Mechanical Stochastic Resonance Therapy Restores Joins and Muscle Injuries on Standard-bred Races Horses

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SUMMARY

Previous studies showed that the Infratonic Stochastic Resonance (SR) therapy increases race performance on standard-bred horses and decreases the risk of inflammation and muscle injury. In the present study we continued investigating the benefits of this therapy on standard-bred racehorses. We expand the research to the analysis of the hyaluronic acid levels and the quality of the synovial fluid within the hocks using the same therapy modality as in the previous study. We also investigated the effects of the therapy on the levels of aspartate aminotransferase (AST) and creatine phosphokinase (CPK) in serum when the therapy was applied from a distance and compare with previous results where the therapy was applied directly to the skin of the horse two times per week. For the therapy from a distance one group of 10 horses was treated from 4 to 11 feet four hours per day, while the second group of 10 horses was used as the untreated control. After 6 weeks of treatment the levels of CPK in serum decrease significantly while the decrease in ASP levels was not significant. Comparing with previous results we found that the changes in CPK and AST levels are more pronounce when the therapy is applied directly to the skin of the horse. On the other hand, we found that the therapy raised the amount of hyaluronic acid within the treated hock within a 15% average, while the untreated hock presented an average decrease of 18%. The therapy also improves the quality of the synovial fluid increasing its volume, viscosity, and clarity. These results indicate that the Infratonic SR therapy normalizes cellular activity in stressed tissue increasing the production of hyaluronic acid and improving the quality of the synovial fluid. We also demonstrated that the Infratonic SR therapy is effective when applied a few feet distance from the horses, finding a statistical significant decrease of CPK in serum.

INTRODUCTION

The Infratonic device is a FDA registered massager that has being used by a number of medical health professionals on athletes and not athletic humans and animals for sport injuries and also by patients after surgery to reduce inflammation, to accelerate wound healing and recovery. Sports injuries decrease functional activities requiring complete or partial immobilization that restricts the athletes or the animals from participating in training and/or sports events. The Infratonic device is an alternative and noninvasive therapy that helps reduce and treat these conditions. Standardbred racehorses provide an excellent model to study inflammation and muscle and joint injuries. These horses represent a homogeneous population that is genetically similar, eat the same daily food and undergo the same daily training. During their normal training and racing regimes they experience constant tissue strain and trauma that affects their entire biomechanical musculoskeletal structure. One of the structures that consistently manifest inflammation is the hocks, which offers an excellent opportunity to achieve consistent results with relatively small numbers of participants.

The Infratonic SR therapy has already proven to be effective in the relief of inflammation and pain in standardbred racehorses (M. Gonzalez and R. Lee, 2013). This previous study was conducted utilizing direct application of the Infratonic device to the dermal layers of the animals. Direct application over specific anatomical areas resulted in a reduction in the infrared thermal gradient within the dermal tissues, indicating a decrease in inflammation, and a reduction in the levels of the enzymes aspartate aminotransferase (AST) and creatine phosphokinase (CPK) in serum, two enzymes used as markers of muscle damage. Other veterinarians have being using this therapeutic device in horses with the same outcome in findings.

The Infratonic device produces mechanical stochastic resonance (SR) that is composed of oscillatory stimulations characterized by a coherent wave, which is superimposed with random noise (for SR review see Hänggi, 2002; Moss et al., 2004 and McDonnell, 2009). The Infratonic device generates low frequency mechanical noise with an acoustical energy below 20 Hz in a chaotic fashion. The Infratonic device dominant frequency range is between 8 and 14 Hz with 80 dB of amplitude (Yount, 2004). The origin of this therapy came from the National Institute of TV and Electro-Acoustics in Beijing, China, while they were researching the amplitude and frequency spectrum of energy emitted from the palms of qigong healers. After measuring the healers' energy the engineer Professor Lu Yan Fang developed a device that simulates their emission. Prof. Lu Yan Fang's device has been enhanced by Infratonic Inc. to include chaotic mechanical noise in the same range of 8 to 14 Hz. The new device is call Infratonic (for human subjects) or Equitonic (for animals) and forms a deep penetrating simulated Qi energy, which is easily applied to meridian points as a type of acupuncture therapy. Because it is an electromechanical device, it allows the study of emitted Qi therapy without involving practitioners, which permits controlled protocols such as the protocol presented here.

SR has been reported in a wide range of systems (Douglas et al., 1993; Braun et al., 1994; Levin et al., 1996; Collins et al., 1996). In humans the SR in the form of electrical vibrating devices producing sub-sensory mechanical noise had been applied via vibrating insoles in patients with diabetic and stroke impairments (Priplata et al., 2006). Other investigators found that SR therapy improves postural stability and tactile sensation (Gravelle et al, 2002; Priplata et al, 2002, 2003; Collins et al 1996, 2003; Dhruv, et al., 2002; Khaodhiar et al., 2003). The SR therapy also helps people with ankle sprains, improving their postural stability (Ross SE, 2007; Ross et al 2004, 2006, 2007), as well as helping to reduce the size of hard-to-heal wounds (Ricci and Afaragan, 2010). The Infratonic SR therapy is safe to apply and has the advantage that can be applied directly to the skin or through cloth or a blanket and it does not include the use of electrodes, thus it does not produce skin irritation.

The mechanism of action of the Infratonic SR therapy is not well understood. Rachlin, Moore and Yount (2012) found that the Infratonic SR stimulation causes changes in membrane permeability. These investigators evaluated cell permeability *in vitro* using the U98 cell line and the fluorophore calcein as a surrogate marker, and analyzed the results by

FACS and by fluorescent microscopy. They found a relative increase of 69% in mean calcein fluorescence intensity within the cells receiving Infratonic SR mechanical noise. It is also important to note that the Infratonic device does not cause any apparent cytotoxicity to cells *in vitro*, as assessed by phase contrast imaging of morphological characteristics (Rachlin et al., 2012), and does not produce genetic damage (Yount et al., 2004). Other investigators using different SR devices found that noise stimulation of peripheral receptors can lead to stochastic-resonance-type effects on the central nervous system (Hidaka et al., 2000 and Manjarrez et al., 2003). It has also been found that mechanical noise stimulates the tissue causing fluctuations in the trans-membrane potential through changes in the permeability of ion channels (Charles et al., 1991; Priplata et al., 2006; Shi et al., 2012), and that the vibratory noise may add mechanical energy to the vibratory stimulus enhancing the vibration transmission through the dermal tissue (Priplata et al., 2006). More research is necessary to expand and clarify the mechanism of action of the Infratonic devices.

The goal of this research was to continue investigating the physiological effect of this therapeutic device on standardbred racehorses. We expanded the study to the analysis of additional biological substances, such as the quality of the synovial fluid and amount of hyaluronic acid within the hocks during direct application of the device to dermal tissues. We also investigate the possibility of applying the Infratonic SR therapy from a distance, specifically its effects on muscle injury through the analysis of the enzymes CPK and AST. The distant therapy consists of placing the device at the back of the treatment stall on the top board aiming to the horses at a distance of 4 to 11 feet four hours per day. The results obtained when the Infratonic wavelengths were emitted over a longer period of time from a distance were compared with a previous study where the therapy was applied directly to the dermis of the animal for 50 minutes two times per week. The advantage of applying the Infratonic SR therapy from a distance is the reduced in time and labor for the therapist on each animal. Most physical therapy modalities have the common characteristic that they are very labor intensive, they require an administering therapist, trainer, groom or owner to expend 45 to 60 minutes or longer on each animal at least once a day. We show that the Infratonic SR therapy improves the wellbeing of the treated animals.

MATERIAL AND METHODS

Infratonic Stochastic Resonance (SR) therapeutic Device. The therapeutic device used was the mechanical SR Equitonic QGM 4.0 unit (equine version of the Infratonic). The Infratonic device is a registered FDA massager used by a number of medical health and veterinarian professionals. The Infratonic and the QGM 4.0 devices were developed by Richard H. Lee (Infratonic Inc., San Juan Capistrano, California).

Animals. Three and four years old genetically similar Standardbred racehorses being fed with the same diet and undergoing the same training programs (daily training and weekly racing) were selected for this project. The horses did not have history of any intra-articular injections within the last thirty days and did not have anything topically applied such as blisters, liniments or poultices within the last two weeks. All horses received a general physical and lameness exam at the initiation of the study. The horses selected did not have current lameness issues and did not have any other physical therapy modality currently being used or which had been used within the 4 days previously to the study. The horses selected were sound and healthy at the initiation of each study. If, during any of the experiments in this study, a horse presented any sign of extreme muscle soreness or lameness, and/or had difficulty training, they were given time off from the normal exercise routine, treated, and/or removed from the study for rehabilitation.

Therapy applied directly to the animal. Ten horses were selected for the hyaluronic acid and synovial fluid analysis. The right side of the horse was treated while the left side of the same animal was use as control. The Infratonic SR therapy was applied twice a week during 6 weeks. The treatment involved the application of the Infratonic device to 12 specific acupuncture points that have an effect within the tissues of the hock: the bladder, stomach, spleen, gall bladder, kidney and liver meridians (for details see Gonzalez and Lee, 2013). These acupuncture points were stimulated only on the right side in a sequence starting at the cranial portion of the animal, proceeding caudally and ending on the distal portion of the right hind limb. The duration of the treatment was about 50 minutes, with 3-4 minutes spent on each of the 12 acupuncture points.

Therapy applied from a distance. Twenty horses were selected for the distance study. These animals were randomly divided into two equal groups; one being a control and the other a treatment group. The exercise schedule proceeded as normal for each individual animal according to the trainer's discretion. Treatment stalls were built by placing the Infratonic device transducer head on the top board in the middle of the back of the stall. The transducer was affixed at a 45-degree angle to the floor of the stall and ten feet above the floor. This positioning would allow the Infratonic SR therapeutic beam to be approximately 4 to 5 feet above the lumbar and gluteal regions of the horse as the animal roamed about its stall on its own free will. When blood samples were taken, the distance from the lumbosacral joint to the transducer would vary from 4 to 11 feet. The treatment stalls were placed in a completely different barn than that which housed the control group. The treatment continued during the 6 weeks period of the experiment as described below. The animals from the treatment group were placed in the treatment stall for a period of four hours each day. This was after they completed their individual exercise regimen for the day. The exercise regimen consisted of one of the three typical protocols: 1) The animal was jogged four miles, bathed, dried and placed in the treatment stalls for 4 hours. 2) The animal was warmed up for one to two miles, trained a mile at close to racing speed, bathed, dried and placed in the treatment stall for 4 hours. 3) The animal rested this day and received treatment for four hours within the treatment stall after hand walking or turnout for a period of time that varied. On the day of the race, the animal received 4 hours of therapy, which would always end at least four hours before racing.

Cytological Examination and quality of the Synovial Fluid. Synovial fluid samples were collected once a week. The samples were collected into tubes containing salts of EDTA to allow cytological examination. 1) Total erythrocyte and leukocyte were counted. 2) An examination for bacteria was made in each sample collected. 3) Total protein was also analyzed. 4) The physical appearance the synovial fluid was performed by inspection of its color and opacity. 5) The Mucin Clot test of the synovial fluid was performed by mixing one part of synovial fluid with four parts of 2% glacial acetic acid (Cohen et al., 1975). A clot forms immediately as a result of the precipitation of the HA and the synovial fluid proteins by the acid. The quality of the clot formed reflects the degree of HA polymerization. When the mucin (hyaluronic acid-protein complex) is normal, a firm tight mass forms in a clear solution. This is described as a "good" mucin clot. Formation of a softer, less compact mass with shreds in a cloudy to turbid solution is described as a ”fair" mucin clot. A "poor" clot is one that is friable, easily broken up, and surrounded by a flocculent cloudy fluid (Perman V., 1980).

Blood Count and Serum Chemistry. Blood was taken from the group of horses participating in the treatment from a distance. Blood samples were taken initially before starting the treatment on all twenty animals and after that twice a week for six weeks. Serum chemistry analysis was conducted on all samples to evaluate for both aspartate aminotransferase (AST) and creatine phosphokinase (CPK) levels present within the blood’s serum.

Statistical Analysis. The statistical calculation for the hyaluronic acid research was performed using the t-test analysis with two tailed distributions and paired samples. The pair sample t-test was used because the same animal was considered as the treated and control variables. By using the same horse as control we eliminate variability between animals. For the CPK and AST serum study we have two groups of horses, one treated and the other the control. The data in this case was analyzed calculating the differences between the pre-treatment and the post-treatment points, allowing easy determination of changes in the enzyme levels in serum. Each weekly point represents the average points from the group of horses participating. A t-test analysis was used to calculate if the difference between the treated and control group were statistically significant. In this case we performed an independent two-sample t-test with equal sample size because we had two separate groups of 10 horses each. We considered that when the t-test value was $P \leq 0.05$ the difference between the samples were statistically significant.

RESULTS

Hyaluronic Acid (HA) levels within the tarsocrural joints (hocks).

The concentration of the HA within the synovial fluid becomes significant in that a lower concentration results in a less efficient lubrication within these joint systems. During the 6 weeks of therapy with the Infratonic device eight of the ten treated hocks experienced an increase in hyaluronic acid concentration within the synovial fluid. By contrast, nine of the ten hocks in the control group experienced a decrease in the hyaluronic acid concentration within the synovial fluid. **Figure #1** shows the average levels of hyaluronic acid within the treated and untreated hock during the 6 weeks treatment. While the hyaluronic acid concentration increases steadily in the hocks treated, it declines continuously within the untreated hocks. The average increase in the hyaluronic acid concentration within the synovial fluid collected from all of the treated hocks during of the duration of the study is 0.15 mg/ml. The average decrease in the hyaluronic acid concentration within the synovial fluid samples collected from all of the untreated hocks is 0.18 mg/ml (**Figure #1**). The differences in concentration in hyaluronic acid between the treated and untreated hock are statistically significant starting on week 3 (**Table #1**) with a P value of 0.014. The difference is more pronounced at the end of the treatment, at week six, with a P value of 0.0005 (**Table #1**).

It is important to notice that only the right half of the animal was treated which correlated with a definite decrease in discomfort that the horse experienced within the musculature along the lumbar spine along the treated side only. This was determined through digital palpation of these anatomical areas. The differences in discomfort were especially evident the day after a hard training session when the right side (treated) palpated as being less painful than the left side (untreated).

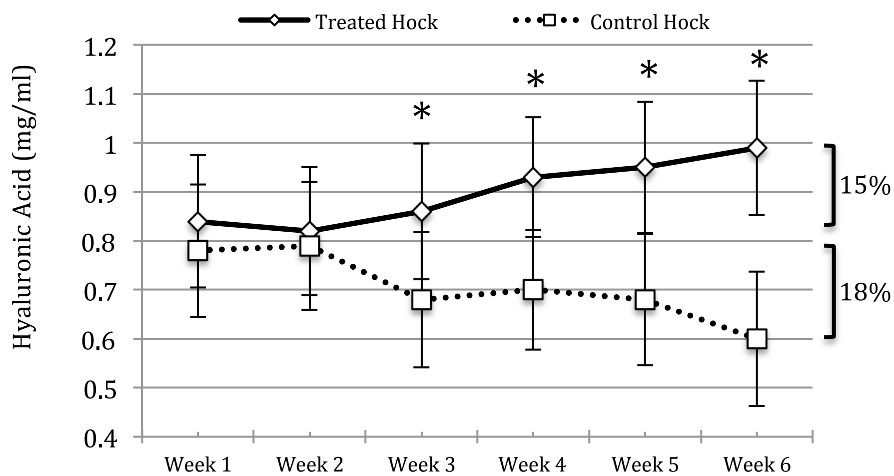

Figure #1. Hyaluronic Acid (HA) concentration within the hocks. A group of 10 standard bred race horses in active training were treated with the Infratonic device. The right hock was treated (solid line) while the left hock (dot line) of the same horse was used as un-treated control. The Infratonic treatment increases HA within the treated hock, while it decreases within the control hock. The (*) symbol indicates that the differences between the treated and control hocks are statistical significant on weeks 3, 4, 5 and 6 (see **Table #1**). The average increase in HA within the treated hock is 15%, while the control hock presented an average decrease of 18%.

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
P values	0.0811	0.2788	0.0140	0.0016	0.0021	0.0005

Table #1. Hyaluronic Acid statistical analysis. A student T-test was applied between the treated and control hocks. The results are statistical significant from week 3 to week 6, with P values lower than 0.05.

<u>Hocks fluid Analysis.</u>

a) *Total protein concentration within the synovial fluid.* Synovial fluid is a filtrate of the blood plasma where, under normal conditions, large molecular weight proteins are excluded. The results showed that the treated hock of five out of ten animals experienced an immediate drop in protein levels within the first week of the study while the decrease in the protein level on the other five was more gradual throughout the six-week duration (**Table #2**). Those animals that started with total protein concentration levels within the normal limits of 5-12 mg/ml experienced a lesser drop than those animals with higher levels at the initiation. The untreated hock of seven out of ten animals underwent an increase in the total protein concentration values during the six weeks therapy, while three animals out of ten experienced a slight decrease in protein concentration values (**Table #3**). These decreases could be theorized as fewer traumas to these hocks due to the animal

Horse	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
1	8.6	8.4	8.42	8.5	8.45	8.5
2	14.6	12.4	12.6	11.5	12.4	12
3	18.4	20.2	16.4	14.8	15.4	17.3
4	10.4	10.8	10.6	10.4	10	10.2
5	13.6	11.2	12.8	12.4	13.2	13.6
6	9.4	8.6	8.8	7.8	7.8	8.2
7	20.5	18.6	19.5	16.4	18.4	18.8
8	15.6	15.2	16.2	15.4	14.6	14.8
9	17.4	17.2	15.9	15.6	16.8	16.4
10	14	14.6	14.2	13.7	13.6	13.2

Table #2. Total protein levels within the right hock synovial fluid of 10 horses. The right hock was treated with the Infratonic.

Horse	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
1	8.6	8.4	8.42	8.5	8.45	8.5
2	14.6	12.4	12.6	11.5	12.4	12
3	18.4	20.2	16.4	14.8	15.4	17.3
4	10.4	10.8	10.6	10.4	10	10.2
5	13.6	11.2	12.8	12.4	13.2	13.6
6	9.4	8.6	8.8	7.8	7.8	8.2
7	20.5	18.6	19.5	16.4	18.4	18.8
8	15.6	15.2	16.2	15.4	14.6	14.8
9	17.4	17.2	15.9	15.6	16.8	16.4
10	14	14.6	14.2	13.7	13.6	13.2

Table #3. Total protein levels within the left hock synovial fluid of 10 horses. The left hock was not treated with the Infratonic.

placing more weight upon the treated limb. **Figure #2** shows the average in protein concentration within the synovial fluid of the hocks of the 10 horses during the 6 weeks therapy. The figure reveals an average decrease in the total protein concentration within the synovial fluid content in the treated right hock. On the other hand, the average in the amount of protein within the synovial fluid of the control hock does not improve during the 6 weeks period. **Table #4** shows the statistic T-test analysis P values, which reveal that the differences in the amount of proteins within the synovial fluid between both hocks on weeks 4, 5 and 6 are statistical significant. These results indicate that the Infratonic SR therapy decreases inflammation as indicated by an improvement in the quality of the synovial fluid.

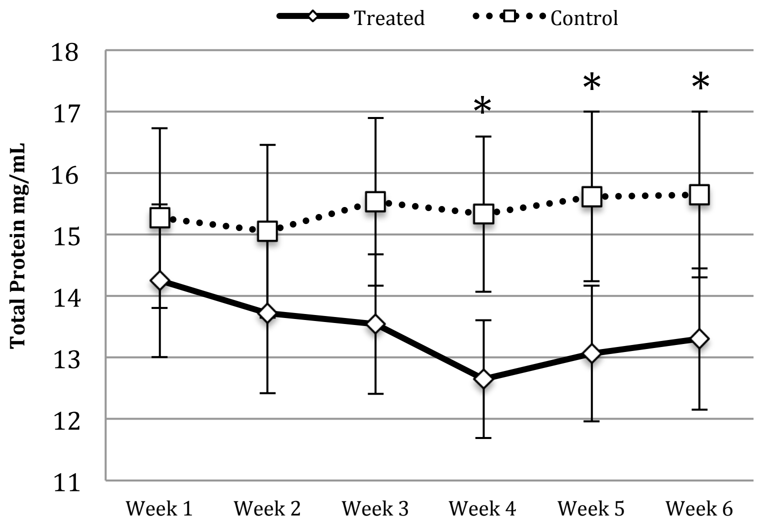

Figure #2. Total protein levels within the synovial fluid. Changes in protein level within the synovial fluid of 10 standard-bred racehorses. The solid line with diamonds represents the treated hocks while the dot line with squares represents the control hocks. The average of the total protein concentration on the treated hock decrease, while the average on the control does not improve during the 6 weeks period. The symbol (*) indicates statistical significant differences.

Time	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
P- Value	0.151	0.115	0.0501	**0.0009**	**0.0006**	**0.0003**

Table #4. Statistic analysis of protein content in synovial fluid. The student statistical T-test analysis was applied to determine the significance from the treated and control hocks. The results show that the differences obtained on week 4, 5 and 6 are statistical significant, with $P<0.05$.

b) *Physical appearance or quality of the Synovial fluid*. Synovial fluid is a lubricating fluid that supplies nutrients and oxygen to cartilage. Total erythrocyte and leukocyte counts were made from each sample. In all the samples taken, the total number of leukocytes present was within the normal limits. In those samples that contain blood, the source of the erythrocytes was from the rupture of sub-synovial capillaries that occurred during the aspiration technique. None of the samples depicted blood that was from an acute arthritic condition. An examination for bacteria was made in each sample collected and there were no bacteria found in any of the samples throughout the study.

A healthy equine synovial fluid is either clear or pale yellow in color with no opacity or suspended particulate material. **Figure #3** shows that six out of 10 of the synovial fluid samples collected from the treated hock improved in color and appearance during the duration of the study. Most of this change in appearance occurred within the first three weeks of the study (**Figure #3A**). By contrast, only one of the samples collected from the control tarsocrural joint changed in appearance **(Figure #3B)**. **Figure #3C** summarizes the

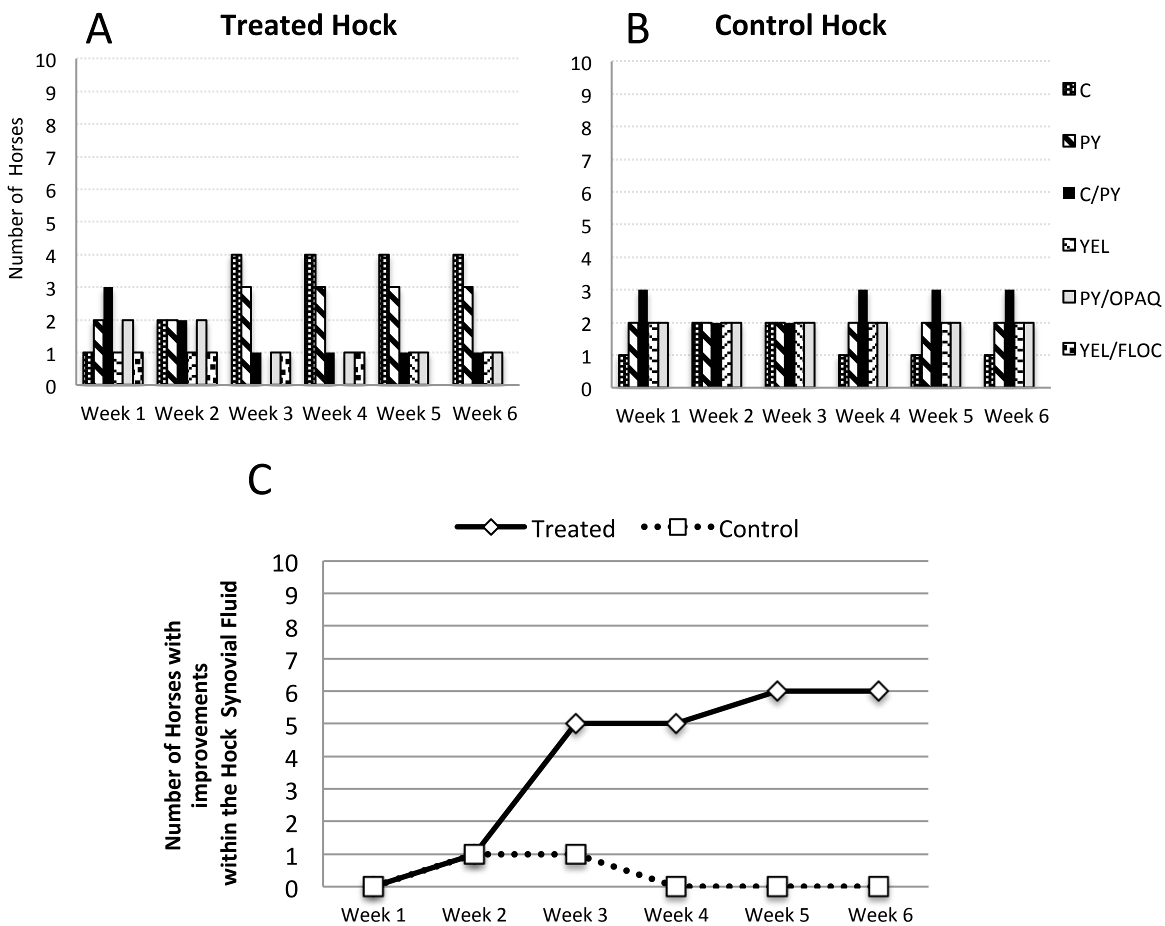

Figure #3. Quality of Synovial Fluid. Appearance of the synovial fluid within the hocks of 10 standard bred race horses in active training. Their right hock were treated with the Infratonic device (**Graph A**) while the left hock was untreated and used as a control (**Graph B**). Clear "**C**" or pale yellow "**PY**" with no opacity or suspended particulate material are normal equine join synovial fluid. When there are problems in the synovial fluid its color changes to yellow "**YEL**" and become opaque "**OPAQ**". If its condition keeps worsening it will contain flocculent material "**FLOC**". **Graph C** shows the number of horses with improve appearance of the synovial fluid within their hocks. The solid line with diamonds are the hocks treated with the Infratonic and the dot line with squares are the control hocks. The results indicate that the Infratonic treatment improves the quality of the synovial fluid.

results showing clearly an increase in the number of horses with improvements within the synovial fluid in the treated hock, while the control hocks maintain the same condition. The results indicate that the Infratonic SR therapy affects the joint synovial fluid in a favorable way.

c) *Mucin Clot Quality of the synovial fluid*.

The quality of the clot formed is a representative indicator of the viscous property of hyaluronic acid. The compactness of the clot and the clarity of the supernatant fluid are the criteria on which the result is based. In general, the more inflamed is the joint the poorer the test results. When the mucin is normal, a firm tight mass forms in a clear solution. This is described as a "good" mucin clot. Formation of a softer, less compact mass with shreds in a cloudy to turbid solution is described as a ”fair" mucin clot. And "poor" clot is one that is friable, easily broken up, and surrounded by a flocculent cloudy fluid. Six out the ten animals improved the mucin clot quality obtained from the treated hock during the duration of the study (**Table #5A**). This indicates that the Infratonic SR treatment improves

Table A

Horse	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
1	G	G	G	G	G	G
2	F	F	G	G	G	G
3	F	F	F	F	G	G
4	G	G	G	G	G	G
5	F	F	G	G	G	G
6	G	G	G	G	G	G
7	P	P	P	F	F	F
8	F	F	F	F	F	F
9	F	F	G	G	G	G
10	F	G	G	G	G	G

Table B

Horse	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
1	G	G	G	G	G	G
2	F	F	F	F	F	F
3	F	F	F	F	F	F
4	G	G	G	G	G	G
5	F	F	F	F	P	P
6	G	G	G	G	G	G
7	P	P	P	P	P	P
8	F	F	F	F	F	F
9	P	P	P	P	P	P
10	F	F	F	F	F	F

Table #5. Analysis of Mucin Clot formation from the synovial fluid of the hocks of horses. A group of ten horses participated in this study and their right hock was treated with the Infratonic (**Table A**) while the left hock was not treated and used as control (**Table B**). Good (**G**) mucin formation indicates a normal HA-protein complex, and it forms a firm tight mass in a clear solution. When the mucin formation is softer, less compact with shreds in a cloudy to turbid solution is described as a ”**fair**” (**F**), and a "**poor**" clot is one that is friable, easily broken up, and surrounded by a flocculent cloudy fluid (**P**).

the viscous property of the hyaluronic acid within the synovial fluid. On the other hand, the horses did not show improvements in the mucin clot formation in the untreated hock and only one of the samples (animal #5) revealed a decrease in the quality of the mucin clot, indicating a decrease in the quality of the hyaluronic acid in this animal (**Table #5B**). The results are summarized in **Figure #4A**, this figure clearly shows an increase in the number of horses with good clot formation and a decrease in the number of horses with fair or poor clot formation on the treated hock. On the other hand, the quality in clot formation in the untreated hock does not improve as shown in **Figure 4B** where, the horses keep the same condition on the left hock throughout the 6 weeks study. The results show that the Infratonic SR therapy improves the quality of the clot formation indicating a decrease in inflammation in the hocks.

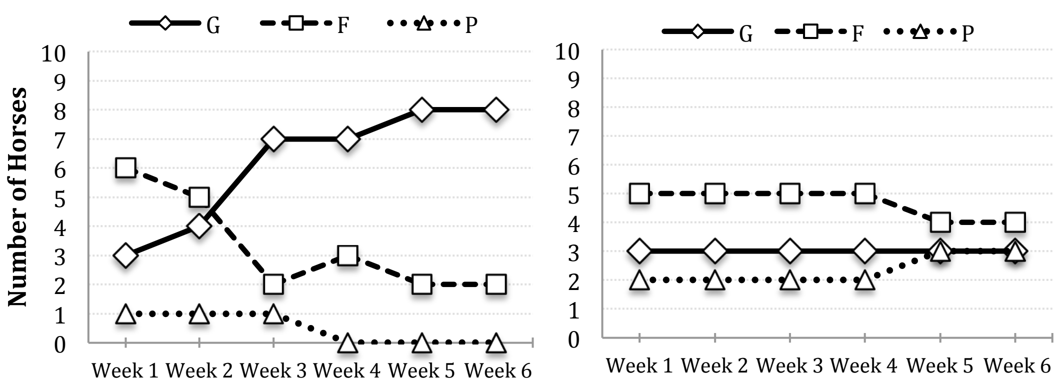

Figure #4. Mucin Clot Quality. A group of 10 standard bred racehorses in active training participated in this study and the formation of the mucin clot from both hocks was analyzed. The horses right hock was treated with the Infratonic (**Graph A at the letf**) while their left hock was not treated and used as the control (**Graph B at the right**). "**G**" good, "**F**" fair and "**P"** poor mucin formation. The number of horses with improvements in mucin formation within the treated hock increase, while their untreated hock does not change significantly throughout the 6 weeks.

<u>Changes in CPK and AST levels during the long distance treatment.</u>

The group of horses treated with the Infratonic device experienced a continuous decrease in CPK levels in serum during the duration of the study, with an average of 53u/L more CPK at pre-treatment time than at week 6, the difference was statistically significant ($P \leq 0.043$). By contrast, the control group depicts an almost constant level of CPK throughout the 6 weeks (**Figure #5A**). The levels in CPK between the treated and untreated group were statistically significant throughout the 6 weeks period (**Table #6**), indicating a healing effect due to the therapy. However, the differences in the AST levels between the treated and control group of horses were not statistically significant (**Figure #5B, Table#6**). Similar to the CPK, the AST levels in the control group were almost constant during the 6 weeks experiment, but the treated group shows a steady decrease in values with an average of 34 u/L less AST at week 6 than at pre-treatment, although the differences were not statistically significant. The lowering of the CPK levels in the treated group can be attributed to a decrease in the inflammatory response within the muscle tissues even during hard training.

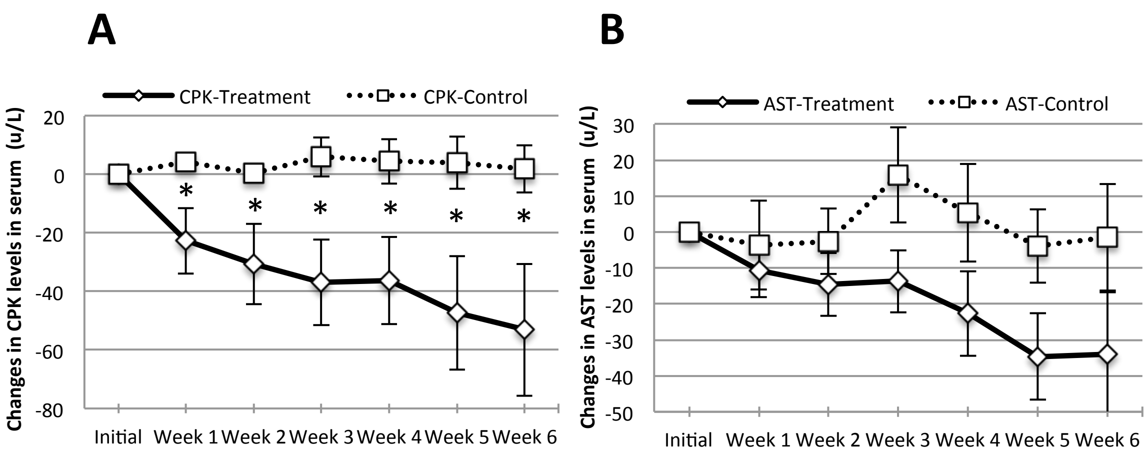

Figure #5. Long distant treatment with the Infratonic. Two groups of 10 standardbred racehorses in active training participated in this study. One group received long distance treatment with the Infratonic to a minimum distance of 4 to 5 feet (solid line with diamonds). The second group was not treated (dot line with squares). Measurements in CPK levels (**Graph A**) and AST levels (**Graph B**) in serum. The symbol (*) indicates that the differences between the treated and untreated groups are statistical significant (see **Table #6**).

Enzymes	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
CPK	**0.028**	**0.0406**	**0.0162**	**0.0261**	**0.0277**	**0.0342**
AST	**0.6225**	**0.3534**	**0.0788**	**0.1349**	**0.0668**	**0.1702**

Table #6. T-test statistical analysis showing the P-values for CPK or AST on serum after a long distant treatment with the Infratonic. The differences in CPK between the treated and control group are statistical significant with values < 0.05. The differences in AST are not statistical significant, but there is a trend line showing a tendency to decrease over time.

DISCUSSION

In the present study we found that the Infratonic SR therapy increases the production of HA within the hocks of standard bred racehorses with an average of 15%, while the untreated hocks underwent an 18% reduction. The amount of HA diverged by 33% between the hocks, a difference that is statistically significant. In addition, the Infratonic SR therapy also improves the quality of synovial fluid within the treated hocks increasing its volume, viscosity, and clarity. These results suggest that the treatment prevents tissue trauma and correlates with a previous study where we found a reduction in inflammation measured by infrared thermography, as well as a reduction in tissue damage, measured by the decrease in levels of CPK and AST in serum (Gonzalez and Lee, 2013). In the present study we also found that the application of the Infratonic SR therapy from a distance has beneficial effects in the well being of the animals, finding a significant reduction in the CPK enzyme in serum. The effect of the therapy from a distance is lower than when the device is applied directly to the dermis of the horse, in which case both CPK and AST decrease significantly (Gonzalez and Lee, 2013). These results together indicate that the Infratonic SR therapy helps to prevent joint and muscle injury and helps regenerate damaged tissue more quickly.

Lubrication of the equine hock joints depends upon two systems, the soft tissue and the cartilage system. Both of these systems rely on molecules of hyaluronate that are found within the synovial fluid. Hyaluronic acid (HA) or hyaluronate along with other fluids within the synovial fluid, form a boundary between these anatomical structures. The synovial fluid (SF) is a filtrate of the plasma and regulates many important physiologic functions in the body. The SF viscosity is due to the presence of HA (Radin et al., 1970, 1971). The structure of the HA was determined by Meyer and coworkers in 1958, and it consist of multiples units of the disaccharide glycosaminoglycan composed of glucoronic acid and N-acetilglucosamina (review Laurent and Fraser 1992, Necas et al., 2008). The HA polysaccharide is high concentrated in the synovial fluid that bathes the joints. HA polymer functions as lubricant, shock absorber, nutrient carrier and filter, regulating the transport of cells and large plasma proteins within the joint tissue (Ogston and Stainer, 1953; Laurent and Frases, 1992; Bell et al 2006). Schmidt and collaborators (2007) found that HA contributes in a dose dependent manner to the boundary lubrication function. Therefore, a decrease in the amount of HA impairs joint health. In our study we found that the Infratonic SR treatment has the effect of increasing the amount of HA within the treated hock, while within the untreated hocks the HA decreased. The average difference of HA between the treated and untreated hock was statistical significant from week 3 to week 6. The results indicated that untreated hocks have a higher propensity to develop traumatized synovial lining in the joins. The decrease in HA is probably a consequence of the production of inflammatory chemicals that break down the HA resulting in poorly lubricated joints. Different investigators had found lower concentrations of HA in traumatized and injured joins (Antonacci et al., 2012), and adding HA to deficient equine synovial fluid restored lubrication function (Kawcak et al., 1997). HA has been used therapeutically for a number of years. Therapeutic injections of HA into the hocks of horses have been common therapy for sore and inflamed joints (Kawcak et al., 1997). HA has also been used by doctors for pain relief in patients. For example patients with osteoarthritis are successfully treated by direct injection of high-molecular-weight HA into the synovial space of an affected joint (Strachan et al., 1990). The mechanism of action is complex and probably involves both the viscoelastic properties of the polymer as well as effects on synovial cells in the joint capsule (Balaz et al., 1974). It has been found that, besides the lubricating action the HA polymer also suppresses cartilage degeneration, reduces pain perception (Strachan et al., 1990, Balaz et al., 1974) and suppress prostaglandin E2 and IL-1 production, which in turn can affect proliferation of synovial cells (Yasui et at., 1992). Thus, the increase in the amount of HA in the hock of horses improves lubrication and healing within the tissue.

The synovial fluid (SF) matrix is a filtrate of the plasma with much lower protein content than in the serum (Balaz et al., 1974). Vascular permeability and synovial membrane permeability are altered by inflammation, which accounts for protein content changes. In the normal SF the large molecular weight proteins and some smaller proteins are completely absent and some other proteins are present in traces (see review in Balaz et al., 1974). However, in all inflammatory joint conditions the concentration of proteins in SF increases, and the missing plasma protein start to appear (Kushner, 1971). This happens because inflammation within the synovial membranes affects the filtration process, becoming "leaky", and the quantity and molecular weight of proteins escaping from the plasma into the synovial fluid increases. This process is called effusion, finding a direct correlation between the increase of the total protein content of the synovial fluid and the amount of inflammation within the synovial membrane. In this study we found statistical significant differences in protein content within the SF between the treated and untreated hocks from week 4 to week 6. The protein content within the SF treated hock decrease while within the untreated hocks increase. These results suggest that the treatment contributes to maintain the integrity of the synovial membrane reducing its damage and inflammation.

One more important diagnosis to measure injury or inflammation within the joints is the appearance of the SF, which is characterized by its color, turbidity, viscosity, and ability to clot. A healthy equine SF is either clear or pale yellow in color with no opacity or suspended particulate material. When there are problems in the synovial fluid its color changes to opaque, and if its condition keeps deteriorating the SF will contain flocculent material. Dark yellow may indicate chronic hemorrhage, and turbid/opaque samples of varied colors are commonly associated with joint inflammation, turbidity is usually due to cells, fibrin, or other debris (Perman, 1980). The group of horses that participated in this study did not show the presence of bacterial within the hocks, indicating that the joints where free of infectious diseases. The results showed that the treated side of the animals improved the appearance of the SF from yellow, opaque and flocculent to pale yellow and clear. The improvements started to show at week 3, indicating that a continuous treatment is important, which corroborate our previous results (Gonzalez and Lee, 2013). By contrast, there were not improvements in color and turbidity within the hocks of the animals that did not received treatment. The viscosity of the SF was also analyzed by its capacity to form a mucin. When there is inflammation within the joint space the HA polymers become damaged, shortening its size and reducing its viscosity and capacity to lubricate. The mucin clot test is a qualitative assessment of the degree of polymerization of the HA-protein complex within the SF and the type of clot formed reflects the degree to which the polymerization of HA has been affected (Cohen, 1975; Perman, 1980; Schumacher, 1977). We showed that the hocks on the treated side of the horses improved the clot mucin formation from poor/fair quality to good quality, while the not treated side did not showed improvements. The results obtained with the color, turbidity and mucin clot formation of the SF indicated that treatment with the Infratonic SR therapy clearly improved the SF appearance indicating a reduction in damage and/or inflammation within the joints.

It is important to mention that after physical examination the horses showed a dramatic desensitization within the musculature along the lumbar spine along the treated side only. This was determined through digital palpation of these anatomical areas. This was especially evident the day after a hard training session when the right treated side palpated as being less painful than the untreated side. This is one more evidence that the Infratonic SR treatment helps to relax the tissues improving the capacity to heal faster. One more important point is that the conditions of the hocks on the untreated side were almost constant throughout the study without dramatic increase in damage and deterioration of the tissue in spite of hard training and racing. It is possible that the treatment in one side has beneficial effects in the other side of the horse, as was found in the previous study (Gonzalez and Lee, 2013). It could be theorized that the animal was placing more weight on the treated right hind limb resulting in lesser amounts of trauma to the left. Therefore, the inflammatory response on the left was lessened to some degree by treatment upon the right. One of the possible explanations for these results is that the analgesic and anti-inflammatory effect upon the treated side allowed the animal to take some of the strain of training off the left side. This effect has been found before where horses stress a different side or part of their body trying to compensate for the primary join or musculoskeletal problem (Seem M. 1993, Schoen AM, 2000). An additional explanation could be that the Infratonic SR mechanical noise and massaging stimulates muscle fibers, sensory receptors and/or afferent nerves, and transmits the signal through the nervous system, releasing components that have their subsequent effects throughout the body. In this way, a treatment applied on the right side of the body might also alleviate the stress or trauma on the left side. In addition, it is also possible that the increase in the HA production in one side of the body have additional benefits to the rest of the body of the animal by the transport of the HA molecules throughout the body. More research will be necessary to confirm these hypotheses.

In this study we also found that the application of the Infratonic SR therapy from a distance has an improved measurable effect in the muscle condition of the animal. We studied the effectiveness of the treatment from a distance analyzing the levels of CPK and AST in serum and comparing the results with a previous study where the treatment was applied directly to the skin of the animal. Plasma CPK and AST are used to diagnose muscle injury arising from myofibrillar and cell disruption (Fallon et al., 1999, Clarkson et al. 1992;

Kuiper, 1994; Noakes, 1987, Janssen et al, 1989). Muscle cell disruption contributes to strength deficits and low performance (Suzuki et al., 2006), thus an increase on these enzyme markers in serum is an indication of muscle damage and inflammation. Increased amounts of CPK and AST in serum has been reported in horse runners, sled dog runners, human marathon runners and humans after eccentric exercises (Child et al., 1999; Evants et al., 1986; Hong et al., 1984; Peterson et al., 2008; Kim, 2007; Marlin et al., 2002, Piercy et al., 2000). We found that the serum CPK levels in the group of horses treated with the Infratonic device showed a continuous decrease and that the changes detected between the treated and untreated groups were statistically significant. In this study we found that the average decrease of CPK in serum was about 53 u/L, while in previous study when applying the therapy directly to the skin the decrease in the levels of this enzyme was 3 times higher (150u/L). Although the AST levels in the treated group also showed a steady decrease in serum the differences between the treated and control groups were not statistically significant. In this study the results showed an average decrease in AST of 34u/L, and in the previous study when the treatment was applied directly to the dermis the average decrease was about 3 times higher (92u/L) and was statistically significant. These results together indicate that the direct application of the Infratonic device on the dermis is more effective than its application from a distance. Based on these results we will be able to improve the therapy from a distance, for example the treatment time period can be more frequent or can be increased to more than 4 hours, and also more than one Infratonic device can be arranged in the stalls to reduce the distance from the horses. The new Equitonic 9 includes a feature that causes the device to turn on automatically four times per day for 3 hours each, which will potentially increase the effectiveness of the treatment. This protocol has not been investigated yet in a controlled study.

This research validate previous findings and together demonstrates that the Infratonic SR therapy helps to release trauma and inflammation in the tissue of horses in active training. Joint discomfort and muscle damage in horses decrease their athletic performance and quality of life, causing interruption in training, culling, animal loss and economic burden (Gonzalez and Lee 2013, Rossdale et al., 1983; Kidd et al., 2001; and Dyson et al., 2008). The intense exhaustive exercise may set the stage for a chronic inflammatory condition and predisposition to infection, and/or onset of chronic diseases (Lamprecht and Williams, 2012). Any improvement in prevention and treatment is useful to reduce these health disorders. Standard bred racehorses in active training and racing provide a realistic, controlled and repeatable model of muscle and joint dysfunction under dynamic physiologic stress (Padalino et al., 2007). In this report we show that treatments with the Infratonic device improve animal welfare and longevity. This study and the previous research with the Infratonic device in standard bred racehorses show that this therapy is effective in reducing the inflammatory response within the tarsocrural joints, increases the amount of HA within the hocks and improves the quality of the synovial fluid, increasing its volume, viscosity, and clarity. It also decreases the enzymes markers of tissue damage CPK and AST in serum. These are compelling evidence that Infratonic SR therapy normalizes cellular activity in traumatized cells. Moreover, we demonstrate that this therapy can also be applied from a distance having the effect of reducing the CPK levels in serum. The benefits of the Infratonic SR therapy can be expanded to other athletic animals and human athletes, and for muscle and bone injuries and after surgery as an adjunct therapy.

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