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Load-Deformation Changes in Plantaris Muscle after Exposure to Different Ultrasonic Frequencies

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Abstract: The purpose of this study: was to investigate the load–deformation response of skeletal muscle under the effect of different frequencies of continuous ultrasound. Thirty New-Zealand rabbits weighting 2-2.5 kg and aging 5-6 months were used and divided into three groups. Group A: were treated with 1-MHz continuous ultrasound for single dose, Group B: were treated with 3-MHz continuous ultrasound for single dose and Group C: control group. Then a dissection of plantaris muscle in rabbits was done and taken for the mechanical testing experiment. A tensile testing machine (Instron instrument serial no.53479) was used to measure the load subjected to the specimen at high yielding point and its corresponding deformation. The result of the study showed that deformation of the samples in response to the applied load at the high yielding point revealed a significant difference between group (A) and control group (C), non statistical significant difference between group(B) and control group (C). Non statistical significant difference between group (A) and group (B). Conclusion: it can be concluded that application of ultrasound prior to stretching (regardless its frequency) has significantly influence on the extensibility of the muscle.

Key words: Continuous Ultrasound · Ultrasound Frequencies · Mechanical Load · Tensile Strain · Skeletal Muscle

INTRODUCTION

The muscle and connective tissue tightness is a common clinical problem. The tightness may be due to scarring or adaptive shortening of the muscle related to disease, injury or immobilization. An important goal in any treatment or rehabilitation program is to attain full range of motion. So clinicians frequently use heat and stretching to treat muscle tightness. Heating is often accomplished with continuous wattage ultrasound which is the method of choice for heating dense connective tissue (fascia and muscle sheaths) [1-4].

The therapeutic effects of ultrasound are classified as thermal and non thermal. The non thermal effects of ultrasound are desired when treating acute soft tissue injuries where heating should be minimized. The non thermal effects of ultrasound increase the cellular diffusion and membrane permeability, as well as fibroblastic activities, such as protein synthesis, that speeds up the tissue regeneration [5-7].

The thermal effects of ultrasound are desired in treating chronic soft tissue injuries that result in inflammation. Like other heating modalities, ultrasound changes nerve conduction velocity, increase enzymatic activity, changes contractile activity of skeletal muscle, increases the collagen tissue extensibility, increases local blood flow, increases pain threshold and reduces muscle spasm [8].

Previous authors agree that the factors affecting the absorption of ultrasound energy in tissue high in collagen include frequency and intensity of ultrasound, duration of treatment, movement speed of the transducer, coupling agent used, type of tissue being treated and size of treatment area [9, 10].

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A typical "heat and stretch" procedure involves placing the targeted connective tissue on stretch during, or immediately following the application of ultrasound. The rationale for adding heat to static stretching is based on the premise that heating alters the viscoelastic properties of connective tissue, making it more extensible, thereby potentiating the effects of stretching [3, 11].

Connective tissue is a viscoelastic structure capable of plastic and elastic changes. The viscous properties of connective tissue allow it to go through a permanent change in structure. Elastic properties refer to the connective tissues ability to regain its original length. When an applied stretch to a connective tissue is removed, the elastic components recover their original length and the viscous component remains deformed. The amount of elastic and viscous deformation can vary considerably, depending on the amount of applied force, duration of applied force and tissue temperature [12-14]. A number of researchers suggested that an increase in temperature alters the behaviour of collagen when subjected to stress and, therefore, is of value prior to the application of passive or active stretch designed to mobilize scars or lengthen contractures [15].

The connective tissue makes up as much as 30% of muscle mass. This tissue allows the muscle to change in length. During passive motion, the sum of the muscle's fascia accounts for 41% of the total resistance to movement. Hence the fascia represents the second most important factor limiting the range of motion. Thus when the muscle is stretched, the connective tissues become progressively taut [16, 17].

It was suggested that the increase in maximal attainable joint range of motion was related to the subject's tolerance to the stretch, rather than the mechanical properties of the muscles [17].

So it is important to know how to augment the effect of stretching exercise. This study can guide the physical therapists to choose 1MHz or 3MHz continuous ultrasound and augment the effect of stretching by gaining the deep heating effect of ultrasound and reach to results about the efficacy of ultrasound in increasing the muscle extensibility.

MATERIALS AND METHODES

30 white New-Zealand rabbits with a body weight range of 2-2.5 kg and age range of 5-6 months old were used. The animals were kept in Cellulose and Paper Department at the National Research Center of Egypt in separate plastic restrain cages, with water and food.

Fig. 1: The tensile testing machine

The animals were randomly distributed into the following experimental groups: Group A: 10 animals had their right plantaris muscle submitted to 1MHz frequency of continuous ultrasound with an intensity of 1.5W/cm^2, then measuring its mechanical strain. Group B: 10 animals had their right plantaris muscle submitted to 3MHz frequency of continuous ultrasound with an intensity of 1.5W/cm^2, then measuring its mechanical strain. Group C: Control 10 animals were not submitted to treatment and kept in cages.

Instrumentations: Tensile testing machine: (Instron instrument serial no. 53479, capacity 5000N, Model 25/9-107, USA) which is computerized, valid and calibrated unit with a range of 0 to 500 Kg per cm^2 was used to measure the load at the yielding point (before failure) and its corresponding deformation (extension) Fig (1).

Ultrasonic apparatus: A common therapeutic ultrasound generator was used to apply continuous wattage ultrasound (Phyaction 190 i,CA)

Experimental Procedures: The skin over and around the plantaris muscle of the rabbit was shaved and covered with a standard coupling gel in order to achieve contact between the ultrasound applicator and the skin. The ultrasound applicator was set to pulsating continuous wattage ultrasound. The intensity of ultrasound treatment was 1.5 W/cm^2, with a frequency 1-MHz (for group A), 3-MHz (for group B) and applied for 8 minutes.

Plantaris Muscle Preparation: After finishing the session, the animals in each experimental group were sacrificed and the plantaris muscle was detached from its origin and insertions, avoiding additional injuries. In order

Fig. 2: The plantaris muscle in rabbit while dissection

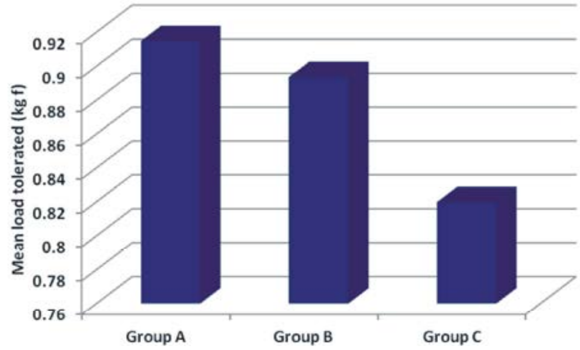

Fig. 3: Mean values of the load(kgf) tolerated by the sample at the upper yield point for the three groups.

to detach the muscle individually, firstly the overlying superficial fascia and fat was removed. Gently the muscle tissue was cut off away using a scalpel or forceps. A blunt metal probe was used to break the surrounding connective tissue. The muscles were separated at the cleavage line by inserting the probe between adjacent muscles. The specimen was soaked immediately in the saline solution to avoid drying and its retraction during the testing procedure and was taken to the mechanical experiment Fig (2).

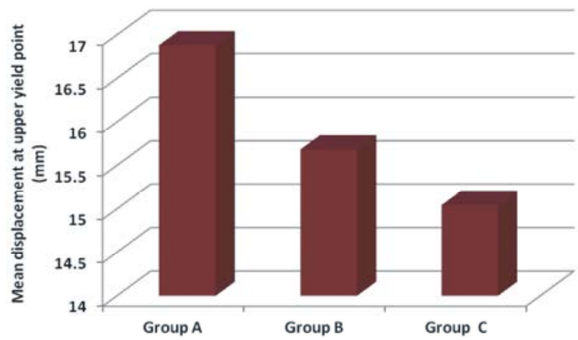

Fig. 4: Mean values of the displacement in (mm) of the sample at upper yield point for the three groups.

Mechanical Traction Testing: Each sample was taken out of saline solution then dried using filtering papers. The specimen was fixed between two clamps of the tensile testing machine with a space of 4 cm. A tensile force was applied to the sample at a rate of 100 mm per minute till muscle rupture occurred. Four parameters were obtained from the computer unit of the apparatus which were: the load at the upper yield point in (kgf). The extension (displacement) of the sample at the upper yield point in (mm). The load at auto-break point in (kgf). The displacement at auto-break point in (mm). These tests were carried out at room temperature within 15 minutes from the time of dissection. Each sample underwent the same steps of measurement and then the data were statistically analyzed.

Statistics: The statistical analysis was conducted using the Statistical Package for Social Sciences (SPPS) program version 16 for windows. Multivariate analysis of variance (MANOVA) was used to compare between the means of the three groups in each of the research variables. To know which of the three groups are different from each other for each of the dependent variables, multiple comparisons were performed using the LSD (least significant difference) test.

RESULTS

The Load-deformation at the Upper Yield Point: MANOVA revealed that there was a significant difference between the mean values of the load at upper yield between the three groups (P=0.001) Fig (3). The results revealed that there was a significant difference between the mean values of the displacement at upper yield between the three groups (P= 0.02) Fig (4). LSD revealed that there is a significant difference between displacement at upper yield of the group(A) and the group(C) (P= 0.005), where a positive mean difference indicates that the mean displacement at upper yield of the group(A) is higher than the mean displacement at upper yield of the group(C). There is no significant difference between the mean displacement at upper yield of the group(A) and the group(B) (P=0.06) (Table 1).

The Load-Deformation at Auto-break Point: There was a significant difference between the mean values of the load at auto-break between the three groups (P= 0.002) Fig (5). In comparison between groups, There is a significant

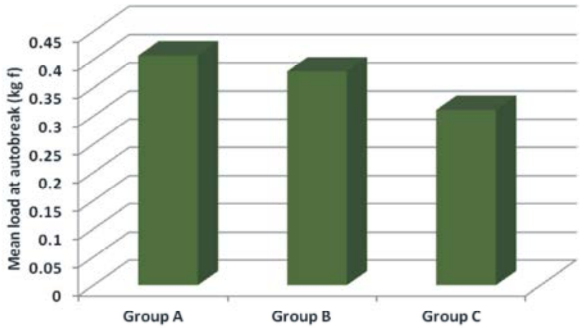

Fig. 5: Mean values of the load (kgf) tolerated by the sample at auto-break point for the three groups.

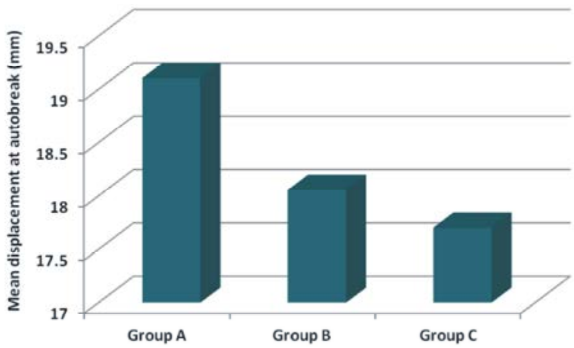

Fig. 6: Mean values of the displacement in (mm) of the sample at auto-break point for the three groups.

Table 1: Descriptive statistics, MANOVA and LSD of the displacement at upper yield point for the three groups.

	Group (A)	Group (B)	Group (C)
Mean±SD	16.88±1.45	15.68±1.55	15.04±1.02
Mean±Standard error of mean	16.88±0.46	15.68±0.49	15.04±0.32
MANOVA Test			
F value = 4.72		P value = 0.02*	
LSD (Multiple comparison test)			
Displacement at upper yield point (mm)		Mean difference	P value
Group A versus group B		1.198	0.06
Group A versus group C		1.84	0.005*
Group B versus group C		0.64	0.30

*Significant.

Table 2: Descriptive statistics and MANOVA of the displacement at upper yield point for the three groups

	Group (A)	Group (B)	Group (C)
Mean±SD	19.11±1.36	18.06±1.46	17.70±1.18
Mean±Standard error of mean	19.11±0.43	18.06±0.46	17.70±0.37
MANOVA test			
F value = 2.99		P value = 0.07	

difference between the mean load at auto break of the group(A) and the group(C)(P=0.001), where a positive mean difference indicates that the mean load at auto break of the group(A) is higher than the mean load at auto break of the group(C). Also there is a significant difference between the mean load at auto break of the group(B) and the group(C)(P=0.01), where a positive mean difference indicates that the mean load at auto break of the group(B) is higher than the mean load at auto break of the group(C). There is no significant difference between the mean load at auto break of the group(A) and the group(B)(P=0.255) Fig (6). Regarding the displacement at auto-break, MANOVA revealed that there is no significant difference between the mean values of the displacement at auto-break point between the three groups. P= (0.067) Table (2).

DISCUSSION

The aim of the current study was to explore the effect of different frequencies of continuous ultrasound on the load at the upper yield point which was tolerated by the plantaris muscle, displacement of the sample at the upper yield point in addition to the load and its corresponding displacement at auto-break point. This may help in supporting whether the ultrasound is an effective modality to be used prior to stretching or not and redirect the attention of the physical therapists to the importance of the modalities in sequence according to effectiveness rather than just including the modality within the program. The findings of the current study revealed that the displacement of plantaris muscle in response to the applied load at the high yielding point was significantly higher for group (A) which was treated with 1-MHz continuous ultrasound.

These findings come in agreement with the results of a study done by Wessling *et al*. [18] on the triceps surae muscle group, which revealed a significant increase in ankle dorsiflexion with the use of ultrasound combined with static stretch compared to static stretch alone. Ultrasound and other deep heating modalities are believed to cause collagen to become more extensible, thus increasing the efficacy of a stretch [19, 20].

The effect of temperature on the mechanical properties of the soft tissues has received more attention in animal models, the viscoelastic properties of tendon and muscle-tendon complex were altered with elevated temperature. Barker *et al*. [23] revealed that the elastic modulus decreased and hysteresis increased significantly in canine Achilles and patellar tendons when temperature was raised from 23°C to 49°C.

Morrow *et al*. [25], studied the passive properties of skeletal muscle along the longitudinal material direction. In order to ensure the accuracy of the predictions of computational models of skeletal muscles, the tensile three-dimensional material properties of muscle tissue is used.

Frieder *et al*. [26] and Jackson *et al*. [27] have also shown a stimulating effect of continuous ultrasound on the healing of partial tendon tenotomy in rats was studied by more than one researcher. The intensity of the ultrasound treatment was 1.5 w/cm^2, with a frequency of 1-MHz, applied for 4 minutes daily and the tendons were examined at 2,5,9,15and 21 days after injury for measurement of tendon breaking strength and at 3 and 5 days post injury for analysis of collagen synthesis. The breaking strengths of ultrasound-treated tendons were significantly greater than the strengths of the untreated tendons 5,9,15 and 21 days post-injury. Collagen synthesis was elevated in the treated tendons compared with the untreated tendons 5 days after injury. Healing tenotomized Achilles tendons of rats were treated with pulsating or continuous ultrasound at an intensity 0.5 w/cm^2, frequency 1-MHz, 5 minutes daily for 14 days. The pulsating mode resulted in the best organization and aggregation of collagen fiber bundles [28].

The results of the current study disagree with Reed and Ashikaga [29] who conducted their study in an attempt to determine the effects of heating with ultrasound on knee joint displacement, they used a continuous ultrasound (1-MHz, 1.5 W/cm^2, 8 min.) in order to treat ligamentous tightness as a pioneer study to test ligament extensibility in vivo. They concluded that the use of heating with ultrasound at common clinical intensities made some knee ligaments slightly more extensible in normal subjects, although the magnitude of the effect was not clinically significant.

Robertson [30] reported that heating was not only of connective tissue but also muscle and nerve, which implies that the extensibility increases or observed were either a result of changes in the mechanical stiffness of muscle tissue, changes in afferent nerve fiber activity, or a combination of these factors.

The continuous mode of ultrasound produces heat in the muscle if the intensity is high enough. The muscle has a high heat capacity. The elastic fibers are responsible for elasticity, collagen is responsible for tensile strength and inextensibility(which is present in intramuscular connective tissue).

The continuous ultrasound with a frequency 1-MHz at an intensity of 1.5 w/cm^2 for 7 to 8 min was sufficient to raise the tissue temperature of the muscle in humans and resulted in viscoelastic changes of collagen. Vigorous heating or an increase of 4°C alter the viscoelastic properties of collagen. When stressed, collagenous tissue is fairly rigid but when heated, becomes much more yielding.

Raising the temperature induces a thermal transition in the microstructure of collagen which significantly enhances the viscous stress relaxation of collagenous tissue allowing greater plastic deformation when stretched. The intermolecular bonding becomes partially destabilized, enhancing the viscous flow properties of collagenous tissue. This thermal destabilization of intermolecular bonding allows elongation to occur with less structural damage so, the continuous ultrasound can augment the effect of stretching and can be considered as a beneficial modality in cases of muscle tightness.

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