



# Effect of PNF Stretching Training on Stem Cells and Growth Factors in Performance Soccer Players

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## ABSTRACT

Aim of the study: is to evaluate the effect of PNF stretching training on stem cells, growth factors and strength tests of soccer players. 15 soccer players volunteers were selected for to this study, PNF stretching training was performed for 6 weeks by each subject independently 5 times a week for 6 weeks through contract – Relax – antagonist – contract, stretching the plantar flexors in a standing wall position. Afterward, to contract the antagonistic dorsiflex onmuscles, the procedure wasrepeateol 4 times during each stretching session in a total period 144 s. for each muscle. The results indictated a significant elevation of Stem cells CD34+, Growth factors (IGF1-b-fgf) and strength tests positively affected. The Research reach It is concluded that the PNF Stretching training led to the integration action of both stem cells and growth factors leading to strengthening of the muscles of the Soccer Players and Performance.

**Key Words:** PNF Stretching training, stem cells, growth factors.

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## INTRODUCTION

Soccer players are in need of a high physical level owing to the hard physical demand of this sport which duration last (90) minutes without extra time, soccer players need different training methods to face the high physical load they encounter during competition matches, they perform a big effort so as to prepare their muscles to train in the extreme situations througho  
ut the duration of training or competitions, as they need the speed in their performance and physical work with the highest exertion in the least duration

which occurred through acceleration the heart rate and increased energy production which is proportional to distance covered during the game which vary from the midfield player to defense players [1, 2].

Skeletal muscle comprises about 40% of the body mass in human, the regulation of muscle mass is of interest to the diverse types of sports, so as to increase muscle strength. [3,4].

The mechanisms regulating muscle mass maintenance are widely studied due to its importance to health and performance, as increased load on muscle results in an

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increased mass or hypertrophy [5] growth hormone Insulin growth factor (IGF) and (b-FGF) basic fibroblast growth factor have a key role in the regulation <sup>(1)</sup> of muscle mass and hypertrophy and muscle regeneration [6]

Two factors contribute to hypertrophy: sarcoplasmic hypertrophy which focuses on increasing glycogen storage and myofibrillar hypertrophy which focuses on increased myofibril size [7].

During exercise, muscle not only produce more IGF than the liver but also more circulating IGF1, Insulin-Like growth factor <sup>(1)</sup> has been shown to induce hypertrophy in both auto crine and paracrine manner and exerts its effects in multiple ways [8].

Satellite cells incorporated in muscle are the mayor donors of new nuclei for muscle development, regeneration or hypertrophy in response to exercise training and growth factors stimulations, so satellite cells contribute to muscle fiber growth by providing new myonuclei, an increase in myofiber size can occur by changes in protein turnover with satellite cell activation in some models of muscle hypertrophy [9].

Asahara and Isner [10] reported that stem cells have two defining properties, the ability to differentiate into other cells, or the potential to develop into other cells and the ability to self-regenerate and to divide and produce more stem cells.

Heshmat and Roshdi [11] added that CD<sup>34+</sup> is an expressed protein, and the first indicator marrow cells derived from the blood and bone and the expression (CD34<sup>(+)</sup>) was only shown the first of the characteristics of stem cells vessels(HSC), which is the main source for the production of blood cells.

Amani Behairy [12] added that exercise may act on the regeneration of tissues by altering the ability to generate new stem cells and differentiated cells.

Stem cells differ from other kinds of cells in the body by being unspecialized. However, unspecialized stem cells can give rise to specialized cells, including skeletal muscle cells, blood cells or nerve cells.

An acute bout of maximal exercise in adult athletes can increase circulating stem cell in the blood and affects muscle functions [13;14].

Laura Bilek [15] investigated the effect of physical activity on stem cells for adults, and related the increased numbers of stem cells as a result of the physical activity affective health and performance of participants.

Proprio-Neuromuscular facilitation (PNF) is defined as methods of promoting or hastening the response of the neuromuscular mechanism through proprioceptors. They act as safe guards that sense changes in muscle tension. There are three proprioceptors involved in stretch inhibition:

1- The golg tendon organ (GTO), located at the musculo-tendonous junction, that detects the mechanical stress, when the stress increased the GTO may induce shut off muscle contraction from the brain.  
 2- Pacinian corpuscles are small bodies near GTO, they are sensitive to deep pressure and quick movement.

3- Muscle spindle is a specialized muscle fiber, detect excess stretch within the muscle, muscle spindles are responsible for fine movements of the muscles [4;16].

Research Problem

In order that the football player achieve the highest level of performance during competitions, he should pay attention to many aspects such as the physiological, biological sides together with the study of genetics and most important stem cell function and the integration relationship of genes and stem cell in muscle hypertrophy and regeneration .

This could be achieved through knowing the effect of different physical loads and rationing training loads on a sound scientific basic at the beginning of training season.

Konrad et al. [16] reported that stretching method such as proprioceptive neuromuscular facilitation (PNF) are used for short term ( repeated stretching training for 3-8 weeks) as stretching are able to increase muscle functions through proprioceptive action and induce gain in structural parameters of the muscle from muscle stiffness, tendon stiffness, fascicle length and pennation angle. This leads the researcher to the study, as a method to enhance performance.

#### AIM OF THE STUDY

This study aims to evaluate the effect of PNF stretching training on stem cells (CD<sup>34+</sup>), growth factors (IGF1, b-FGF) and strength tests of soccer players.

#### RESEARCH HYPOTHESIS

1- There are statistically significant differences on (CD34<sup>(+)</sup>) between pre-post PNF stretching training in favor of post measurement.

2- There are statistically significant differences on growth factors between pre-post PNF stretching training in favor of post measurement.

3- There are statistically significant differences on strength tests between pre-post PNF stretching training in favor of the post measurement.

#### RESEARCH PROCEDURES

Research method, the researcher used the experimental method due to its suitability to the nature of the study.

Research Sample

15 soccer players of different clubs in Suez Canal province volunteers to participate to the study, 12 players for the main study and (3) for the pilot study. The main study was done at April 2017.

**Table (1):** Homogeneity and equivalence of the sample

variables	Mean	±SD	Skewness
Age(years)	22.7	4.31	0.254
Height(cm)	171.4	9.4	1.3
Weight(kg)	68.9	5.61	0.362
Training experience	9.4	0.91	2.01



Table (1) reported that skewness was between ( $\pm 3$ ) indicating homogeneity of the sample.

**Data Collection Tools**

- Height using restameter.
- Weight using medical scale.
- Syringes for blood sample.
- Test tubes containing anticoagulant (EDTA).
- Coleman Plus ice for blood separation.
- kits for IGF1, b-FGF for Elisa (DRG, USA) determination in clinical lab.
- Body composition analysis (FFM) using Tanita analyzer.
- Determination ( $CD^{34+}$ ) stem cells using Flow cytometer, using flurochrome conjugated antibody to identify and numerate cell population expressing the CD34 antigen present in human biological samples.

**Pilot Study**

The researcher conducted the pilot study composed of (3) players from outside the sample and from the same research community, this aimed to:-

- Explain the tests used for assistants and trained on the measurement and registration.
- To train them on the speed and accuracy of measurements.
- Adjust the tools used to ensure the safety and suitability.
- Determine the best ways to carry out measurements and data recording.
- To know the difficulties that can encountered during training and measurements.
- Ensure the players to accommodate the training and tests used and the ease of its application.

This has resulted on the safety and validity of the tools and used devices to accommodate the players to the training used measurements and recording.

PNF stretching training program five times a week for 6 weeks, in the morning outside the ordinary normal program of each soccer player in his club, subjects had to keep a diary of the stretching performance, several parameters were determined before and after PNF program.

The PNF stretching training was performed independently so that no assistance is needed.

**PNF stretching program**

The participants of this program of soccer players were instructed to undertake a (contract-relax-antagonist-contract) after Sharman et al. [17] for the planter flexor muscles.

Stretching was performed 5 times a week for 6 weeks.

The participants undertake the stretching of the plantar flexor in a standing wall push position and to stretch until a point of discomfort was reached.

One stretching consisted of a 15-s.static stretch of the lower leg, followed by an isometric contraction of the stretched muscle for 6 s.

The subjects were instructed to contract the antagonistic dorsi flexor muscle for another 15 s. [18;19] to induce another stretch for the plantar flexors. This was repeated 4 times during each stretching session, alternating both legs with no rest.

Resulting is a total stretch period of 144 s. for each muscle.

Statistical data analysis using (SPSS) including

- Arithmetic mean.
- Median
- Standard deviation.
- Skewness
- T test  $P < 0.05$

**RESULTS**

Table (2) reported statistical significant differences between pre-post (PNF) stretch training in  $CD^{34+}$  numbers.

Table (3) reported statistical significant differences between pre-post (PNF) stretches training in growth factors.

Table (4) reported statistical significant differences between pre-post (PNF) stretches training in different variables.

**Table (2)**  $CD^{34+}$  before and after PNF stretch training of participants

	Before		After		
Variables	PNF stretch		PNF stretch		T test
$CD^{34+}$ (no)	M	$\pm SD$	M	$\pm SD$	Sig.
	158.3 $\pm$	16.2	263.7 $\pm$	19.3	

**Table (3)** arithmetic mean and standard deviation of basic. Fibroblast growth factor and insulin like growth factor before and after PNF stretching training.

	Before		After		
Variables	PNF stretch		PNF stretch		T test
b-FGF pg./ml	M	$\pm SD$	M	$\pm SD$	Sig.
	13.6 $\pm$	1.2	27.8 $\pm$	2.5	
IGF1ng/ml	160.3 $\pm$	12.6	312.7 $\pm$	13.8	Sig.



**Table (4)** Arithmetic mean and standard deviation in stretch tests before and after stretch training

Variables	Before		After stretch		T test
	Training		Training		
Vertical jump (cm)	M	±SD	M	±SD	Sig.
		190.4±	12.1	195.1±	
Isotonic leg muscle strength (k)	78.3±	4.1	86.3±	5.1	Sig.
Strength speed (m)	53.1±	2.4	56.1±	3.2	Sig.
FFm (kg)	58.1±	3.3	58.7±	3.0	Sig.
BMI kg/m <sup>2</sup>	22.9±	0.63	20.3±	0.51	Sig.

## DISCUSSION

The data presented in table (2) revealed a significant increased CD34+ after 6 weeks PNF stretching training. These results are in accordance with that of Jung et al. [20] and Sarah Witkowski [21] and Assaf et al. [22] who suggested that the stress condition might stimulate catecholamine action and chemokines and cytokines together with proteolytic enzymes and osteoclasts in bone marrow leading to the stimulating effect on the increased stem cells (CD<sup>34+</sup>) in the circulating blood. This indicated the positive effect of the PNF stretching training in increasing stem cells in the blood and in different organs of the body.

The proprioceptive neuromuscular facilitation (PNF) used for 6 weeks as stretching are able to induce gain in the structural parameters of the muscle and in fibers which leads to better achievement of the muscle and the blood stress induce on the muscle led to the stimulating effect on the stem cell in the niche of bone marrow leading to its increase in the blood and stimulation of satellite cells in the muscle to perform its action together with the growth factors.

Collins et al. [23], Adams and Scadden [24], Choumeriano et al. [25] reported that the stimulating effect of (CD34+) on satellite cells display a range of cell surface markers different potentials for cell renewal and differences in differentiation potential both along myogenic and non-myogenic lineages. Immune cells can infiltrate beneath the basal lamina and populate the satellite cell compartment.

Asakura et al. [26] indicated that non-myogenic activities such as fibrogenic osteogenic or adipogenic of skeletal muscle satellite cells instead arise from distant populations of cells that cohabit the satellite cell niche.

Table (2) indicated increased stem cells (CD<sup>34+</sup>) numbers after PNF stretching training for 6 weeks this increased stem cells (CD34+) revealed a positive results due to the stretching procedure of proprioception.

The results of this research are in accordance with that of Laufs et al. [27], Rehman et al. [28], Shaffer et al. [29].

Marni Boppart [30] determined that an adult stem cell present in muscle is responsible to exercise, a discovery that may provide a link between exercise and muscle health. The finding could lead to new therapeutic techniques using these cells to rehabilitate injured muscle and prevent or restore muscle loss with age.

He added that mesenchymal stem cells in skeletal muscle have the potential to release high concentration of growth

factors into the circulating system during exercise also may provide a critical link between enhanced whole body health and participation in routine Physical activity. It is worth willing to say that such increased stem cells during exercise may be utilize effectively to preserve muscle mass in the face of atrophy of the skeletal muscle.

Kuang and Rudnicki [31] conclude due to action of exercise on stem cells (CD34+) leading to stimulate interstitial stem cells which in turn stimulate satellite cell in skeletal muscle underneath the basal lamina have been shown to be necessary and sufficient for muscle growth and repair.

They also added that investigations into the mechanisms regulating the quiescence, activation, proliferation and differentiation of the various types of myogenic stem cells stimulated by the hematopoietic stem cells will lead to successful cell based therapy for muscle diseases and muscle aging.

Data presented in table (3) indicated an increased concentrations of growth factors (Insulin growth factor(1) and b-fibroblast growth factor), both IGF, and b-FGF increased significantly after stretch training which indicated an anabolic action of both growth factors IGF1, and b. FGF.

This result was also supported by many scientific research studies.

Gordon et al. [32] Charge and Rudnichi [33], Teven et al. [34] stated that IGF activates both myoblast proliferation and differentiation crucial processes for successful regeneration and hypertrophy of skeletal muscle. Clearly administration of IGF and other growth factors have the potential to accelerate regeneration and hypertrophy muscle.

Barrett et al. [8] explained the mechanism of action of growth hormone on growth through new information. As growth hormone was originally thought to produce growth by a direct action on tissues, then later was believed to act through its ability to induce somatomedins (IGF). A current hypothesis explains that growth hormone stimulates stem cells and IGF that lead to a dual action of both stem cells and IGF to induce growth, so growth hormone and IGF can act both in cooperation and independently that stimulate path ways that lead to growth, so growth hormone increases circulating IGF, which in turn stimulate satellite cells of skeletal muscle and together act on increase muscle mass and hypertrophy.



Mougiou [35] reported the mechanism of exercise induced hypertrophy of skeletal muscle resulting from enhanced protein synthesis. The increase in gene expression inside the muscle fibers is partly due to a higher DNA content thanks to the addition of muscle derived from satellite cells. Mechanical loading lead to the division of satellite cells, one of the two daughter cells then fuses with the muscle fiber, then providing an addition muscles, the other cell remains a satellite cell. Also, IGF of the muscle helps in the mitosis of satellite cells, and with the integration action of IGF, growth factor and b-FGF together with the fusion of satellite cell into the myofiber, this increase the cross sectional area of the muscle fiber and increase muscle mass and leading to hypertrophy of the muscle.

One of the best practical applicants of the integration of both stem cell and growth factor in muscle regeneration and function is the research of Hwang et al. [36] by using a combination of stem cells and basic fibroblast growth factor in muscle regeneration, Hwang et al.[36] added that although many researchers have realized the therapeutic potential of stem cells in the study of muscle regeneration, also research indicates that growth factors could provide the optimum circumstances for stem cells and enhance stem cell proliferation.

Basic fibroblast growth factor (b-FGF) contributes to survival and differentiation of stem cells and enhance muscle function and regeneration.

Table (4) indicated stretch tests before and after proprioceptive stretch training, vertical jump, muscle stretch testing, strength speed, FF mass, BMI.

The results indicated a statistical increase in the different tests which revealed the positive effects of the stretch training which is in coordination with the research of Metthew et al. [37], reported that both growth factors of IGF, b-FGF are prime candidate for influencing muscle mass and strength, Also Mougiou [35 2006] added the effect of satellite muscle stem cell which integrate with the function of growth factors leading to hypertrophy and increasing muscle strength and mass.

Buzi et al. [38 2008], added that growth promoting agents that purported to increase size of existing muscle fibers together with satellite cells proliferation and differentiation crucial process for successful muscle growth.

The proceeded discussion indicated that the research hypothesis has been realized.

## CONCLUSION

It may be concluded that PNF stretching training may induce the following:

- 1- Triggers stem cells in muscle.
- 2- Stimulate growth factors (b-FGF and IGF)
- 3- Both stem cells (CD34+) and growth factors integrate together for increasing muscle mass and strength.
- 4- Stretch tests indicated a positive effect of PNF stretching training of soccer players.

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