OA Medical Hypothesis
Open Access

Hypothesis

Biomedical Sciences

Diacutaneous fibrolysis versus passive stretching after articular immobilisation: muscle recovery and extracellular matrix remodelling

WR Martins[1]*, MM Carvalho[1], Mota MR[2], GFB Cipriano[1], FAS Mendes[1], LR Diniz[1], GC Junior[1], RL Carregaro[1], JLQ Durigan[1]

Abstract

Introduction

Atrophy and muscle shortening due to articular immobilisation are common problems in musculoskeletal rehabilitation. Muscle stretching mechanical stimuli might be considered as the golden standard procedure to improve muscle flexibility in rehabilitation. Muscle stretching generates mechanotransduction, potentiating specific gene expression and promotes sarcomerogenesis and extracellular matrix remodelling on shortened and atrophied muscles.

Hypothesis

Diacutaneous fibrolysis, like stretching, uses an external force to stress connective and muscle tissues mechanically to treat muscle shortening; thus, it is widely used in clinical practice even if there is no evidence to support it. Considering this subject, we have hypothesised that diacutaneous fibrolysis can generate mechanotransduction, affecting muscle hypertrophy and extracellular matrix remodelling after immobilisation.

Evaluation of hypothesis

We have designed a laboratory experimental study with a sample of 50 rats. The sample was randomly divided into five groups: Control group (n = 10) with non–immobilised rats; 3–week immobilisation group (n = 10); 3–week immobilisation/3–week non–immobilisation group (n = 10); 3–week immobilisation/3–week stretching group (n = 10); and 3–week immobilisation/3–week diacutaneous fibrolysis group (n = 10). All rats had their left tibiotarsal joint immobilised in maximum plantar flexion with the orthotics for 3 consecutive weeks. After the immobilisation period, the intervention groups received their respective intervention on their left triceps suralis for 3 weeks. Dependent variables of the study were sarcomere analysis, polymerase chain reaction, connective tissue density, collagen birefringence and matrix metalloproteinases. Statistical analysis was performed using analysis of variance and Duncan *post hoc* test was applied for differences between groups. For all calculations, a 5% ($p < 0.05$) significance level was established.

Conclusion

If the hypothesis is confirmed, the present study might provide evidence to support the use of this physical therapy resource widely used to treat muscle dysfunctions.

* Corresponding author
Email: wrmartins@me.com

[1] Division of Physical Therapy, University of Brasilia, Brasilia, Distrito Federal, Brazil
[2] College of Health Sciences, University Center of Brasília, Brasília, Brazil

Introduction

Muscle plasticity is a remarkable mechanical property; it is the ability of muscle cells to alter their structure and function in accordance to different stimuli. Articular immobilisation leads to muscle atrophy and rigidity, characterised by decrease in muscle fibre protein content and size[1] and increase in their connective tissue[2].

Data show that during the first 6 hours of articular immobilisation, the synthesis of muscle protein is reduced, and within 72 hours, it might reduce the muscle mass up to 30% of its original size[3,4]. Seven days of immobilisation results in loss of muscle fibres, reduces the number of serial and parallel sarcomeres and leads to muscle atrophy and shortening[5,6]. Evidence shows that the increase in connective tissue diminishes blood flow, water and proteoglycans into muscle fibres; binding of abnormal collagen fibres occurs, which induces rigidity and loss of flexibility of the connective tissue[7,8].

Passive muscle stretching is considered as an effective resource and is often used to increase muscle and joint flexibility. Previous studies have showed that muscle stretching promotes sarcomerogenesis (contractile protein synthesis triggered by specific muscle gene potentiation)[9–12] by mechanotransduction (mechanical stimulus conversion into chemical activity)[13–15]. During muscle stretching, mechanical stimuli are first transmitted to the extracellular matrix (ECM), and the integrins on their membrane detect those stimuli and transmit them to the cell interior, activating a series of nuclear proteins responsible for modifying the specific gene transcription that regulates sarcomerogenesis[16].

Stretching–induced mechanotransduction affects connective tissue remodelling through matrix metalloproteinases (MMPs) because they degrade ECM components. MMPs play a role in both tissue function and development, which include pathological processes. Coutinho et al.[2].

FOR CITATION PURPOSES: *Martins WR, Carvalho MM, Mota MR, Cipriano GFB, Mendes FAS, Diniz LR, et al.* Diacutaneous fibrolysis versus passive stretching after articular immobilisation: muscle recovery and extracellular matrix remodelling. OA Medical Hypothesis 2013 Dec 21;1(2):17.

Competing interests: none declared. Conflict of interests: none declared.
All authors contributed to conception and design, manuscript preparation, read and approved the final manuscript.
All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.

OA Medical Hypothesis
Open Access

Demonstrated that daily sessions of muscle stretching for 3 weeks were enough to rearrange collagen bands of rats' immobilised muscle, showing positive outcomes of muscle stretching in ECM remodelling.

Diacutaneous fibrolysis[17,18] is a non–invasive physiotherapeutic method to treat musculoskeletal disorders and movement restriction[19] that uses a stainless steel hook to generate mechanical stimulus[20] from lateral traction movement of the muscle belly. This traction technique is performed manually with smooth and precise anatomical knowledge. Despite the extensive use of diacutaneous fibrolysis in physical therapy clinical practice to release adherences between muscle, aponeurosis and tendons[21], we found no evidence of its effects on muscle plasticity.

Hypothesis

To the best of our knowledge, no previous study has described diacutaneous fibrolysis in skeletal muscle adaptations. But, Veszely et al.[22] analysed the effects of diacutaneous fibrolysis on human skeletal muscles through the use of surface electromyography, confirming that the traction technique presented neural and mechanical effects similar to those observed in studies which assessed passive muscle stretching. We obtained the significant T_{max}/M_{max} (mV) reflex reduction and passive mechanical tension (N_m) of the triceps suralis (TS) muscle of young adults 30 minutes after diacutaneous fibrolysis.

Diacutaneous fibrolysis is an external force that stimulates muscle and connective tissues mechanically. This hypothesis is based on the affirmative that the traction technique produces enough muscle tension to provoke mechanotransduction. Thus, we hypothesise that the diacutaneous fibrolysis traction technique affects muscle plasticity, promoting sarcomerogenesis and ECM remodelling in muscle atrophy and shortening, based on the work of Veszely et al.[22].

Figure 1 shows the traction technique on the muscle belly of the biceps braquialis of a patient presenting muscle shortening, which shows that the greater curve of the hook fits the muscle surface involving it. Figure 2 shows the traction technique adapted to rats.

Figure 1: Traction technique on the muscle belly of the biceps braquialis of a patient presenting muscle shortening.

Therefore, the objective is to assess diacutaneous fibrolysis effects on muscle atrophy signalling pathways, sarcomerogenesis and ECM remodelling of muscles in disuse and compare them to the effects of passive muscle stretching by applying the same analysis.

Evaluation of Hypothesis

The authors have referenced some of their own studies in this hypothesis. The protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed. Animal care was in accordance with the institution guidelines.

The authors have designed an experimental research because of the research problem. The research was conducted in accordance with the Guide for Care and Use of Laboratory Animals of the University of Brasília.

A sample of 50 rats (*Wistar* lineage *Rattus norvegicus*) was selected from the University of Brasília's vivarium. These animals were randomly divided into five groups: Control group (CG, n = 10) with non–immobilised rats; 3–week immobilisation group (IG3, n = 10); 3–week immobilisation/3–week non–immobilisation group (INIG3, n = 10); 3–week immobilisation/3–week stretching group (STR, n = 10); and 3–week immobilisation/3–week diacutaneous fibrolysis group (DF, n = 10). All rats had their left tibiotarsal joint immobilised in maximum plantar flexion with the orthotics described by Coutinho et al.[23] for 3 consecutive weeks. After the immobilistion period, STR and DF groups received their respective intervention on their left TS for 3 weeks.

The STR group had the TS passively stretched by maintaining a maximum tibiotarsal dorsiflexion for 1 minute in accordance with the

FOR CITATION PURPOSES: *Martins WR, Carvalho MM, Mota MR, Cipriano GFB, Mendes FAS, Diniz LR, et al.* Diacutaneous fibrolysis versus passive stretching after articular immobilisation: muscle recovery and extracellular matrix remodelling. OA Medical Hypothesis 2013 Dec 21;1(2):17.

Competing interests: none declared. Conflict of interests: none declared.
All authors contributed to conception and design, manuscript preparation, read and approved the final manuscript.
All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.

OA Medical Hypothesis
Open Access

Figure 2: Details of the traction technique adapted to rats.

protocol of Coutinho et al.[2]. Each rat was subjected to 3 sessions/day with 1 minute interval between each session, and 5 days/week. The authors chose manual passive stretching because it is most commonly used in humans[24,25]. The DF group received the traction technique at the left TS with the tibiotarsal joint in the neutral position, and the traction was performed with the hook's greater curve, following the protocol: (1) 20 traction movements of the medial portion of the muscle belly; (2) 20 traction movements of the lateral portion. The hook always fits the muscle surface with slow and smooth movements.

The INIG3 rats had their tibiotarsal joints immobilised for 3 weeks and after the joint release, they walked freely inside the cage for 3 weeks, with no intervention. The IG3 group was subjected to euthanasia after orthotics removal; and the CG group was also subjected to the same procedure after 3 weeks of free walk inside the cage.

All animals subjected to immobilisation and/or intervention received an intraperitoneal injection of Xylazine (12 mg/kg) and Ketamine (95 mg/kg) for anaesthesia. All were euthanised with anaesthetic overdose. Then, the soleus muscle was removed and fixated in 5% paraformaldehyde aqueous solution for 4 hours at ambient temperature. Then, they were dehydrated by sequential immersion in alcoholic solutions with a decreasing water percentage, diaphonised in Xylol, and embedded in hot paraffin. The paraffin blocks were refrigerated until the microtomic section. For every 250–µm slice, a sample of 5–µm thickness was selected for further analysis. The morphometric study was conducted by only one observer using an optical microscope with topographic magnification and the images were captured by AxioCam (Carl Zeiss, Germany) microscope camera. To isolate muscle fibres, the samples were fixated in polystyrene plates, immerged in 2.5% glutaraldehyde solution for 3 hours and in 30% nitric acid solution for 2 days for connective tissue hydrolysis; after that, they were stored in 50% glycerol solution.

Dependent variables

Sarcomere analysis

We used a stereomicroscope (Stemi DRC, Zeiss) to isolate muscle fibres in a Petri dish with forceps. Histological sections were stained with Gomori's trichrome and were analysed by visible light optical microscopy with an immersion lens (100×). Five muscle fibres from the medial portion were selected from all the slides and sarcomere count was started, dividing the slides into six distinct fields of 50 µm, with a total of 300 µm/muscle fibres. The number of serial sarcomeres along the fibre were estimated by a simple rule of three[26].

Polymerase chain reaction

The fragments were readily stored at −80°C after dissection for further total RNA extraction. The fragments were homogenised with Trizol (1 ml), according to the manufacturer's protocol (Life Technologies Inc., USA). Extracted RNAs were dissolved in Tris–HCL and in ethylenediamine tetra–acetic acid (EDTA) (pH 7.6), and quantified by spectrophotometry. RNA reverse transcription was conducted in a thermoclycler (Eppendorf AG, Germany), using reaction mixture consisting of 1 µg of total RNA, 4 µl of 5× Buffer, 0.8 mM dNTPs (Invitrogen), 4 mM DTT (Invitrogen), 200 µl of reverse transcriptase (Invitrogen).

Primer pairs were coded for the myostatin (GenBank, AF019624), atrogin–1 (GenBank, AF441120), and the transcription factor IID (TFIID; GenBank, D01034) using Primer Express software v2.0 (Applied Biosystems, Foster City, Califórnia). TFIID were used as an internal control because of the constant expression levels in musculoskeletal stretching models. We obtained MyoD muscle–specific transcription from Hill et al.[27] and primers were synthesised by Imprint kit (Sigma–Aldrich Co. LLC.). RNA transcription levels for experimental and control groups were simultaneously analysed in duplicates

FOR CITATION PURPOSES: *Martins WR, Carvalho MM, Mota MR, Cipriano GFB, Mendes FAS, Diniz LR, et al.* Diacutaneous fibrolysis versus passive stretching after articular immobilisation: muscle recovery and extracellular matrix remodelling. OA Medical Hypothesis 2013 Dec 21;1(2):17.

Competing interests: none declared. Conflict of interests: none declared.
All authors contributed to conception and design, manuscript preparation, read and approved the final manuscript.
All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.

OA Medical Hypothesis
Open Access

Hypothesis

using SYBR green fluorescent stain (Applied Biosystems) in a sequence detection system (GeneAmp 5700, Applied Biosystems)[28].

Connective tissue density analysis
The microscope slides were stained with Masson's trichrome. We used a dot reading planimeter; and the counting was performed in squares of 2500 μm^2 with 56 intersecting straight lines. Coinciding dots from the endomysium and perimysium were counted in 5 fields/section of 5 sections/animal, a total of 1400 dots/animal. Thus, connective tissue density (relative area) was the result of the total coinciding dots on intersecting straight lines divided by the total number of dots[2].

Collagen birefringence analysis
Microscope slides of the soleus muscle section of 10 µm were embedded in distilled water (n_D = 1,333) for 30 minutes, and were covered with cover slips containing water at each interface.

Connective tissue birefringence was measured using Zeiss microscope with polarised light using a 10× objective lens with monochromatic light and 1/4 λ Sénarmont compensator. The optical delaying between the polarised lines that represents the macromolecular aggregation, and orientation state of the collagen fibres was also assessed. One hundred measurements at different areas of each muscle section were obtained by considering the embedding solution to provide collagen fibres heterogeneous distribution in each muscle. Collagen fibres were oriented at 45° for measurement[2].

Analysis of matrix metalloproteinases
Muscle samples were treated as described by Cleutjens et al. The samples were homogenised and incubated in 0.5 ml of extraction buffer [cacodylic acid 10 mM with pH 5.0; NaCl 0.15 M; $ZnCl_2$ 1 mM, $CaCl_2$ 20 mM, NaN_3 1.5 mM; Triton X–100 0.01% (v/v)] at 4°C for 24 hours, and were centrifuged (10 minutes, 13,000 × g at 4°C). The samples containing a pool of five separated areas of each muscle received 10 µg total proteins to increase their concentration, and resolved by SDS 10% and gelatin 1 mg/ml concentration gel electrophoresis. To remove the SDS after electrophoresis, the gels were washed in 2.5% Triton X–100 and incubated in a substrate buffer (Tris–HCl 50 mM pH 8.0; $CaCl_2$ 5 mM, NaN_3 0.02%) at 37°C for 20 hours. Gels were stained with Coomassie Blue Brilliant R–250 (Bio–Rad) for 90 minutes and destained in methanol:acetic acid:H_2O (4:1:5, v:v:v). Gelatinolytic activity was observed as light bands in the stained gel. These gels were photographed with Canon G6 Power Shot 7.1 mega pixels digital camera and the mean band intensity was analysed using the Gene Tools software. Data were expressed as MMP–2 active form and concentration (total integrated optical density for intermediate and active MMP–2 proenzyme).

Statistical analysis
Statistical analysis was performed using SPSS software v17.0, and the Shapiro–Wilk test was used for data normality. If the normality premises are confirmed, analysis of variance (ANOVA) and Duncan *post hoc* test were used to study the differences between groups. For all calculations, a 5% ($p < 0.05$) significance level was established.

Discussion

Muscle atrophy and shortening are common problems in traumatology and orthopaedic rehabilitation. Despite the lack of evidence concerning its effects, diacutaneous fibrolysis has been used to treat such conditions. Therefore, further research is necessary to understand diacutaneous fibrolysis molecular and morphological mechanisms that affect muscle plasticity and ECM remodelling.

Diacutaneous fibrolysis might be more beneficial than passive stretching in clinical practice due to two aspects: (i) the procedure does not produce pain or discomfort; and (ii) does not promote joint movement. These aspects might be relevant for muscle stimulation in early treatment of intra–articular fractures, luxation and muscle lesions, when joint movement is counter–indicative.

Conclusion

If the hypothesis is confirmed, the present study might provide evidence to support the use of this physical therapy resource widely used to treat muscle dysfunctions.

Abbreviations list

ANOVA, analysis of variance; CG, Control group; DF, diacutaneous fibrolysis; ECM, extracellular matrix; EDTA, ethylenediamine tetra–acetic acid; IG, immobilisation group; INIG, 3–week immobilisation/3–week non–immobilisation group; MMP, matrix metalloproteinases; STR, stretching group; TFIID, transcription factor IID; TS, triceps suralis.

References

1. Bonaldo P, Sandri M. Cellular and molecular mechanisms of muscle atrophy. Dis Model Mech. 2013 Jan;6(1):25–39.
2. Coutinho EL, DeLuca C, Salvini TF, Vidal BC. Bouts of passive stretching after immobilization of the rat soleus muscle increase collagen macromolecular organization and muscle fiber area. Connect Tissue Res. 2006;47(5):278–86.
3. Gamrin L, Berg HE, Essén P, Tesch PA, Hultman E, Garlick PJ, et al. The effect of unloading on protein synthesis in human skeletal muscle. Acta Physiol Scand. 1998 Aug;163(4):369–77.
4. Urso ML. Disuse atrophy of human skeletal muscle: cell signaling and potential interventions. Med Sci Sports Exerc. 2009 Oct;41(10):1860–8.
5. Lima SC, Caierão QM, Durigan JLQ, Schwarzenbeck A, Silva CA, Minamoto VB, et al. Short–term immobilization causes morphometric and mechanical alterations in rat muscles. Rev bras fisioter. 2007 Jul–Aug;11(4):297–302.

FOR CITATION PURPOSES: *Martins WR, Carvalho MM, Mota MR, Cipriano GFB, Mendes FAS, Diniz LR, et al.* Diacutaneous fibrolysis versus passive stretching after articular immobilisation: muscle recovery and extracellular matrix remodelling. OA Medical Hypothesis 2013 Dec 21;1(2):17.

Competing interests: none declared. Conflict of interests: none declared.
All authors contributed to conception and design, manuscript preparation, read and approved the final manuscript.
All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.

OA Medical Hypothesis
Open Access

Hypothesis

6. Gomes AR, Coutinho EL, França CN, Polonio J, Salvini TF. Effect of one stretch a week applied to the immobilized soleus muscle on rat muscle fiber morphology. Braz J Med Biol Res. 2004 Oct;37(10):1473–80.
7. Aquino CF, Viana SO, Fonseca ST. Comportamento Biomecânico e resposta dos tecidos biológicos ao estresse e imobilização. Revista Fisioterapia em Movimento. 2005 Jun;18(2):35–43.
8. Caieirao QM, Teodori RM, Minamoto VB. A influência da imobilização sobre o tecido conjuntivo muscular: uma revisão. Revista Fisioterapia em Movimento. 2007 Jul–Sep;20(3):87–92.
9. Goldspink G, Scutt A, Loughna PT, Wells DJ, Jaenicke T, Gerlach GF. Gene expression in skeletal muscle in response to stretch and force generation. Am J Physiol. 1992 Mar;262(3 Pt 2):R356–63.
10. Goldspink G. Selective gene expression during adaptation of muscle in response to different physiological demands. Comp Biochem. Physiol Biochem Mol Biol. 1998 May;120(1):5–15.
11. Goldspink G. Changes in muscle mass and phenotype and the expression of autocrine and systemic growth factors by muscle in response to stretch and overload. J Anat. 1999 Apr;194(Pt 3): 323–34.
12. Goldspink G, Williams P, Simpson H. Gene expression in response to muscle stretch. Clin Orthop Relat Res. 2002 Oct;(403 Suppl):S146–52.
13. Salvini TF, Durigan JL, Peviani SM, Russo TL. Effects of electrical stimulation and stretching on the adaptation of denervated skeletal muscle: implications for physical therapy. Rev Bras Fisioter. 2012 Jun;16(3):175–83.
14. Kaneko D, Sasazaki Y, Kikuchi T, Ono T, Nemoto K, Matsumoto H, et al. Temporal effects of cyclic stretching on distribution and gene expression of integrin and cytoskeleton by ligament fibroblasts in vitro. Connect Tissue Res. 2009;50(4): 263–9.
15. Rittweger J. What is new in neuro–musculoskeletal interactions: mechanotransduction, microdamage and repair? J Musculoskelet Neuronal Interact. 2007 Apr–Jun;7(2):191–3.
16. De Deyne PG. Application of passive stretch and its implications for muscle fibers. Phys Ther. 2001 Feb;81(2):819–27.
17. Ekman K. Eine neue methode der fibrolyse zur unterstutzung der manuellen therapie. Manuelle Medizini. 1972; 10:3–6.
18. Aiguadé R, Camps PP, Carnacea FR. Techniques de crochetage instrumental myofasciale. Kinesither Rev. 2008 Mar;8(75):17–21.
19. Barra López ME, López de Celis C, Fernández Jentsch G, Raya de Cárdenas L, Lucha López MO, Tricas Moreno JM. Effectiveness of diacutaneous fibrolysis for the treatment of subacromial impingement syndrome: A randomised controlled trial. Man Ther. 2013 Oct;18(5):418–24.
20. Colombo I, Ekman K. La fibrolisi diacutanea: nuovo mezzo diagnostico e terapeutico in fisiatria. European Medicophys. 1968;4:29–36.
21. Barra ME, López C, Fernández G, Murillo E, Villar E, Raya L. The immediate effects of diacutaneous fibrolysis on pain and mobility in patients suffering from painful shoulder: a randomized placebo–controlled pilot study. Clin Rehabil. 2011 Apr;25(4):339–48.
22. Veszely M, Guissard N, Duchateau J. Contribution à l'étude des effets de la fibrolyse diacutanée sur le triceps sural. Ann Kinésithér. 2000;27(2):54–9.
23. Coutinho EL, Gomes AR, Franca CN, Salvini TF. A new model for the immobilization of the rat hind limb. Braz J Med Biol Res. 2002 Nov;35(11):1329–32.
24. Guissard N, Duchateau J. Neural aspects of muscle stretching. Exerc Sport Sci Rev. 2006 Oct;34(4):154–8.
25. Decoster LC, Cleland J, Altieri C, Russell P. The effects of hamstring stretching on range of motion: a systematic literature review. J Orthop Sports Phys Ther. 2005 Jun;35(6):377–87.
26. Volpi FS, Casarolli LM, Pudell C, Menon T, Ciena AP, Alves EPB, et al. Efeitos da Remobilização em duas semanas com natação sobre o músculo sóleo de ratos submetidos à imobilização. Revista Brasileira de Medicina do Esporte. 2008;14:168–70.
27. Hill M, Wernig A, Goldspink G. Muscle satellite (stem) cell activation during local tissue injury and repair. J Anat. 2003 Jul;203(1):89–99.
28. Peviani SM, Gomes AR, Moreira RF, Moriscot AS, Salvini TF. Short bouts of stretching increase myo–D, myostatin and atrogin–1 in rat soleus muscle. Muscle Nerve. 2007 Mar;35(3):363–70.

FOR CITATION PURPOSES: *Martins WR, Carvalho MM, Mota MR, Cipriano GFB, Mendes FAS, Diniz LR, et al.* Diacutaneous fibrolysis versus passive stretching after articular immobilisation: muscle recovery and extracellular matrix remodelling. OA Medical Hypothesis 2013 Dec 21;1(2):17.

Competing interests: none declared. Conflict of interests: none declared.
All authors contributed to conception and design, manuscript preparation, read and approved the final manuscript.
All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.