THERAPEUTIC ULTRASOUND DID NOT INCREASE THE MECHANICAL PROPERTIES OF FLEXOR TENDONS AFTER THEIR REPAIR

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ABSTRACT

Objective: Experimental study idealized to investigate the mechanical properties of deep flexor tendons of rabbits that underwent the tenotomy followed by tenorrhaphy and early application of therapeutic ultrasound with different intensities, in comparison to tendons submitted to tenorrhaphy only. Material and Method: Forty-four rabbits were divided into four experimental groups according to the ultrasound application. They were all submitted to a section of deep flexor tendon in zone 2 and immobilized with an orthosis maintained throughout the experiment. Group A received ultrasonic treatment with an intensity of 1.4 W/cm², group B with 0.6 W/cm², both in continuous mode, group C with 0.6 W/cm² SATA, in pulsated mode at 50% and group D did not receive any ultrasonic treatment. The ultrasonic frequency employed was 1 MHz. After euthanasia, the tendons were dissected and submitted to the mechanical test of traction and qualitative histological analysis. The evaluated mechanical properties were: maximum force, deformation in maximum force and stiffness. Results: There were no statistically significant differences among the experimental groups. Conclusion: Therapeutic ultrasound did not improve the mechanical properties of the flexor tendons after repair.

Keywords: Ultrasonic therapy. Tendon injuries. Biomechanics.

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INTRODUCTION

Therapeutic ultrasound is one of the physical agents used the most in physiotherapy for the recovery of soft tissue lesions.[1] According to Ramirez et al.[2], this resource stimulates cell division, increases fibroblastic activity and collagen synthesis during the active stage of tissue repair, which starts around the third day after the lesion.[3]

A large portion of the many studies that investigated the effects of ultrasound on soft tissue repair, was geared specifically toward tendon healing[4-10], and although there are strong signs that ultrasound favors the healing of this tissue, these studies still exhibit contradictory results.

Roberts et al.[4] reported that the application of pulsed ultrasound at 0.8 W/cm², five times a week, for six weeks, resulted in a detrimental effect on the healing process of the flexor tendon. Stevenson et al.[5], Turner et al.[6] and Romano[7], did not find significant differences in the mechanical properties of the flexor tendon treated with ultrasound. However, Cunha et al.[8] compared the effects of continuous and pulsed ultrasound in the healing of Achilles tendons of rats. The results showed that ultrasound in the pulsed mode presented a better effect in the organization and aggregation of collagen bands than in the continuous mode. More recently, studies demonstrated beneficial effects of continuous ultrasound on the maximum resistance of calcaneal

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tendons (Achilles), both with doses of 1.0 W/cm^2 and of 2.0 W/cm^2, suggesting that ultrasound accelerated the healing process of these tendons.[9,10] With similar results, Yeung et al.[11] found an improvement both in the mechanical properties maximum load and stiffness, and in the quantity of fibroblasts and collagen alignment in Achilles tendons of rats irradiated with ultrasound in the pulsed mode at an intensity of 0.5 W/cm^2. On the other hand, Larsen et al.[12], did not find an improvement in mechanical properties while employing the same ultrasonic intensity on rabbits' calcaneal tendons.
Therefore, based on the review of literature, the aim of this study was to investigate the mechanical properties of deep flexor tendons of rabbits that underwent tenotomy followed by tenorrhaphy and early application of therapeutic ultrasound employing different intensities.

MATERIAL AND METHODS

Subjects

The study subjects were 44 female rabbits of the White New Zealand breed, with body weight around 2.5 kg, kept in individual cages, fed with standard rabbit feed and water ad libitum. This survey was approved by the Commission of Ethics in the Use of Animals (CEUA) of Universidade de São Paulo.

Surgical procedure

The animals were anesthetized via an intramuscular injection of acepromazine maleate (1.0 mg/kg), ketamine chlorhydrate (30.0 mg/kg) and xylazine chlorhydrate (5.0 mg/kg) for the surgical procedure, and a dose of pentabiotic (40000IU/kg) was applied after surgery.
All the animals were submitted to a complete section of the deep flexor tendon of the third toe of the right forepaw, at the base of the middle phalanx, which corresponds to the region of zone 2 of the human hand. After this tenorrhaphy was performed following the Kessler technique, using monofilament polyester thread (Prolene®) gauge 6/0. The suturing of the subcutaneous tissue and skin was performed using the same thread.

Immobilization

After the surgical procedure, the operated paws were immobilized with an orthosis of thermoplastic material, made up previously, keeping the wrist flexed at approximately 70°, the metacarpal–phalangeal joints with flexion of 50º and the proximal and distal interphalangeal joints in extension, a position that is similar to orthoses used on humans (Figure 1). The orthosis was fixed with the help of plaster, and kept in place throughout the experiment.

Experimental groups and application of therapeutic ultrasound

The 44 animals were divided into four experimental groups (A, B, C and D), with 11 animals each, and the animals from groups A, B and C were submitted to ultrasound application sessions with frequency of 1 MHz, for 6 minutes, in 10 consecutive sessions, from the fourth postoperative day. In the animals from group A, the intensity was 1.4 W/cm^2, while in those of group B it was 0.6 W/cm^2, both in the continuous mode, and in those from group C 0.6 W/cm^2 SATA in the pulsed mode at 50%. The animals from group D did not receive any ultrasonic treatment.

Figure 1 – *Orthosis of thermoplastic material, keeping the wrist flexed at approximately 70°, the metacarpal-phalangeal joints with flexion of 50° and the proximal and distal interphalangeal joints in extensio*

The equipment used in the applications was the Sonacel III® (S. 00217) equipped with an especially customized head, 0.5 cm in diameter, for better coupling on the narrow space of the rabbits' toe. Ultrasound was applied by the direct method, with uniform circular movements, using gel as a coupling medium.

Euthanasia and preparation of the tendons

All the animals were euthanized on the thirty-first postoperative day, by means of an intramuscular injection of an overdose of anesthetic. After the euthanasia, the deep flexor tendon of the third toe was carefully dissected and dried. For the tendons that underwent the traction test, the musculotendinous junction and the distal segment, comprised of phalange and nail (Figure 2), were maintained for adequate fixation to the accessories of the universal testing machine (Figures 3A, 3B and 3C). The tendons designated for histological analysis, after being dissected, were sectioned 1.0 cm proximal from and 0.5 cm distal from the suture site.

Figure 2 – *Deep flexor tendon dissected with preservation of the musculotendinous junction and the distal segment (phalange and nail).*

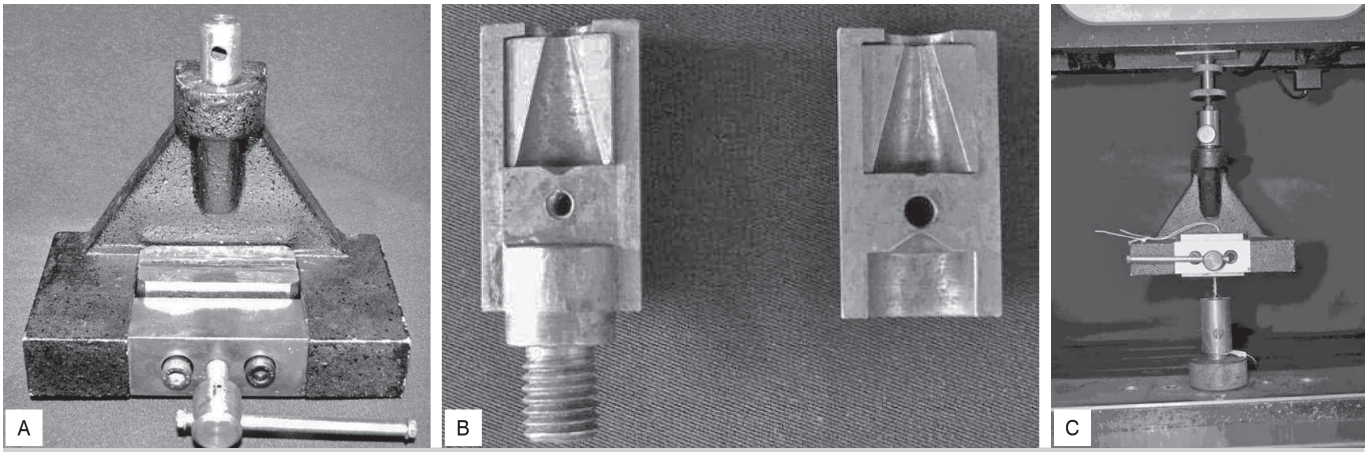

Figure 3 – A) *Superior accessory for fixation of the musculotendinous junction to the universal testing machine.* **B)** *Inferior accessory for fixation of the distal segment (phalange/nail) to the universal testing machine.* **C)** *Fixation of the tendons to the universal testing machine.*

Mechanical test of traction

Ten tendons from each group were submitted to the mechanical test of traction using the universal testing machine (EMIC®, model DL 10000) from the Bioengineering Laboratory of Faculdade de Medicina de Ribeirão Preto of Universidade de São Paulo. The machine was coupled to a microcomputer endowed with Tesc 3.13® software, responsible for commanding the equipment and for plotting the force versus deformation graph. A pre-load of 2 N was applied at the beginning of all the tests, with accommodation time of 60 seconds. The load application speed was 5 mm/min and was recorded by a 50kgf load cell. For each test the force versus deformation graph was plotted and the following mechanical parameters were obtained: maximum force, deformation in maximum force and stiffness.

After the mechanical tests, specifically for the mechanical property maximum force, four reference values (8, 16, 20 and 24 N) were established, and it was attempted to verify the percentage of tendons from each experimental group that supported a load equal to or above each one of these values.

Qualitative histological analysis

One tendon from each group was randomly designated for the qualitative histological analysis. After having been dissected they were kept in 10% formaldehyde for 24 hours, embedded in paraffin blocks and submitted to longitudinal sections 5 μm thick, then died with Hematoxylin-Eosin-Phloxine. The histological evaluation was blind, employing a Zeiss® light microscope (model Axiophot II), equipped with a Sony® digital camera (model SSC-DC 54) coupled to a microcomputer endowed with Snappy 4.0 software for the storage of digital images. The fibroblastic activity, collagen deposition and vascularization were observed at the lesion site.

Statistical analysis

The ANOVA test was used for simultaneous comparison among the experimental groups of the mechanical properties maximum force, deformation in maximum force and stiffness. Fisher's exact test was used to compare the percentages ratio of tendons from each experimental group that supported the four values of maximum force established as reference, both with level of significance established at 5% ($p=0.05$).

RESULTS

Overview

In general, the animals tolerated well the anesthesia and the surgical procedure, as well as the confinement period, the immobilization of the operated limb and the ultrasound application. Of the 40 tendons that underwent the mechanical test of traction, three were discarded (one from each group: B, C and D) due to the considerable variation in the curves obtained in the force versus deformation graphs. During the tests all the tendons failed due to rupture, which occurred at the lesion site.

Mechanical properties

Figure 4 shows the mean values and standard deviation for the maximum force of the four experimental groups and although the mean value, in absolute numbers, was higher for the tendons from group B, the statistical analysis showed that there was no significant difference among groups ($p=0.13$).

Figure 5 shows the mean values and standard deviation for deformation in maximum force of the four experimental groups, while there was no statistically significant difference among groups ($p=0.15$).

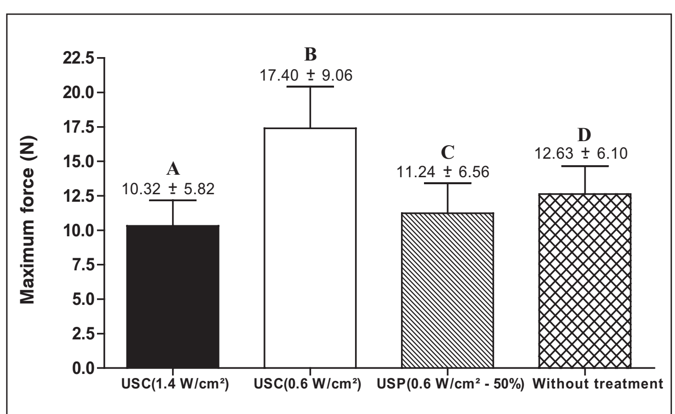

Figure 4 – *Mean values and standard deviation of maximum force of the four experimental groups.*

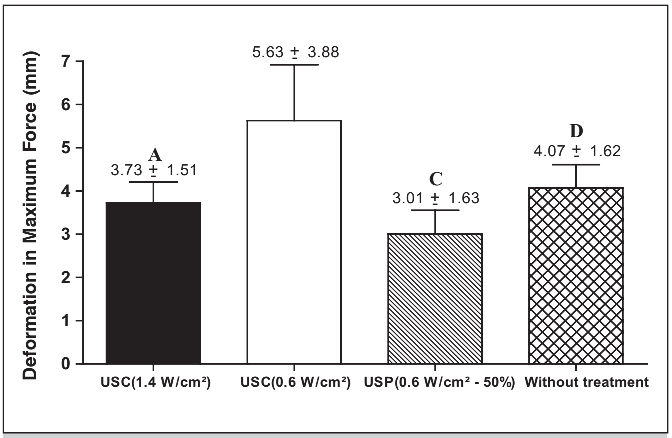

Figure 5 – *Mean values and standard deviation of deformation in maximum force of the four experimental groups.*

Figure 6 shows the mean values and standard deviation for stiffness of the four experimental groups, and as is the case of the other parameters, there was no statistically significant difference among groups (p=0.48).

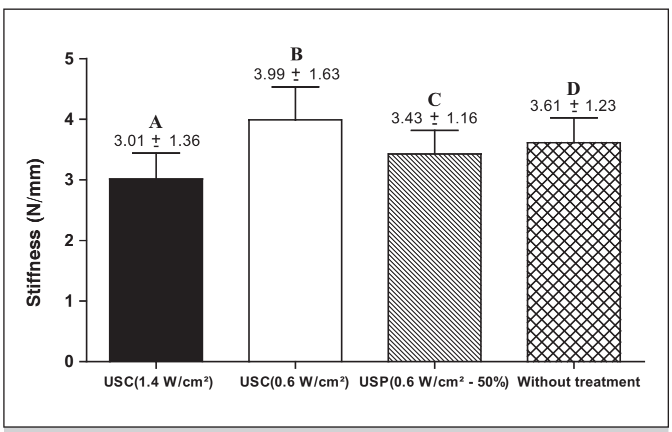

Figure 6 – *Mean values and standard deviation of stiffness of the four experimental groups.*

The analysis of results of the percentage of tendons from each experimental group that supported a load equal to or higher than each one of the values established for the mechanical property maximum force (Table 1), shows that the percentage values of tendons from each group referring to maximum force higher than or equal to 8 N are relatively close. Now the values for maximum force above or equal to 16, 20 and 24 N, are always higher for group B (USC – 0.6 W/cm^2), which might indicate that the tendons from this group tolerate a higher load than the tendons from the other groups. However, there were no statistically significant differences among groups for the four values of maximum force established, although the statistical analysis indicates a strong tendency for group B to have a significantly higher percentage of values in maximum force, higher than or equal to 16 N (p=0.07), higher than or equal to 20 N (p=0.09) and higher than or equal to 24 N (p=0.09), than group A (USC – 1.4 W/cm^2).

Table 1 – *Distribution of percentages of tendons from the four experimental groups in keeping with the values established for maximum force.*

Maximum force (N)	Group A n (%)	Group B n (%)	Group C n (%)	Group D n (%)
< 8	4 (40)	2 (22)	4 (45)	2 (22)
≥ 8	6 (60)	7 (78)	5 (55)	7 (78)
< 16	8 (80)	3 (33)	6 (67)	7 (78)
≥ 16	2 (20)	6 (67)	3 (33)	2 (22)
< 20	10 (100)	6 (67)	8 (89)	7 (78)
≥ 20	0 (0)	3 (33)	1 (11)	2 (22)
< 24	10 (100)	6 (67)	9 (100)	9 (100)
≥ 24	0 (0)	3 (33)	0 (0)	0 (0)

Qualitative histological analysis

The results of the histological analysis revealed that the findings, at the extremities of the tendinous tissue, were similar for all the tendons, where fibrocytes were found arranged in parallel, interspersed with collagen and little vascularization. In the central region of this tissue, which corresponds to the suture site, for the tendons from groups A, C and D they found fibroblastic proliferation with deposit of collagen fibers arranged in parallel with either dense or slack characteristics. Now for group B (USC – 0.6 W/cm^2 SATA - 50%), in addition to fibroblastic proliferation, intense vascular reaction was also observed with collagen fibers arranged in parallel in a totally dense manner, which might suggest a better organization of this collagen.

DISCUSSION

The results presented showed that, although the tendons from group B presented in absolute numbers higher values for the mechanical property maximum force, there were no statistically significant differences among the experimental groups, demonstrating that therapeutic ultrasound did not interfere in the mechanical properties of the flexor tendon. These findings coincide with those demonstrated by Stevenson et al.,[5] Turner et al.[6] and Romano,[7] who did not find an improvement in the mechanical properties in flexor tendons of rabbits irradiated with ultrasound either.

However, it is worth emphasizing here some differences in absolute numbers, for the mechanical property maximum force, which was higher for group B, irradiated with continuous ultrasound at an intensity of 0.6 W/cm^2, which might suggest that these tendons became more resistant, as they support a higher load. These results point in the direction of the findings of the study of Enwemeka et al.,[13] who found an improvement in the mechanical properties with intensity of 0.5 W/cm^2 in continuous mode, an intensity very close to that used in the tendons from group B. It can be assumed that the heat produced by intensities around 0.5 W/cm^2 might have contributed to the improvement of the mechanical properties, by having allowed an increase of the extensibility of collagen, a fibrous protein that provides resistance to the tissue. This assumption is based on the study of Backer et al.[14] who, starting from an extensive review of literature, indicate that both the thermal and the non-thermal effects can increase fibroblastic activity, improve collagen extensibility and facilitate mast cells degranulation, contributing to tissue repair. Nonetheless, it is important to emphasize that there are studies that evidence an improvement in the quality of collagen, employing ultrasonic intensities in the pulsed mode, including those of Cunha et al.[8] and Gan et al.[15] On the other hand, the values for maximum force were lower for the tendons from group A, irradiated with ultrasound in the continuous mode at an intensity of 1.4 W/cm², suggesting that these tendons became less resistant. It is possible that an intensity of 1.4 W/cm² in the continuous mode generated excessive heat on the tissue, inhibiting the repair process. It is worth emphasizing, however, that this result found in the current study is in opposition to those presented by Jackson et al.,[16] which evidenced an improvement in the mechanical properties in tendons irradiated with ultrasonic intensity of 1.5 W/cm² in the continuous mode and also to the findings of Ng et al.[9] and Ng et al.,[10] who employed intensities of 1.0 and 2.0 W/cm². Although the studies of Yeung et al.[11] have demonstrated an improvement in the maximum load and stiffness in tendons irradiated with intensities of 0.5 W/cm² SATA, in the pulsed mode, the results of this study showed that the tendons submitted to the application of pulsed ultrasound, with intensity of 0.6 W/cm² SATA, exhibited a biomechanical behavior inferior even to the tendons of the animals from group D, which did not receive any ultrasonic treatment at all. These findings coincide with those demonstrated by Romano,[7] who did not find an improvement in the mechanical properties in flexor tendons of rabbits irradiated with ultrasound in the pulsated mode at 20%, with an intensity of 0.8 W/cm², either. For the parameters deformation in maximum force and stiffness, the mean values obtained by the four experimental groups were very close, suggesting that ultrasound did not interfere in these properties. Now Larsen et al.[12] found in their study a significant decrease of stiffness with the gradual increase of the ultrasonic intensities employed.

Specifically for the mechanical property maximum force, an effort was made to verify the percentage of tendons from each experimental group that supported a load above or equal to 8, 16, 20 and 24 N. The results showed that the percentage values of tendons for maximum force above or equal to 16, 20 and 24 N were always higher for group B, indicating that the tendons of this group tolerated a higher load than the tendons from the other groups. Additionally, although the statistical analysis has not shown significant differences among the experimental groups, the tendons from group B (USC – 0.6 W/cm²) tended to present a superior performance to that of group A (USC – 1.4 W/cm²), in terms of resistance. As far as the histological findings are concerned, we can observe in this study that in the tendon of group B the collagen fibers appeared totally dense, contrary to what was observed in the tendons of the other experimental groups, whose fibers were sometimes dense and sometimes slack. This finding might perhaps explain the better biomechanical behavior of these tendons during the traction tests, considering that there are studies evidencing a relationship between collagen quality and improvement in mechanical properties.[15,17] Finally, it is worth emphasizing the importance of the continuity of scientific studies involving ultrasonic therapy in the tissue repair process, since the results of the various studies in the area are still contradictory. The findings of this study demonstrate that, even though there are no statistically significant differences among the experimental groups, there was an improvement in the resistance of the tendons irradiated with ultrasound. The results found by other authors also indicate satisfactory effects originating from the therapeutic ultrasound in the tendon repair process,[3,8-13,15] but there are also those studies that did not demonstrate an improvement in the mechanical properties in flexor tendons of rabbits irradiated with ultrasound.[5-7]

CONCLUSION

Under the experimental conditions of this survey, we conclude, based on the statistical analysis, that ultrasound did not interfere in the mechanical properties of the flexor tendon after repair.

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REFERENCES

1. Dyson M. Mechanisms involved in therapeutic ultrasound. Physiotherapy. 1987;73:116-20.
2. Ramirez A, Schwane JA, McFarland C, Starcher B. The effect of ultrasound on collagen synthesis and fibroblast proliferation in vitro. Med Sci Sports Exerc. 1997;29:326-32.
3. Enwemeka CS. The effects of therapeutic ultrasound on tendon healing: a biomechanical study. Am J Phys Med Rehabil. 1989;68:283-7.
4. Roberts M, Rutherford JH, Harris D. The effect of ultrasound on tendon repairs in the rabbit. Hand. 1982;14:17-20.
5. Stevenson JH, Pang CJ, Lindsay WK, Zuker RM. Functional, mechanical, and biochemical assessment of ultrasound therapy on tendon healing in the chicken toe. Plast Reconstr Surg. 1986;77:965-72.
6. Turner SM, Powell ES, Ng SS.The effectof ultrasound on the healing of repaired cockerel tendon: is collagen cross-linkage a factor? J Hand Surg Br. 1989;14:428-33.
7. Romano CVG. Os efeitos do ultra-som terapêutico aplicado na fase precoce da cicatrização do tendão flexor. Estudo biomecânico em tendões de coelho [dissertação]. São Carlos: Escola de Engenharia de são Carlos, Universidade de São Paulo; 2001.
8. Cunha A, Parizoto NA, Vidal BC. The effect of therapeutic ultrasound on repair of the Achilles tendon (tendo calcaneous) of the rat. Ultrasound Med Biol. 2001;27:1691-6.
9. Ng CO, Ng GY, See EK, Leung MC. Therapeutic ultrasound improves strength of achilles tendon repair in rats. Ultrasound Med Biol. 2003;29:1501-6.
10. Ng GY , Ng C O , See E K. Comparison of therapeutic ultrasound and exercises for augment tendon healing in rats. Ultrasound Med Biol. 2004;30:1539-43.
11. Yeung CK, Guo X, Ng YF. Pulsed ultrasound treatment accelerates the repair of Achilles tendon rupture in rats. J Orthop Res. 2006;24:193-201.
12. Larsen A, Kritensen G, Thorlancius-Ussing O, Oxlund H. The influence of ultrasound on the mechanical properties of healing tendons in rabbits. Acta Orthop. 2005;76:225-30.
13. Enwemeka CS, Rodriguez O, Mendosa S. The biomechanical effects of low-intensity ultrasound on healing tendons. Ultrasound Med Biol. 1990;16:801-7.
14. Backer KG, Robertson VJ, Duck F. A review of therapeutic ultrasound: biophysical effects. Phys Ther. 2001;81:1351-8.
15. Gan BS, Huys S, Sherebrin MH, Scilley CG. The effects of ultrasound treatment on flexor tendon healing in the chicken limb. J Hand Surg Br. 1995;20:809-14.
16. Jackson BA, Schwane A, Starcher BC. Effects of ultrasound therapy on the repair of Achilles tendon injuries in rats. Med Sci Sports Exerc. 1991;23:171-6.
17. Parry DA, Barnes GR, Craig AS. A comparation of the size distribuition of collagen fibrils in connective tissues as a function of age and a possible relation betweeen fibril size distribuition and mechanical properties. Proc R Soc Lond B Biol Sci. 1978;203:305-21.