; \* Significant at P<0.001

(pain, LEFS) in 3 stages (pretest, posttest, & 3 months after the protocol completion). Besides, the Bonferroni test was used to determine the stability level of protocols' effect and compare the mean of stages in the experimental group. In this research, the significance level was considered at 0.05, test power at 95%, and effect size at 0.7, and SPSS was applied for all data analyses.

### Results

In Table 2, the study subjects' demographic information, including age, height, weight, and Body Mass Index (BMI) are presented as well as their previous information on the duration of pain existence, exercise background, the number of training sessions per week and the duration of training per session.

Mauchly's Test of Sphericity indicated that the assumption of sphericity in both variables had not been violated (P<0.05) (Table 3). The results of repeated-measures ANOVA presented in Table 4 signify a significant interaction between group and time for pain (P=0.0001,  $\eta^2=0.337$ ); the same significant interaction was detected between group and time in LEFS by repeated-measures ANOVA (P=0.0001,  $\eta^2=0.892$ ).

The two-by-two comparison of variables with Bonferroni post hoc test for pain indicated a significant difference between the values of pretest and posttest and the 3-month follow-up stages in the subjects of the experimental group (P=0.0001). However, there was no such significant difference between the posttest and follow-up steps (P=0.948). This result indicates the durability of

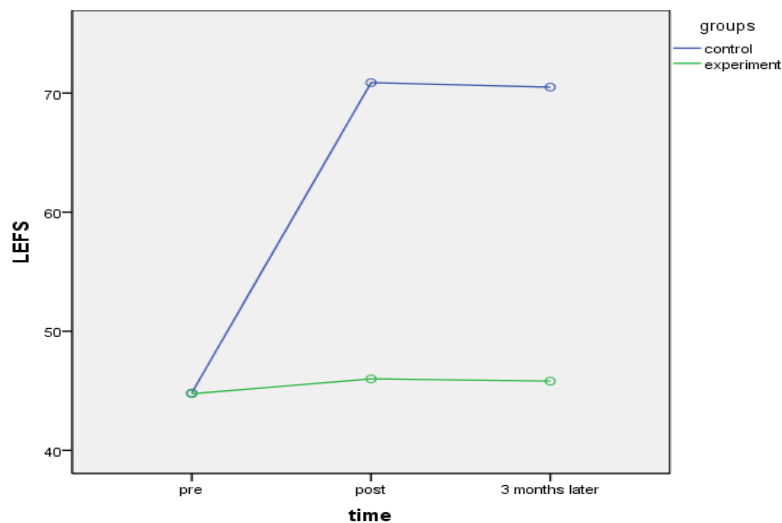


Figure 1. Comparison of pain (VAS) in 3 stages between the study groups

the training protocol effect in the experimental group, 3 months after the completion of the treatment program. The two-by-two variables' comparison of lower extremity function by using Bonferroni post hoc test also suggested no significant difference between the stages of the posttest and follow-up in the subjects of the experimental group ( $P=0.210$ ) (Table 5, Figures 1 & 2).

### Discussion

The present study examined the effects of a strengthening exercise program for hip abductor muscles based on NSCA protocol on the extent of pain and lower extremity function in elite distance runners with ITBS. The obtained results reflected the significant effects of these

exercises on pain reduction and lower extremity function increase among the experimental subjects. We also observed the persistency of used treatment programs' effect 3 months after the study completion. The present study results were consistent with those of Frederickson et al., to some extent. They found that by rehabilitating the runners through traditional stretching exercises in the hip, knee, and pelvis, their pelvic rotational power and hip area increase and get stronger, respectively; this improvement can cause the return of subjects to pain-free running phase. However, the main focus of the present study was on hip abductor muscles strength, through a special strengthening exercise which had a much higher effect than Frederickson's research [21].

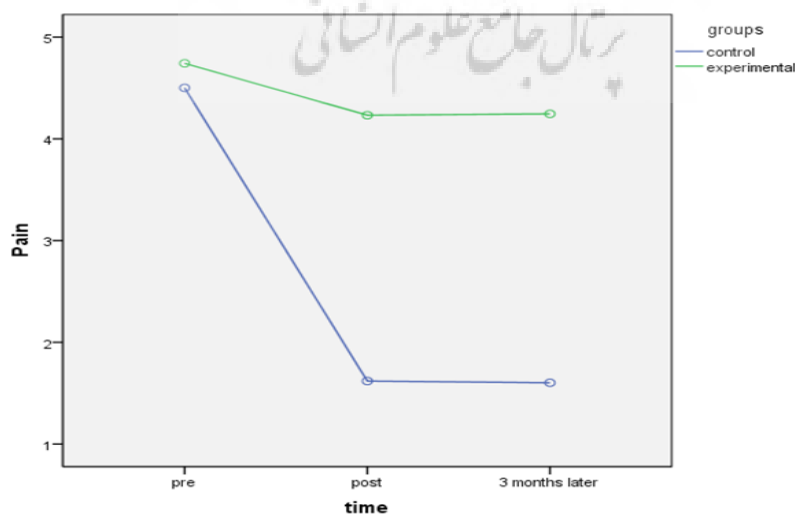


Figure 2. Comparison of lower extremity function (LEFS) in 3 stages between the study groups

On the other hand, the present research results were inconsistent with those of Ellis and associates. In their study, the effect of traditional protocols was described on ITBS at a moderate and limited level. The reason for the difference between the two studies can be attributed to the type of exercises, i.e., used by the researchers; while Ellis' exercise had no strengthening function on the hip abductors and had more focus on increasing the length and flexibility of ITB, the new protocol, i.e., used in the present study signified an impressive effect on improving the pain and lower extremity function of the patients with ITBS, by simultaneously strengthening exercises for two-joints and one-joint muscles [33].

The current investigation findings were also compatible with those of Beers and colleagues. These researchers had observed a significant increase in the power of hip abductor muscles, as a result of their treatment protocol and declared that hip abductor strengthening, causes the increase in capacity and physical function of lower extremities and prevents various injuries of the lower body [34]. They also expressed that the strength of hip abductors, hip flexors, and external rotation power in the injured leg of athletes with lower extremity injury, was significantly lower than their non-injured leg [35, 36].

However, Allison et al. presented a different opinion; they argued that as one of the main hip abductor muscles in female runners with ITBS, GM muscle does not present gross strength disorders, but it is less resistant to fatigue. These researchers suggested that for implementing a treatment program in ITBS, the corrective exercises specialists must consider the strength and endurance of hip abductor muscles [37].

The present study data were also to some extent inconsistent with those of McKay et al.; they have concluded that traditional stretching exercises are not significantly different from other common methods in confronting ITBS, despite its effect on the lower extremity. This can be attributed to the combination of different methods, especially traditional ones in their research. They also stated that difficulty in identifying the reason of ITBS causes it to prevent its effective treatment; while there are standard methods for its evaluation. Thus, the treatment protocols can provide different results because there is a lack of cognition about the main reason for a person's ITBS and an inability to remove it [5].

It is useful to apply the movements that target the weak muscle groups within the motor chain, in exercise therapy protocols, because these muscle groups will limit the production of strength and speed of multi-

joint movements during the competition. The literature indicated that most researchers used different stretching exercises combined with other methods, such as ultrasound, massage, as well as the techniques of release and manipulation. Moreover, their findings were effective in some cases; however, in some others, they were not so. Usually, applying the stretch to the tissue that got short and stiff is the most primary treatment method for a musculoskeletal disorder. Based on the joint and muscle chain approach for recovering the balance of muscle power and alignment of tissue length in a joint, especially in the lower extremity. However, it is important to consider simultaneously, the strengthening of weak muscles in all body parts, even in the segments far from the injured area, besides the application of other functional rehabilitation techniques [29-32].

In this regard, the positive effect of applied exercise protocol in the present study on its variables can be critical; the study results highlighted an increase of hip abductors power due to the strengthening exercise program in 8 weeks, i.e., the main factor in reducing pain and increasing the lower extremity function of distance runners with ITBS. Thus, the strength level of abductors can be a predicting factor for ITBS. Furthermore, a possible relation between the weakness of the hip abductor muscle and ITB hardness can be described, according to the findings of the present study. In other words, ITBS usually occurs in the lower hip area, due to the additional loading or repetitive compressive movements during long activities [38]. Hip joint abductors play an important role in lower extremity alignment during the frontal and transversal plane movements; thus, the weakness of these muscles in the hip joint may lead to the changes in hip movements, which present a serious effect on lower extremity performance [39, 40].

The distance runners perform more hip joint extension and internal angular rotation during the movements which are done on one leg; therefore, if the abductor's muscles get weak, high pressure will be exerted to ITB, and the band's stiffness will increase. Thus, a runner with the weakness of hip abductors is more vulnerable to large external forces, applied to their gluteal muscles and trunk; accordingly, it reduces the ability of stabilization in these parts of the body [26].

The correlation between hip abductors' strength and transversal plane movements can be potentially explained by the function of the GM muscle. The GM muscle in the hip area makes the external rotation of the hip. Besides, if the muscle gets weak or tired, the hip



will rotate internally and this rotation will cause more hardness of ITB and increases the band's abrasion [26].

Some of the exercises used in this study, such as one leg squat and flexion or lying down abduction, caused the strength and activation of hip abductor muscle and increased lower extremity strength and performance of the experimental group subjects. In general, strengthening the lower extremity muscles especially abductors plays a very valuable role in pain reduction and a performance increase of runners with ITBS, and the exercise protocol of this study, modeled from the NSCA treatment method, completely covers this category. Studies considering the corrective exercises, especially hip abductors strengthening programs for ITBS treatment are scarce [5, 36, 41-45]. However, Miriam et al. focused on strengthening hip muscles and ITB by 6 weeks of physiotherapy through ultrasound shear wave and elastography. Their results reflected the significant increase of muscle strength and the decrease of ITBS' side effects in the runner subjects. Unlike the present study, the subjects of Miriam's research did not completely cure and some symptoms of complication were almost seen after 6 weeks of treatment program in their runners [46].

The importance of strengthening exercises in the NSCA program can provide a new approach for future studies by researchers in the ITBS treatment area. However, an important point for the ITBS rehabilitation is getting the complete history of the patient on the age of activity start, gender, weight, previous injuries, level of education, exercise program, recovery program, appropriate weight loss before the match, the abrasion of the shoe sole, the level and the mileage of running in short or long times. Accordingly, a biomechanical evaluation of each compensatory pattern or motion asymmetry, caused by previous injuries or healthy musculoskeletal mechanics, must be performed. These general assessments will lead to the optimal diagnosis of ITBS; which can determine the extension of disorder in muscular bar tissue and instability of movement in ITBS. After this diagnosis, the right action can be done to eliminate the syndrome.

## Conclusion

As running is developing at different levels and distances, it is essential to identify the related injuries to this sport, like ITBS. In this regard, the present study results indicated that corrective exercises, i.e., based on abductor's muscles strengthening and are modeled of NSCA training protocol significantly affect pain reduction and increasing motor function of runner subjects; the findings also revealed that the effect of this program was

persistent, even 3 months after the program completion. Thus, the exercise program of this study can cause the prevention of injury and may lead to the improvement of subjects by increasing their muscular strength. Therefore, it is highly suggested to use this program for reducing the pain and improving the lower extremity function in elite distance runners with ITBS.

## Ethical Considerations

### Compliance with ethical guidelines

All experiments on these subjects were performed with their sufficient understanding and their signature on written consent.

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### Authors' contributions

Both authors were equally contributed to preparing this article.

### Conflict of interest

The authors declared no conflicts of interest.

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