An Investigation into the Nature of Inflammation in the Hocks of Horses with Mechanical Stochastic Resonance Acupuncture Therapy

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Summary

Healthy racehorses in active training, as well as human athletes, experience constant musculoskeletal stress and trauma that affect their performance. In this study we evaluated the effectiveness of a mechanical stochastic resonance (SR) device in treating inflammation and muscle injury in standardbred racehorses. Although inflammation is often a part of the body's natural response to tissue injury which can initiate a healing process, inflammation is frequently painful, can impede healing, and often leads to hypersensitivity to mechanical stimuli, which is difficult to treat clinically. The Infratonic device generates a low frequency mechanical noise, or SR, able to treat this condition without pain. We measured the temperature on the hocks of 10 horses in active training by infrared thermography before and after Infratonic treatment. The results show that Infratonic therapy is able to decrease the temperature in the treated hocks, indicating a reduction in inflammation. Moreover, this result correlates with our finding of a decrease in the amount of serum enzymes, markers of muscle damage, aspartate aminotransferase (AST) and creatine phosphokinase (CPK). We demonstrate that Infratonic therapy is effective in reducing inflammation and muscle damage in standardbred racehorses and suggest that it might be also effective in human athletes.

Introduction

Sports injuries decrease functional activities in athletics. Some sports injuries include muscle damage that results in swelling or inflammation, soft tissue injuries, ankle sprains, knee injuries etc. Most of these injuries require complete or partial immobilization that restricts the athletes from participating in training and sports events. Sports injuries affect humans as well as animals. In this research we study muscle injury and hock (ankle) inflammation in standardbred racehorses, and investigate a stochastic resonance (SR) therapy that we postulate might prevent and alleviate these injuries.

The therapy consists of applying mechanical stochastic resonance (SR) using the Equitonic QGM device [equine version of the Infratonic]. SR consists 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 generates low frequency mechanical noise with an acoustical energy below 20 Hz in a chaotic fashion. The Infratonic dominant frequency range is between 8 and 14 Hz with 80 dB of amplitude (Yount, 2004). The origin of this therapy was conducted at the national Institute of TV and Electro-Acoustics in Beijing, China, which measured the amplitude and frequency spectrum of energy emitted from the palms of qigong healers. The Infratonic device, which simulates this emission, 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 study of emitted Qi therapy without involving practitioners, which permits controlled protocols such as the protocol presented here. This stimulation is safely applied directly on the skin and also can travel through cloth or a blanket. The Infratonic does not include the use of electrodes, thus it has the advantage of not producing skin irritation.

SR has been reported in a wide range of systems. In biology specifically, SR has been demonstrated experimentally in various sensory neural systems, including crayfish (Douglas et al., 1993), shark (Braun et al., 1994), cricket (Levin et al., 1996), and humans (Collins et al., 1996). In humans, for example, the application of SR in the form of sub-sensory mechanical noise to the soles of the feet via vibrating insoles enhanced balance in patients with diabetic and stroke impairments (Priplata et al., 2006). SR therapy also improves postural stability and tactile sensation in healthy young and elderly individuals (Gravelle et al, 2002; Priplata et al, 2002, 2003; Collins et al 1996, 2003; Dhruv, et al., 2002). SR in the form of low level electrical noise applied to neuropathic diabetic patients improves their ability to perceive sensory stimuli (Khaodhiar et al., 2003) and reduce the size of hard-to-heal wounds (Ricci and Afaragan, 2010). It has also been found that SR helps people with ankle sprains, improving their postural stability (Ross SE, 2007; Ross et al 2004, 2006, 2007).

The biological process by which the SR therapy improves physical symptoms is not clearly understood, but various mechanisms have been proposed. Hidaka et al. (2000) and Manjarrez et al. (2003) found that noise stimulation of peripheral receptors can lead to stochastic-resonance-type effects in the central nervous system. Other investigators 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). It has also been proposed that vibratory noise may add mechanical energy to the vibratory stimulus enhancing the vibration transmission through the dermal tissue (Priplata et al., 2006). The mechanism of action of the Infratonic therapy could be one, or a combination of these postulates. Rachlin, Moore and Yount (2012) found that the Infratonic 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 in cells receiving Infratonic 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).

Standardbred racehorses provide an excellent model to study inflammation and muscle injury. They 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 hock, which offers an excellent opportunity to achieve consistent results with relatively small numbers of participants. During this study inflammation in the hocks was evaluated using infrared thermography. Infrared thermography is a noninvasive technique that measures heat emitted from the body surface as infrared radiation (Turner, 1991). It gives images of temperature gradients showing areas where the heat increases or decreases making it useful for the detection of inflammation (Latif Emrah, 2007; Tracy Turner, 1998). In addition, we measure muscle injury by analyzing serum chemistry with specific attention to enzyme markers for muscle damage, the enzymes aspartate aminotransferase (AST) and creatine phosphokinase (CPK). Inflammatory conditions and muscle damage in horses would account for a decrease in performance. Therefore, the purpose of this study was to determine the effectiveness of the Infratonic therapy in reducing inflammation and muscle damage in standardbred racehorses.

Material and Methods

Animals. Three year old genetically similar Standardbred horses being fed 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 treatment to the hocks within the last three months and did not have anything topically applied on the hocks for the last two weeks. All horses received a general physical and lameness exam at the initiation of 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, it was given time off from the normal exercise routine, treated, and/or removed from the study for rehabilitation.

Therapy. The therapy was applied using the mechanical stochastic resonance (SR) acupuncture stimulator, Equitonic QGM 4.0 unit (equine version of the Infratonic), Infratonic Inc., San Juan Capistrano, California. The therapy consisted of the direct application of the Infratonic over acupuncture points on the skin of the horse. The treatment varied depending on the experiment. Basically, the Infratonic was applied directly on the hock for 10 or 20 minutes at 5 different acupoints for 2 or 4 minutes each. A different treatment involved the application of the Infratonic to 12 acupuncture points that have an effect on the hock, for 3 to 4 minutes each point for a total of 50 min treatment (see details below).

Inflammation study using infrared thermography. Inflammation was analyzed by measuring the surface temperature on the hocks by infrared thermography. The thermal images of the horse's hocks were taken from the front legs looking back to the hind limbs. Thus, the thermograph pictures in this study show the left hock on the right side of the image and the right hock on the left side of the image. Four different experiments were conducted to evaluate the effectiveness of the Infratonic to affect the thermal gradient on the hocks. A group of 10 horses participated in each experiment. The right hock of each horse was treated with the Infratonic while the left hock was used as the untreated control.

(**A**) The first experiment consisted of one single treatment where the Infratonic was applied directly on 5 acupoint of the right hock for about 2 minutes each for a total time of 10 minutes. The acupoints on the hock treated were Bl 60, Bl 62, St 41, Sp 5 and Ki 3 (See **Figure #1** and **Table #1** for details). Infrared thermographs were taken of both hocks before the treatment and 0.5, 1, 2, 4 and 6 hours post-treatment.

(B) The second experiment consisted of one treatment where the Infratonic was applied directly on each acupoint on the right hock for about 4 minutes for a total treatment time of 20 minutes. Infrared thermographs were taken from both hocks before the treatment and 0.5, 1, 2, 4, 6, 12, 24 and 36 hours post-treatment.

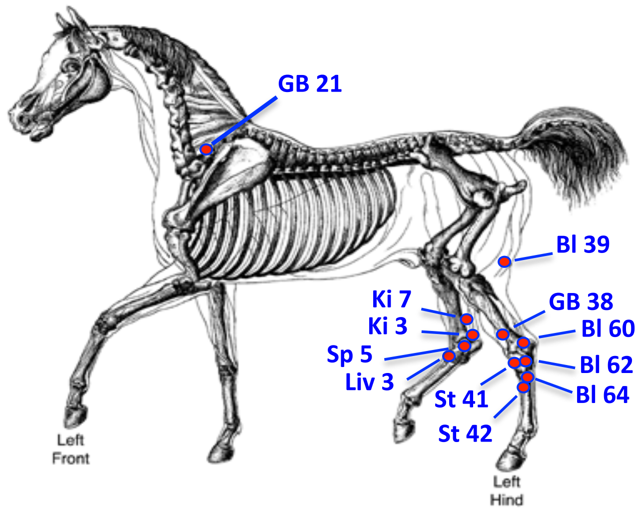

Figure #1. Schematic representation of a horse showing the anatomical locations of the treated acupoints. See Table #1 for acupoints description.

Meridian	Acupoints	Acupuncture Points Anatomical Locations
Bladder	Bl 39	Depression at the end of the groove between the biceps femoris and semitendinosus muscles
	Bl 60	Between the lateral condyle of the tibia and the tuber calcus
	Bl 62	Depression distal to the lateral malleolus
	Bl 64	Caudodistal to the head of the fourth metatarsal bone
Stomach	St 41	Junction of the tarsus and metatarsus, at the level of the lateral malleolus, between the long and lateral digital extensor, proximal to the intermediate retinaculum
	St 42	Distal to the hock point on the craniolateral aspect of the metatarsus, proximal to the distal retinaculum
Spleen	Sp 5	In a depression distal to the medial malleolus, proximal to the tendon of the tibia cranial muscle
Gall Bladder	GB 21	Cranial to first thoracic vertebra, between seven cervical and first thoracic vertebrae, overlying the serratus ventralis muscle, half way craneoventrally on the cranial edge of the scapula
	GB 38	Cranioproximal to the tip of the lateral malleolus of the tibia
Kidney	Ki 3	Within a depression between the medial malleolus and the tendocalcaneus, at the level of the tip of the medial malleolus
	Ki 7	Located proximally to kidney point 3 on the cranial border of the tendocalcaneus
Liver	Liv 3	On the craniomedial aspect of the third metatarsal bone, at the level of the head of the second metatarsal bone

Table #1. Anatomical locations of the acupuncture points used in this research. During the Infrared Thermograph experiments 1, 2 and 3, were treated the acupoints Ki 3, SP 5, St 41, Bl 60 and Bl 62. For the rest of the experiments all the acupoints on this table were treated with the Infratonic device.

(**C**) The third experiment included two 20 minutes treatments, 12 hours apart. The Infratonic was applied directly to the right hock, as in the previous experiment, during both treatments. Infrared thermographs were taken of both hocks at pre-treatment time and 0.5, 1, 2, 4, 6, 12, 24, 36, 48, 72, 96, 120, 144 and 156 hours post-treatment.

(**D**) The fourth experiment involved the application of the Infratonic 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 (see **Table #1**). 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 45 minutes, with 3-4 minutes spent on each of the acupuncture points. Infrared thermographs were taken of both hocks before the treatment and 0.5, 1, 2, 4, 6, 12, 24, 36, 48, 72 and 96 hours post-treatment.

Blood Count and Serum Chemistry (AST and CPK). Two groups of 10 horses were selected for this study. The treatment group received therapy with the Infratonic throughout the study and the control group did not receive any treatment. The treated group received a bilateral treatment involving the standard twelve acupuncture points for the hocks that occur on both sides of the body (see **Table #1** and **Figure #1)**, twice a week. All animals had blood drawn for complete blood count and serum chemistry evaluation (See **Table #2** for detail of parameters evaluated). Two blood samples were taken initially to determine a mean baseline level (pre-treatment), and then 6 more times as follows: upon completion of the Infratonic treatment, and then 1, 4, 6, 12, 24 and 48 hours post-treatment, twice a week. The control group also had blood drawn at the same time intervals as the treated group.

Statistical Analysis. Analysis of thermal gradients was performed calculating the percent change from the initial thermograph. Each column on the graphs from **Figure #2** shows the average and the error bars of each specific group. The statistical calculation 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 study, the data was analyzed calculating the differences between the pre-treatment and the post-treatment points, allowing easy determination of changes of the enzyme levels in serum. During the 48 hours analysis, each time point in the graph represents the average obtained from the group of animals during the 6 weeks of the study. On the analysis during the 6 weeks period, each week point represents the average from the group of horses participating. Although the control group started with 10 horses, the analysis only includes the average of 6 horses because 4 were pulled out of the study between week 4 and week 6. A t-test analysis was used to calculate if the difference between the samples were statistically significant. In this case we performed an independent two-sample t-test because we had two separate groups of horses, the treated and the control group. We also used the t-test formula for an unequal sample size because the treated group had 10 animals, and the control group 6 animals. We considered that when the t-test value was $P \leq 0.05$ the differences between the samples were statistically significant.

Parameters Analyzed from Blood and Serum		
Blood Count	Red Blood cells	Total Erythrocytes
	Total white blood cells	Total Leucocytes
	White blood cells	Lymphocytes
		Granulocytes
		Neutrophils
		Eosinophil
		Basophils
	Platelets	Thrombocytes
	All cell lines	Total cells
	Packed cell volume	Volume
Serum Chemistry	Albumin levels	35-50% of the serum protein
	Albumin/Globulin ratio	Total protein values
	Globulin levels	Total globulin
	Serum protein	Nutritive function
	Aspartate aminotransferase (AST)	Muscle injury
	Creatine phosphokinase(CPK)	
	Blood Urea Nitrogen (BUN)	Renal Function
	Gamma Glutamyl Transpeptidase (GGT)	
	Creatinine	
	Alkaline Phosphatase levels	Hepatic function
	Total bilirubin	
	Icteric Index	
	Lipemic index	
	Sodium	Electrolyte balance
	Potassium	
	Chloride	
	Calcium	Calcium metabolism
	Magnesium	Magnesium metabolism
	Phosphorus	Phosphorus metabolism
	Glucose	Measure to monitor other disease
	Hemolytic Index	A value that may affect other tests
	Nitric Oxide	Release from the vascular walls

Table #2. Parameters measured from the blood test and serum analysis.

Results

Inflammation

To determine the effectiveness of the Infratonic acupuncture therapy in reducing inflammation, we measured the hock surface temperature by infrared thermography. All the horses that participated were in active training, and were evaluated by a general physical exam before starting and during each study. Different Infratonic acupuncture protocols were investigated to establish the best therapy to treat inflammation on the hocks. Four experiments with a group of 10 horses each were conducted. In all horses, the right hock was treated, while the left hock served as a control. By using the same horse as the control, we eliminated variability between animals. The thermograph was set at 0.5ºC per isothermic level. All infrared thermographs were measured and calculated to determine the percent change in the thermal gradient from the initial thermograph.

A) Experiment #1. The application of the Infratonic for 10 minutes created a significant change within the infrared thermograph. As an example from the group of 10 horses, we include here the description for animal #2, the right hock showed a thermal gradient that was more intense over the medial portion of the talus, and then becomes diffuse distally over the medial portion of the hock surface. The left hock initially revealed a thermal gradient that was generalized medially over the entire hock. After only 30 minutes post-treatment, we can observe in **Figure #2A** an average of 22.6% reduction in the temperature of the right hock, while the left hock did not present changes in thermal gradient. The statistical t-test calculation showed a P value of $\leq 1.8 \times 10^{-8}$ at 30 minutes post-treatment and $P \leq 0.0008$ at 4 hours post-treatment, these values indicate that the differences in thermal gradient of the right and left hock are statistically significant. The P values for 1 and 2 hours were also statistically different (See **Table #3**). The effect of the Infratonic therapy was detected almost immediately after treatment (30 minutes). The thermal gradient started to revert back to the initial thermograph after an hour, and at 6 hours post-treatment the temperature was not different than the initial. The untreated left hock retained the initial thermal gradients throughout the entire time frame (**Figure #2A**).

B) Experiment #2. Doubling the Infratonic treatment time to 20 minutes produced a greater effect in the thermal gradient than treating for only 10 minutes (**Figure #2B**). The average percentage in temperature decrease was significantly more, and lasted for a longer period of time when compared with the first experiment (**Figure #2A and 2B**). As an example for this group, we include the description for animal #6. The initial thermograph shows smooth isothermic levels for the right hock with a generalized increase in temperature over the tibiotarsal articulation. The left hock initially revealed a more elevated temperature along the medial surface. As early as 30 minutes post-treatment, we observed a decrease of 49.5% in the average temperature of the right hock. This difference is two times greater when compared with the 22.6% from the 10 minutes treatment in the first experiment (**Figures #2A and #2B**). The left hock did not present changes on its thermal gradient throughout the experiment. The statistical t-test analysis shows a P value of $\leq 4.5 \times 10^{-9}$ at 0.5 hours, $P \leq 5.6 \times 10^{-9}$ at 4 hours, and $P \leq 1.1 \times 10^{-5}$ at 24 hours, indicating that the differences in the thermal gradient of the right and left hock are statistically significant for all these time points. The rest of the time points also presented P values that are statistically significant except for the 36 hours time point where the right hock returned to the original thermal

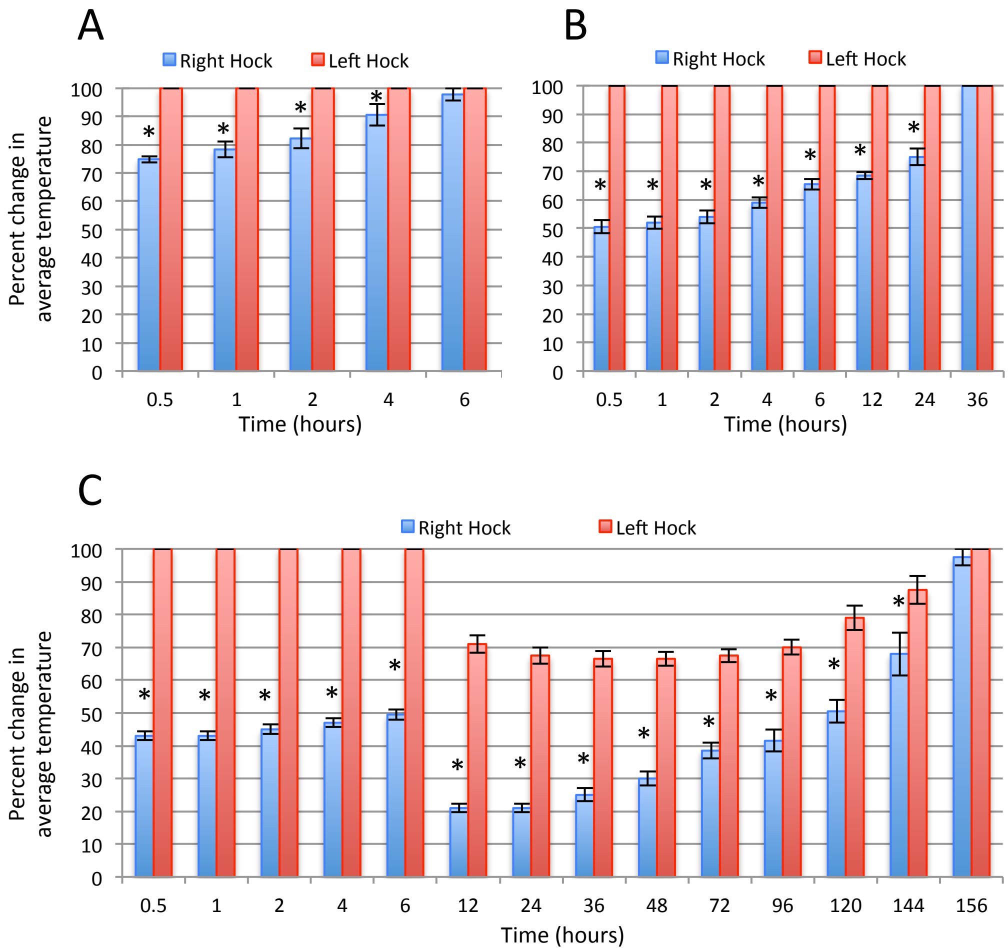

Figure #2. Percent changes in temperature on the right and left hock of standardbred horses as measured by Infrared Thermography. One group of ten horses was evaluated on each experiment. The right hock was treated (blue) and the left hock was used as the untreated control (red). (A) One treatment using the Infratonic directly to 5 acupoints (Ki 3, SP 5, St 41, Bl 60 and Bl 62) on the right hock for two minutes each point and ten minutes total treatment. (B) One treatment to the same acupoints on the right hock as in (A) but for 4 minutes each point and a total of 20 min treatment. (C) Two treatments for 20 minutes each directly to the right hock 12 hours apart, each 20 minutes treatment was applied as on experiment (B). The symbol (*) indicates that the differences between the treated and control hock are statistical significant. The P values are shown in **Table #3**.

gradient, P≤0.34 (see **Table #3**). It is important to note that the thermal gradients started to return to the original temperature after 2 hours, and reverted to their initial readings at 36 hours, which is a longer and better effect than the 6 hours from the first experiment.

	Statistic T-test values		
Hours	**Exp. #1**	**Exp. #2**	**Exp. #3**
0.5	1.85×10^{-8}	4.59×10^{-9}	1.04×10^{-11}
1	6.89×10^{-5}	3.21×10^{-9}	1.04×10^{-11}
2	0.0008	6.41×10^{-9}	2.00×10^{-11}
4	0.036	5.67×10^{-9}	2.00×10^{-11}
6	0.346	2.05×10^{-8}	1.35×10^{-10}
12	NA	1.68×10^{-9}	9.02×10^{-8}
24	NA	1.16×10^{-5}	6.15×10^{-8}
36	NA	NA	2.18×10^{-7}
48	NA	NA	9.45×10^{-9}
72	NA	NA	3.59×10^{-7}
96	NA	NA	4.71×10^{-7}
120	NA	NA	1.30×10^{-5}
144	NA	NA	0.0053
156	NA	NA	0.3434

Table #3. Statistic T-test values obtained from the Infrared Thermography on Experiments #1, #2 and #3. When P values are ≤ 0.05 indicate that the differences from the treated hock and control hock are statistically significant. NA means not applicable or not evaluated.

C) Experiment #3. When the Infratonic therapy was given twice for 20 minutes, 12 hours apart, the decrease in the average of the thermal gradient was more significant, and to a greater extent, than in the first and second experiments. The temperature in the right hock decreased each time the Infratonic was applied and surprisingly, after the second 20 minutes treatment, the left hock also exhibited a decrease in temperature (**Figure #2C**). As an example of the 10 horses of this group, the initial thermograph for animal #7 revealed a greater temperature over the tibiotarsal and intertarsal articulations. This is not an intense thermal gradient but one that is commonly found with a horse in heavy training. The initial thermal gradient on the left hock depicts a generalized higher temperature medially with a greater intensity over the medial portion of the tibiotarsal articulation and the tarsalmetatarsal joint. Only 30 minutes after the first treatment the thermal gradient on the right hock decreased significantly to 57%, and after the second treatment it decreased even more, to 79%. This treatment had a significantly better effect in thermal reduction than the one 10 minute treatment (22.6%) or the one 20 minute treatment (49.5%). The duration of the Infratonic effect was remarkable. The decrease in thermal gradient lasted up to 156 hours, which is a significantly longer period of time than the 6 hours for 10 minutes, or 36 hours for the 20 minute treatment.

Similar to the first and second experiment, the untreated left hock did not change in its thermal gradient during the first treatment period. But unexpectedly the left hock thermal gradient decreased 29% during the first 30 minutes post-second treatment, even though the left hocks of these animals were never treated (**Figure #2C**). The statistical t-test calculation revealed that the differences in the average temperature between the right and left hock were statistically significant for the time points between 0.5 hours to 144 hours, see **Table #2**. The only point that was not statistically different was at 156 hours, where the thermal gradient returned to the original average temperature for both hocks, with a $P \leq 0.34$.

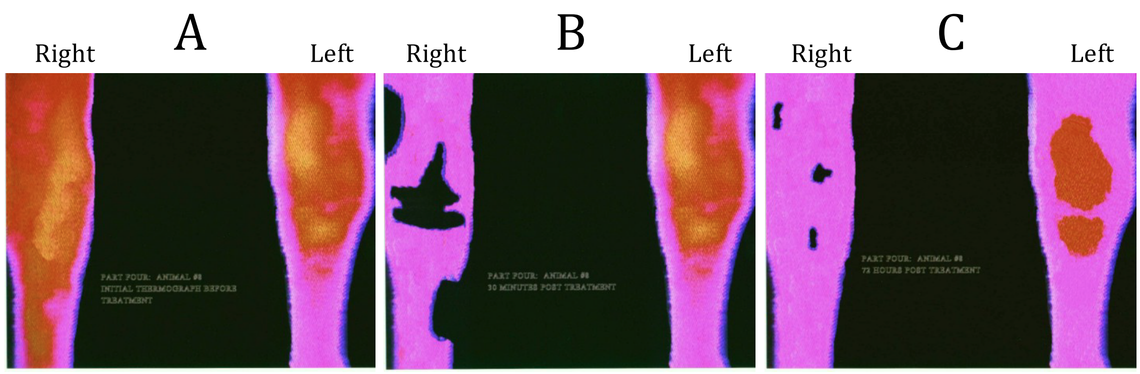

Figure #3. Infrared Thermographs showing the hocks from a horse treated only on the right side. The thermographs were taken from the front legs looking back to the hind limbs, thus the right hock is the hock appearing on the left side of the pictures. The Infratonic was applied for 3-4 minutes on each of the 12 selected acupuncture points. As an example the pictures correspond to both hocks from the racehorse #8. (A) Before treatment; (B) 30 minutes after treatment; and (C) 72 hours after treatment. The black spots on the treated hock (B and C thermographs) indicate that the temperature dropped off the scale.

D) Experiment #4. The last experiment consisted of applying the Infratonic for about 50 minutes covering a greater area of the right side of the animal including the hock (see **Figure #1** and **Table #1** for details). **Figure #3** shows the thermal gradient from horse #8; all 10 animals revealed the same thermograph evidence. Initially, the thermal gradient of the right hock presented a high temperature over the dorsal and medial area of the hock (**Figure #3A**). The left hock contained an increase in the thermal gradient over the medial portion of the hock including the medial aspect of the tibiotarsal articulation and a generalized higher temperature over the tarsalmetatarsal area dorsally (**Figure #3A**). As in previous experiments #1, #2 and #3, the effect of the Infratonic therapy occurred rapidly after treatment, as can be observed after only 30 minutes post-treatment (**Figure #3B** on the left). This effect was observed in all 10 horses and it was so pronounced that the drop in temperature ran beyond the sensitivity of the infrared thermograph. The thermograph was set at 0.5ºC per isothermic level. The black areas in the thermograph picture on **Figure #3B** show a decrease in 3ºC or more within the isothermic level. As a consequence, no thermograph readings were collected, and only the thermograph pictures of horse #8 are shown on **Figure #3** as a representation of the whole group. This type of decrease in temperature was not achieved on experiments #1, #2 and #3, where treatment times were 10 minutes, 20 minutes or twice 20 minutes. After 72 hours, the right hock was still showing a deficit within the thermal gradients (**Figure #3C** left side), although these were now much smaller in area when compared to the thermograph taken at 30 minutes. After 7 days, with the animals still in daily training, the thermal gradients within the right hock had still not even come close to returning to their initial levels. There were also changes in the thermal gradient on the left hock. At 30 minutes post-treatment, there was very little change (**Figure #3A** and **#3B**, on the right), but after 72 hours the thermograph of the left hock revealed a reduction of 75-80% throughout the entire structure (**Figure #3C**). This result correlates with experiment #3, where the left hock also presented a change in the thermal gradient even though this entire side of the animal was never treated.

Serum Chemistry.

The thermograph study showed that the Infratonic therapy has an effect on the surface temperature of the hocks, indicating a reduction in inflammation and healing of the hock tissues. We hypothesized that the Infratonic therapy could also be affecting the blood chemistry and the ability of the horses to stay sound throughout the duration of the study. To answer this question, we investigated the correlation between the thermograph studies and the clinical pathological changes within the serum chemistry in two groups of 10 horses during 6 weeks. One of the groups of horses was the control group and did not receive Infratonic therapy. The treatment chosen for this study was a bilateral therapy spending 3 to 4 minutes per acupoint (12 total from each side) because this was the best result obtained in the previous study. We also compared the physical and athletic abilities of each group, as measured by frequent general exam and by racing and training times.

Horse Performance. The results throughout the 6 weeks study showed that the horses treated with the Infratonic (Group A), were sounder, and demonstrated better attitudes and appetites than the control group (B). It was also found that after racing or a tough training session, all 10 animals in the Infratonic treated Group A recovered much faster than those in the untreated Group B. **Table #4** and **Figure #4** show that all 10 horses in the treated Group A were in active training during the entire study without missing any race or training days. However, in the control Group B, 7 horses were given time off due to lameness or extremely sore muscles. The results show that starting as early as week 3, 2 animals from the control group were given time off (**Table #4**), and this number continued to increase through the end of the study, where seven horses out of 10 were not training. **Figure #4** shows that at the end of the 6 week study, only 30% of the animals in the control group were in active training/racing in comparison with the 100% of the treated animals still training and racing. Moreover, 4 of the animals within the control Group B had to be pulled from this investigation due to various musculoskeletal problems during the study. These horses were treated and given rehabilitation therapy.

Additionally, we found that all horses in the treated Group A were pacing faster, with an average decrease of 1.65 seconds. These animals recovered faster, had a better appetite and were healthier in appearance than those in the control group, which in contrast, had an average increase in training time of 4.07 seconds.

Group A

Horse	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
1	Active Training	Active Training	Active Training	Active Training	Active Training	Active Training
2	Active Training	Active Training	Active Training	Active Training	Active Training	Active Training
3	Active Training	Active Training	Active Training	Active Training	Active Training	Active Training
4	Active Training	Active Training	Active Training	Active Training	Active Training	Active Training
5	Active Training	Active Training	Active Training	Active Training	Active Training	Active Training
6	Active Training	Active Training	Active Training	Active Training	Active Training	Active Training
7	Active Training	Active Training	Active Training	Active Training	Active Training	Active Training
8	Active Training	Active Training	Active Training	Active Training	Active Training	Active Training
9	Active Training	Active Training	Active Training	Active Training	Active Training	Active Training
10	Active Training	Active Training	Active Training	Active Training	Active Training	Active Training

Group B

Horse	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
1	Active Training	Active Training	Active Training	Active Training	Active Training	Active Training
2	Active Training	Active Training	Active Training	Active Training	Active Training	Active Training
3	Active Training	Active Training	Active Training	Active Training	Active Training	Out for Rehab.
4	Active Training	Active Training	Active Training	Active Training	Active Training	Active Training
5	Active Training	Active Training	Two days off	Two days off	Out for Rehab.	Out for Rehab.
6	Active Training	Active Training	Active Training	Active Training	Active Training	Two days off
7	Active Training	Active Training	Active Training	Active Training	Active Training	Two days off
8	Active Training	Active Training	Two days off	Out for Rehab.	Out for Rehab.	Out for Rehab.
9	Active Training	Active Training	Active Training	Active Training	Out for Rehab.	Out for Rehab.
10	Active Training	Active Training	Active Training	Active Training	Two days off	Two days off

Table 4. Two groups of ten 3 years old standardbred racehorses were selected for a 6 weeks study. All horses were in active training and were sound and healthy at the initiation of the study as the general and lameness exam showed. The **Group A** received a bilateral treatment, at the beginning of each week, with the Infratonic on 12 acupuncture points specific for the hocks (Figure #1, Table #1). **Group B** was the untreated control. Some of the horses in **Group B** did not continue with the training and were given two days off or were pulled out of the experiment for rehabilitation.

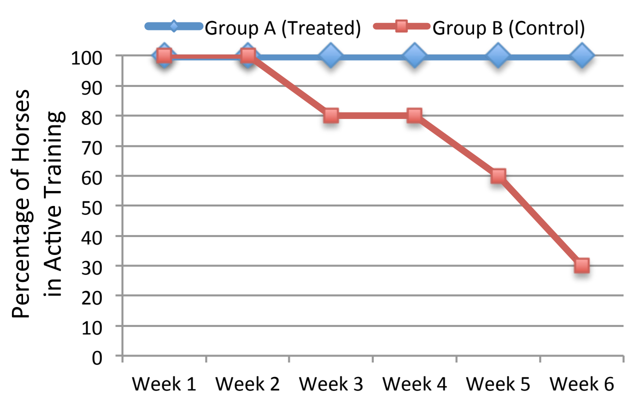

Figure #4. Percentage of horses actively training during the 6 weeks study. The **group A** (Blue) received the Infratonic acupuncture treatment as described in material and methods and the **group B** (Red) was not treated. This indicates that Infratonic treatment allows horses to participate in a grueling workout routine without injury or lameness.

Blood count and serum chemistry. All parameters that were measured in the blood work are shown in **Table #2**. There were not significant differences in the blood count, electrolyte balance, renal, hepatic or in total serum proteins, albumin or globulin between the two groups. These results indicate that the use of the Infratonic does not produce any negative side effects from this treatment and that the Infratonic is a safe device. The changes that were prominent in the serum analysis were the enzymes Creatine Phosphokinase (CPK) and Aspartate Amino Transferase (AST). There were some other changes in the blood test that were temporary conditions of dehydration, slight anemia and electrolyte imbalance, which were not consistent and were not related to the treatment protocol.

Creatine Phosphokinase (CPK). The group of horses treated with the Infratonic experienced a decrease in CPK, while the control group experienced a slow increase (**Figures #5A** and **#5B**). The CPK analysis during the first 48 hours showed that the amount of this enzyme in serum started to decrease during the treatment time, showing a significant change at the post-treatment point, which is about 1 hour after the initiation of treatment, **Figure #5A**. First, the level of this enzyme decreases fast during the first 2 hours, and after 2 hours it decreases more slowly. The average decrease in the enzyme between the pre-treatment point and 48 hours is about 50 u/L, which is statistically significant ($P \leq 8.8 \times 10^{-7}$). By contrast, the control group experienced a significant increase of 20 u/L between the pre-treatment and 48 hours ($P \leq 6.6 \times 10^{-5}$). The differences in CPK levels between the treated and control group of horses are also statistically significant. All the t-test P values are less than or equal to 0.05 (**Table #5A**).

During the 6 week period the CPK enzyme decreased smoothly and continuously in the treated group, while in the control group the CPK level went up (**Figure #5B**). We observed a statistically significant decrease throughout the experiment, with an average of 150 u/L less of the enzyme at week 6 than at pre-treatment level ($P \leq 0.0002$). The control group shows an average increase of 250 u/L at week 6 in relation to the pre-treatment level ($P \leq 0.014$). When comparing the levels of the enzyme CPK between the control and treated group we also find that the differences are statistically significant (**Table #5B**).

A

	Post-Treat	**1 hour**	**4 hours**	**6 hours**	**12 hours**	**24 hours**	**48 hours**
CPK	1.63×10^{-7}	1.52×10^{-10}	4.76×10^{-10}	3.31×10^{-7}	8.26×10^{-7}	1.14×10^{-6}	4.97×10^{-11}
AST	0.57520	**0.02408**	**0.00487**	0.06326	0.07037	**0.00428**	**0.00035**

B

	Week 1	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**
CPK	**0.00048**	**0.00401**	0.07346	**0.014847**	**0.00704**	**0.00088**
AST	0.31663	**0.01560**	**0.00824**	**0.00505**	**0.00020**	**0.00050**

Table #5. Statistical analysis in the levels of the CPK and AST enzymes in serum between the Infratonic treated and the untreated group of horses. Table (**A**) shows the P values at different time points during 48 hours, and Table (**B**) shows the P values during the 6 weeks study. The P values were calculated using the T-test statistic analysis. The samples are statistically significant when $P \leq 0.05$.

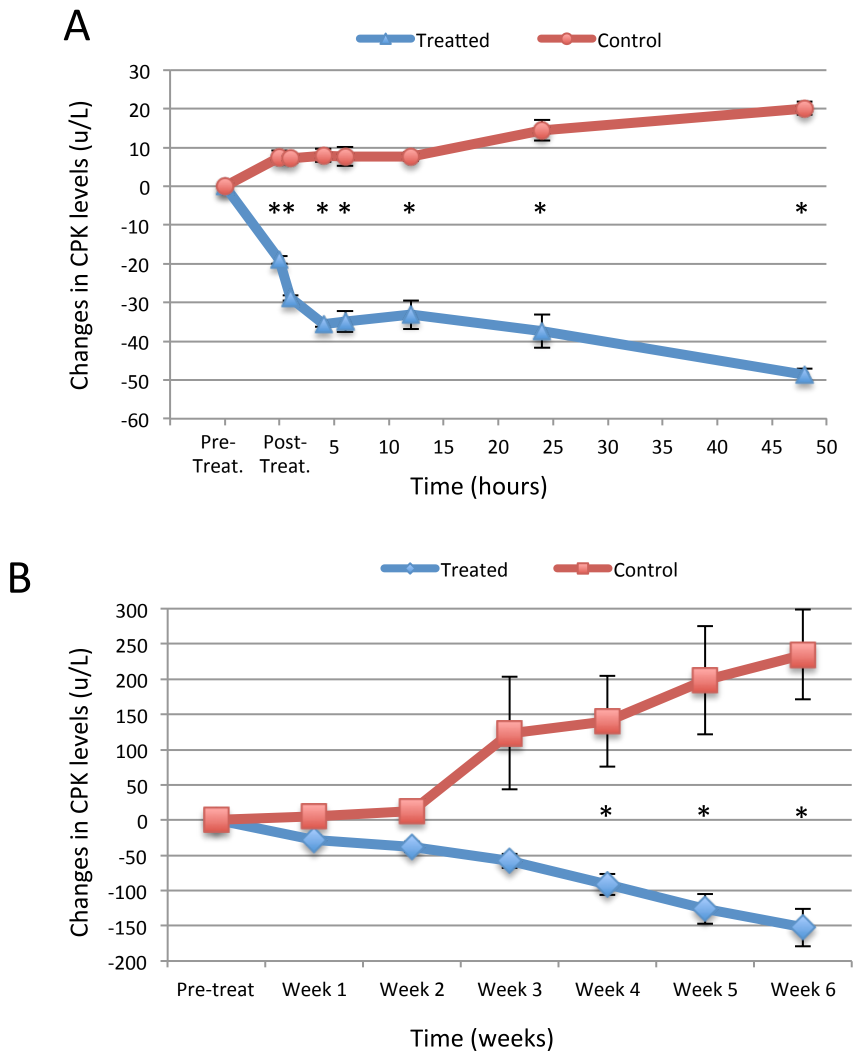

Figure #5. Comparison of CPK levels in units per Liter between a group of 10 horses receiving the Infratonic acupuncture therapy (blue) and a non-treated group of 6 horses as controls (red). (**A**) Changes in CPK levels during 48 hours after starting the treatment. The values are the averages from 6 analyses (one per week) obtained from each group of horses. (**B**) Changes in CPK levels during 6 weeks. The values are the averages of CPK levels from 10 horses on the treated group and 6 horses on the control group. The symbol (*) indicates that the differences between the treated and untreated group of horses are statistically significant (see **Table #5**).

<u>Aspartate Amino Transferase (AST)</u>. We also found significant differences in the AST levels between the treated and control groups of horses (**Figure #6**). When comparing the serum AST levels during the first 48 hours (**Figure #6A**), the treated group revealed a significant decrease of 23 u/L, with a P value of $\leq$0.0002. By contrast, the AST levels in the control group increased very slightly during 48 hours (9 u/L), which is not a statistical difference from the initiation time ($P \leq 0.213$), indicating that the AST stayed at about the same level during the first 48 hours (**Figure #6A**). However, it is important to note that the decrease in the treated group was high enough to show a significant difference in AST levels between the treated and control groups at 24 hours ($P \leq 0.004$) and 48 hours ($P \leq 0.0003$) (**Table #5A**).

A greater and more significant change is found through the entire 6 weeks period, indicating that regular use of the Infratonic causes a more significant effect in the muscle structure. The treated group revealed a gradual decrease in AST levels (100 u/L) in spite of hard racing and exercise, while the control group experienced a gradual increase (**Figure #6B**). The differences in AST levels in the treated group between week 1 and week 5 or 6 are statistically different, with P values of $\leq$ 0.024 and $\leq$0.005. In contrast, the AST levels in the control group increased throughout the study with a peak at week 5, reaching up to 100 u/L more AST enzyme than at the initiation time, which is statistically significant ($P \leq 0.01$). There is a slight decrease from week 5 to week 6, although not statistically significant. Similar to CPK, the differences in AST between the treated group of horses and the control are statistically significant at weeks 2, 3, 4, 5 and 6 (see **Table #5B**). The biggest difference between CPK and AST was that the CPK responded to treatment faster than the AST enzyme.

Discussion

The Equitonic QGM [equine version of the Infratonic] is a stochastic resonance device that generates random mechanical noise frequencies between 8 and 14 Hz. The stochastic resonance signal generated by the Infratonic is used in this study as a type of acupuncture. We found that the Infratonic acupuncture treatment has the effect of reducing inflammation in the hocks of standardbred racehorse in active training. We also found changes in blood chemistry between treated and untreated animals, specifically a decrease in the amount of the serum enzymes CPK and AST. These are diagnostic enzymes that reflect muscle damage when their levels are increased in serum.

Race horses provide an excellent model to study inflammation and muscle injury because during their training and racing regimes they consistently manifest muscle damage and inflammation in their hocks and muscles. When the Infratonic was applied to a hock, the surface thermal gradient on that area decreased, as measured by infrared thermography. Medical thermographs give visual temperature patterns or thermal gradients of the skin and are very useful for the detection of inflammation because heat is the major sign of inflammation (Yanmaz et al., 2007, Turner et al., 1998). Infrared thermographs were evaluated during this study from paired samples, comparing the right and left hock, where the right hock was treated and the left was the un-treated control. Using the same animal as its own control allowed us to truly compare two similar samples without dealing with the variability between animals. Initially, these images were almost

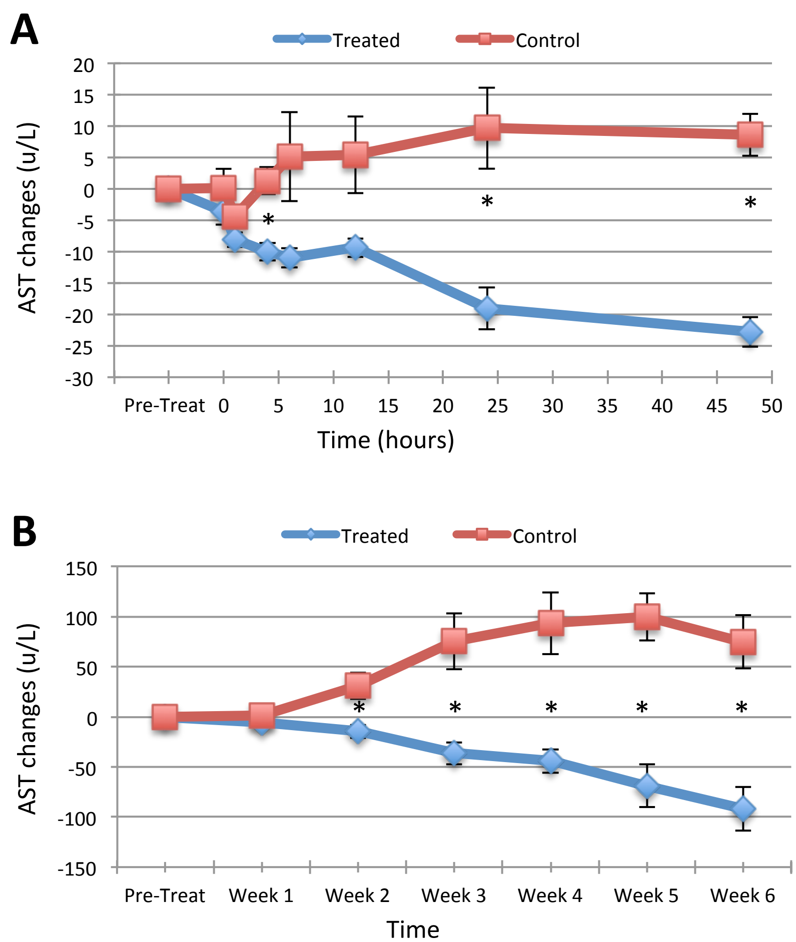

Figure #6. Comparison in changes in the AST levels in units per Liter between a group of 10 horses receiving the Infratonic acupuncture therapy (blue) and a non-treated group of 6 horses as controls (red). (A) Changes in AST levels during 48 hours. The values are the averages from 6 analyses (one per week) obtained from each group of horse. (B) Changes in AST levels during 6 weeks. The (*) symbol indicates that the differences in enzymes levels between groups are statistical significant (see **Table #5**).

identical, and after Infratonic treatment the right hock decreased in temperature when compared to the initial thermograph while the left hock retained the same pattern or changed more slowly over time when compared with the right hock.

When thermographic evaluations show 1ºC difference between 2 anatomically symmetric regions, it is generally considered to indicate inflammation in this region (Yanmaz et al., 2007). In some experiments during this study the skin temperature was reduced below the detection point of the infrared thermograph, it indicated that the thermal gradients had dropped more than 3ºC within the isothermic level. This accounted for a 100% reduction in temperature. Thus, a 50% reduction in the thermal gradient accounted for a decrease of 1.5ºC in the tissue. We found that 10 and 20 minute treatments with the Infratonic substantially reduced thermal gradient on the hocks. The 20 minute treatment was more significant than 10 minutes, reaching to an average of a 50% reduction (about 1.5ºC). This change indicates a diminution in inflammation and implies that this tissue has a lower propensity to be damaged and/or develop chronic inflammation. Although a degree of inflammation is a necessary and appropriate tissue response to initiate repair following tissue injury, inadequate regulation of this response may lead to excessive tissue damage and chronic inflammation.

An important observation is that the second 20 minute treatment of the third experiment, applied 12 hours after the first 20 minute treatment, provided profoundly deeper and longer lasting relief from inflammation, indicating that Infratonic therapy is cumulative, with two treatments providing substantially more benefit than a single treatment. Moreover, after the second 20 minute treatment the left hock experienced a 30% decrease in temperature that lasted up to 96 hours, even though the left hock was never treated throughout this phase. The effect of the Infratonic therapy was even more prominent when the treatment time was extended to 50 minutes covering a broader area on the right side of the animal. This therapy also affected the non-treated left hock, corroborating the results from the previous experiment, where treatment in one side also had beneficial effects in the other side of the horse. One of the possible explanations for these results is that the analgesic and anti-inflammatory effect upon the right hock allowed the animal to take some of the strain of training off the left hock. This effect has been found before, where horses stress a different side or part of their bodies trying to compensate for the primary join or musculoskeletal problem (Seem M. 1993, Schoen AM, 2000). An additional explanation could be that the Infratonic 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. More research will be necessary to confirm these hypotheses.

The results obtained with the Infrared thermograph encouraged us to look for physiologic responses to the Infratonic therapy through blood chemistry analysis. The only change in serum that was significant between the treated group of horses and the control group, were the enzymes CPK and AST. Plasma CPK is a marker signifying muscle injury arising from myofibrillar and cell disruption in muscle tissue (Clarkson et al. 1992; Kuiper, 1994; Noakes, 1987, Janssen et al, 1989). This disruption leads to release of CPK, which in turn contributes to strength deficits and low performance (Suzuki et al., 2006). Increase in serum CPK has been reported for human marathon runners (Kim, 2007), for humans after eccentric exercises (Child et al., 1999; Evants et al., 1986), for horse runners (Marlin et al., 2002) and also for sled dog runners (Piercy et al., 2000). In our study we found an immediate response in CPK serum levels after the Infratonic therapy. On the other hand, AST in serum had a slower response. AST is primarily used to diagnose liver and muscle disorders (Fallon et al., 1999). In this study we found that the hepatic parameters measured from the serum chemistry indicated that the changes in AST levels were not specific for a liver disorder but as a diagnostic for muscle tissue injury. This was evident by the normal levels in Alkaline Phosphatase, lipemic and icteric index and total bilirubin in both groups of horses. The serum levels of this enzyme increase following hard exercise or skeletal muscle injury (Hong et al., 1984; Peterson et al., 2008). Similar to the CPK enzyme, the AST levels decrease in serum in treated horses, and it was elevated in the control group. These results indicate that the Infratonic helps to prevent muscle damage and helps regenerate a damaged muscle more quickly, which is evident by a decrease in CPK and AST in serum, which is likely due to less CPK and AST leaking from the cell membrane.

The reduction in CPK and AST enzymes in serum correlates with the decrease in inflammation found during the infrared thermograph study. These findings indicate an improvement in the muscle integrity and strength in the horses due to the Infratonic therapy and explain why, after six weeks of treatment, the treated horses had a better performance and ran an average of six lengths faster than the control group.

The current study did not address specific mechanisms regarding how inflammation or muscle damage was reduced after Infratonic therapy. Other investigations by Rachlin and collaborators (2012) with the Infratonic device had shown an effect on cell membrane permeability *in vitro*. These researchers evaluated the effect of the Infratonic on cell permeability using the U98 cell line and the fluorophore calcein as a marker. They found a relative increase of 69% in mean calcein fluorescence intensity inside cells receiving Infratonic mechanical noise. Yount and collaborators (2004) also found that the Infratonic does not produce genetic damage in cells *in vitro* and Rachlin and collaborators (2012) found that the Infratonic does not cause any apparent cytotoxicity as assessed by phase contrast imaging of morphological characteristics. These results indicate that the Infratonic is a safe device and that one of the mechanisms of actions is through affecting the membrane cell permeability. Different investigators had found that mechanical noise stimulates tissues 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). The CPK and AST results indicate that the Infratonic stimulation affects muscle fibers and it is possible that it also affects nerve tissues producing a more extensive effect in the body. We had also investigated the effect of the Infratonic on the condition of the hyaluronic acid and in the appearance of the synovial fluid in the hock of these racehorses. We found that the Infratonic treatment also improves these parameters (manuscript in preparation). More research will be needed to determine the biochemical and molecular mechanisms of the Infratonic.

In conclusion, we found that the Infratonic acupuncture therapy increases quality of life and also increases athletic potential while minimizing the risk of inflammation, reducing muscle injury and increasing equine race performance. The beneficial effects of the Infratonic therapy can also be extended to human athletes because, like the horses in this study, they are constantly in training and competition. Athletes might improve performance and prevent muscle stress by using Infratonic therapy on regular basis, as well as reducing muscle injury and inflammation and reducing recovery time from injury.

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