# The University of Reading Department of Agriculture

The effects of Back on Track rugs on equine locomotion

by

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Dedicated to my Grandfather, A. D. Brownstone.

Who always encouraged my pursuit of knowledge

and inspired it with stories of his own.

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ABSTRACT

Back on Track are a company that produce a range of therapeutic products for horses, humans and dogs that work through the utilisation of properties of ceramic particles and infrared radiation. This study was conducted on 44 randomly selected horses, and sought to investigate the changes in locomotion resulting from the use of *"Back on Track"* therapeutic rugs. Locomotion was measured in three ways; fore limb stride length (FLSL), hind limb stride length (HLSL) and hind limb protraction (HLP). During the experimental periods there were highly significant improvements ($P<0.001$) in all three measurements, while there were no significant improvements in any of the three measurements during the control periods, although there were deteriations. The results of this experiment not only provide evidence of the efficacy of the *"Back on Track"* therapeutic rugs, but also lend support to the wellbeing and performance enhancing effects of infrared radiation.

1.0.0 INTRODUCTION

The inspiration for this research stemmed from personal use of *Back on Track* products. Many of the benefits that are described in the literature review, section 2.2, had been observed or experienced first-hand, however the research into these effects in vivo is very limited. The practical applications of research into this area may well have very important implications for the world of equine performance and wellbeing.

According to the British Equestrian Trade Association (BETA), the equestrian industry as a whole is worth an estimated £3.8 billion a year with in the UK alone (BETA, 2011). Approximately 20% of the horses owned in the UK are used for performance purposes such as equestrian competitive sports or hunting (BETA, 2011). The combined value of equestrian sports competitions in the UK (excluding racing), including affiliated and unaffiliated competitions has been estimated by the British Horse industry Confederation (BHIC), to have a combined value of roughly £37 million per year (BHIC, 2006). Economically speaking the horse racing community is the most important industry of the performance based equestrian sectors. It has an economic impact of £2.86 billion within the UK, and generated £288 million in tax revenues for the UK government in 2004/5 (BHIC, 2006). Not only does racing command the largest economic impact in the UK for the equestrian sporting sectors as a whole, but in 2004 the total prize money awarded was £101.3 million in the UK, making it a highly lucrative sport for the owners of winning horses too.

With the industry ever expanding, and the breeding of high performance horses reaching its limits in terms of increasing performance, it will soon be the case that the different sectors of the equestrian industry start to look toward marginal gains. This research will discuss whether these gains can be provide through the use of ceramic textiles and the effects of

the resulting infrared radiation, which could make it highly valuable to those looking to find new ways of boosting the performance of the horses that they own, train or work with.

In addition this research may prove to be invaluable in terms of equine, human and canine wellbeing as a whole. This is because the beneficial aspects of the *Back on Track* technology are not just limited to helping to improve equine performance as they produce products for humans, horses and dogs. It may also provide insights into injury prevention and treatments for sports and non-sports horses alike; treatment for chronic conditions such as arthritis; and finally using the horse as a model to apply the findings to other animals as well.

1.1.0 BACKGROUND OF THERAPEUTIC HORSE RUGS

The therapeutic horse rugs being used in this investigation have been provided by *'Back on Track'*. The company was founded by Dr Erland Breselin who continues to develop their broad product range and support research into the area. The fabric used for the *'Back on Track'* rugs used in this study has been manufactured using textiles that have had a ceramic powder melted onto the fibres.
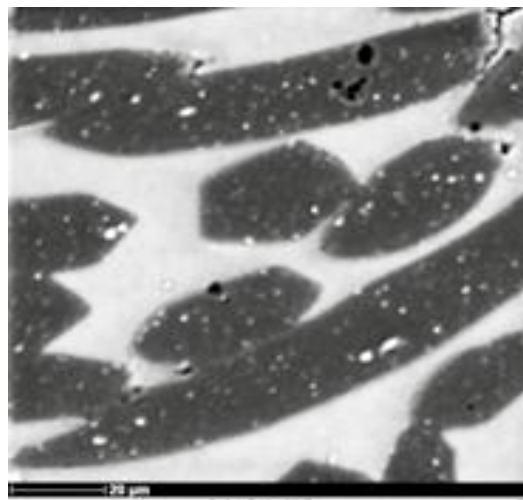
Figure 1 shows an x-ray of the ceramic powder (white flecks) melted onto the base fabric

The ceramic powder melted onto the fabric absorbs the wearer's body heat and re-emits it back at the wearer in the form of infrared radiation. This is due to the properties of the minerals found within the ceramic powder. Unfortunately the exact composition of the minerals with in the ceramic powder was not available when asked for due to commercial reasons.

Other *"Back on Track"* products include joint braces for horses, human and dogs, dog rugs and human clothing garments. All of the products are made with the ceramic infused textiles and advertise the same benefits.

1.2.0   BACKGROUND ON INFRARED RADIATION

The name infrared is derived from the Latin 'infra' meaning below (BioSmart, 2012) and red. This is because while red is the longest wavelength in the visible light spectrum, infrared has a longer wavelength but a lower frequency than visible light, hence the name infrared. The human body emits infrared radiation at a wavelength of 3-50µm through the skin (BioSmart, 2012), which is the basis for thermal imaging technology (Byres, 2008). The emission of far infrared radiation (FIR) (NASA, 2007) from the palms of our hands is thought to be responsible for the first example of infrared therapy; approximately 3,000 years ago palm healing started to be practiced in China and other parts of Asia (BioSmart, 2012). Modern interpretations of this form of healing indicates that the energy referred to as Chi by the healers is in fact FIR which recent studies have shown to have positive effects on wound healing and body conditioning (Toyokawa et al, 2003).

1.3.0 RESEARCH AIMS

The aims of this study are to investigate the effects of "*Back on Track*" therapeutic rugs on equine locomotion through the use of digital gait analysis which is described in the literature review section 2.6.2. The mechanisms through which the locomotion is likely to be affected are also described in the literature review.

2.0.0 LITERATURE REVIEW

2.1.0 DIFFERENT CATEGORIES OF INFRARED RADIATION

According to the International Commission on Illumination (ICE) infrared radiation can be split into 3 categories or bands:

Table 1 CIE classification of IR radiation

Name	Wavelength (μm)	Photon Energy (THz)
Near Infrared Radiation (NIR)	0.7 – 0.14	215 - 430
Mid infrared Radiation (MIR)	1.4 – 3.0	100 – 215
Far Infrared Radiation (FIR)	3.0 - 100	3 – 100

Near infrared radiation has been proven to penetrate approximately 23cm through soft tissue (Whelan, 2001) . The reason that near infrared penetrates so far is primarily because it is not absorbed by haemoglobin (Vladimirov, 2004) or water (ICNIRP, 2006). The greater penetration of near infrared radiation has made it of great interest to photo biologists compared to other ranges in the electromagnetic spectrum such as visible light because of the photochemical interactions that can occur when human tissue is exposed to it (ICNIRP, 2006). According to tests done by an independent laboratory, the infrared radiation emitted by *"Back on Track"* products falls within the bands of short wave through to far infrared wavelengths, 2 - 18μm (Beselin, 2012). The effects of FIR have not been as heavily researched as NIR however more recent research into the benefits of FIR has been the source of photo biological interest.

2.1.1 FAR INFRARED RADIATION

There are discrepancies on the different band widths of infrared radiation, within the literature. However for the purposes of this study we will refer to the bandwidths as described above, where FIR includes all wavelengths between 3 and 100μm (Vatansever and Hambin, 2012). Both *in vivo* and *in vitro* evidence suggests that FIR exposure has beneficial effects on cell and tissue stimulation (Vatansever and Hambin, 2012). FIR can be delivered to the body in a multitude of ways, including speciality lamps, saunas, and textiles that utilise FIR emitting ceramics such as the *"Back on Track"* products. FIR can penetrate up to 4cm into soft tissue (Vatansever and Hambin, 2012), this is less than the penetrative effects of NIR because of interactions between the FIR and water molecules. Levels of FIR that produce both detectable and non-detectable heating effects have both been observed to have biological effects (Vatansever and Hambin, 2012), meaning that both heat bearing forms, such as lamps, and non-heat bearing sources, such as the ceramic textiles can be equally effective (Toyokawa et al, 2003).

2.2.0 THERAPEUTIC BENEFITS OF FAR INFRARED RADIATION

There are four notable therapeutic effects of Infrared exposure which include:

- Pain relief

- Increased circulation

- Injury prevention and muscle relaxation

- Reduced recovery periods post injury

2.2.1   PAIN RELIEF

The pain alleviating effect is achieved through the utilisation of the same metabolic pathway as those used when taking opiates (Burke, 2009). According to a report published by the British Pain Society (BPS), an opioid is a drug that exerts activity by acting as an agonist at the endogenous receptors (opioid receptors), and elicits the characteristic stereospecific actions of natural morphine like glands (BPS, 2010). Opioids have a well-established role in management of acute pain following trauma or illness (BPS, 2010). When an opiate such as morphine is taken, the morphine molecules bind to the nerve cell receptors, which causes a release of nitric oxide (NO) (Ferreira, 1992). NO then goes on to activate a molecule, called Cyclic Guanine Mono-Phosphate (cGMP), that mediates the diminution in pain (Ferreira, 1992). 80% of patients taking opioids experience adverse side effects which can range from constipation through to vomiting (BPS, 2010) and, patients should always be informed of these before the opioids are prescribed. Presently opiates are effective for short to medium term pain relief; although the long term safety and efficacy of opioid treatment is uncertain. This is because prolonged use can lead to problems with insensitivity/tolerance, dependence and addiction (BPS, 2010). However Infrared exposure, in particular FIR exposure causes an increase in NO levels in exposed tissues (Leung et al, 2008) and utilises the same metabolic pathways as those used when opiates are taken. This essentially by-passes the need for drugs with the same (although less potent) results being achieved as those when an opiate is taken (Burke, 2009).

2.2.2   INCREASED CIRCULATION

In addition to pain relief, NO also causes vasodilation (Hassid, 1989) which increases circulation (Klabunde, 2010). The increased levels in NO are believed to be caused by an

increase in levels of NO-synthase (NOS) (Vladimirov, 2004). In particular infrared radiation would appear to stimulate the production and activation of inducible NOS (iNOS) (Vladimirov, 2004), neural NOS (nNOS) and epithelial NOS (eNOS) (Vatansever and Hambin, 2012). Stimulation of iNOS when not being promoted by exposure to infrared radiation occurs during an inflammatory response (Klabunde, 2010). This of course has many benefits for anyone undergoing a form of infrared therapy as blood flow is an important part of any healing process (Lavery, 2003). As a result of increased circulation it has been found that FIR exposure can also cause an increase in muscle tissue oxygenation (McClue, 2005) Increased oxygen profusion has been shown to aid in the increase of energy. Energy produced at the cellular level will accelerate the recovery of muscle tissue after exercise, which is known to induce lactic acid increases, rebuild strength in muscles damaged by exercise, and also reduce the incidence of cramping, oedema, and muscle fatigue post strenuous exercise in athletic conditioning (McClue, 2005).

2.2.3   INJURY PREVENTION AND MUSCLE RELAXATION

Infrared therapy can reduce the chances of incurring injury by aiding the relaxation of skeletal muscles. This benefit is achieved by another effect of the NO and cGMP relationship. The activation of cGMP by NO in skeletal muscle cells causes a relaxation of the skeletal muscle fibres (Stamler, 1994). The relaxation of muscles can prevent injury occurring because although a tight muscle is unlikely to cause more than discomfort, a tight muscle group can lead to muscle imbalance (Woonton, 2012). This is a problem as it will alter the biomechanics of the individual and put excessive strain on opposing muscle groups leading to injuries (Woonton, 2012). In support of this, studies on single sided equine lameness have demonstrated that this type of lameness results in activation of

compensatory mechanisms (Zaneb, 2008) in the muscles of the un-injured side. As the muscle activity of the lame side is affected, this creates and imbalance, which may lead to further injury (Zaneb, 2009).

2.2.4 REDUCED RECOVERY TIME/INCREASED RATE OF HEALING

The reduced recovery periods or increased rate of healing effects of infrared radiation have been much studied. Studies have been conducted on specimens ranging from cell cultures to treating ailments on an animal as a whole. A study conducted by Toyokawa *et al.* (2003) investigated the effects of FIR on wound healing. It aimed to shed some light on the biological effects of FIR on whole organisms by investigating whether the rate of wound healing was increased during periods of FIR exposure. This was achieved by removing a round section of full thickness skin (15mm diameter) from a rat's dorsal area, and then exposing a certain group to constant FIR. The curative effect was expressed as a percentile of wound area (Toyokawa et al, 2003), compared with that on day 0. Measurements were taken from 3 groups: a *Control Group* with no FIR exposure at an environmental temperature of 24°c - 25°c; *Group A* which was constantly exposed to FIR at an environmental temperature of 26.5^{o}c – 27.5^{o}c and, *Group B* which had no FIR exposure, but the same environmental temperature as *Group A.* Measurements were taken on days 0, 4, 7, 12 and 14. Their results showed that the rate of wound healing in *Group A* was significantly greater than that of the *Control Group* and *Group B* (Toyokawa et al, 2003) and that the environmental temperature of each group had no significant effect on the rate of wound healing. Toyokawa et al also measured surface skin temperature during the study and found that the FIR caused no significant increase in the skin temperature.

See figure 2 to see the time course of changes in the relative wound area:
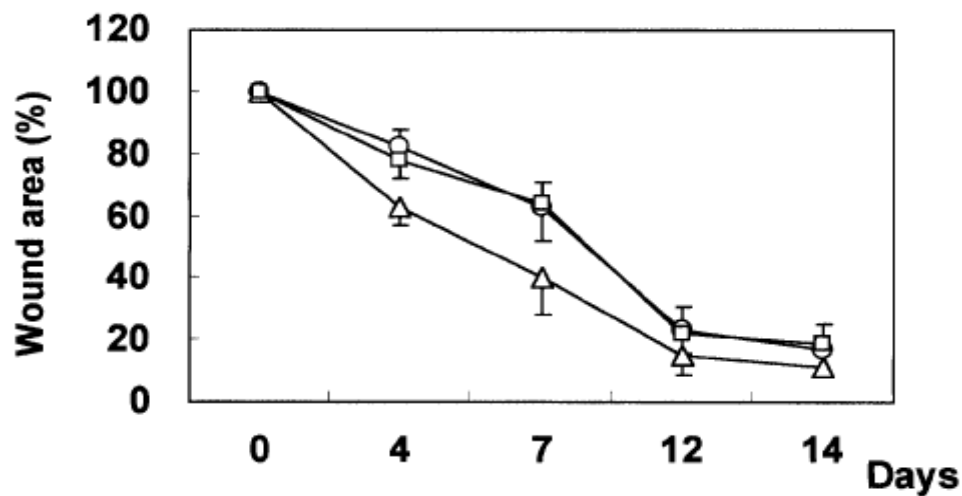
Figure 2 showing the accelerated rate of healing between Group A (Δ) and the Control Group (O) and Group B (□). (Toyokawa et al, 2003).

It has also been found that infiltrating fibroblasts express Transforming Growth Factor beta 1 (TGF-β1) during wound healing (Toyokawa et al, 2003). TGF-β1 is a cytokine that is well known for accelerating healing (Lawrence and Deigelmann, 1994), it can be shown with staining (as shown on the next page in figure 3) and it has demonstrated that during infrared exposure the number of fibroblast expressing TGF-β1 increases (Toyokawa et al, 2003).
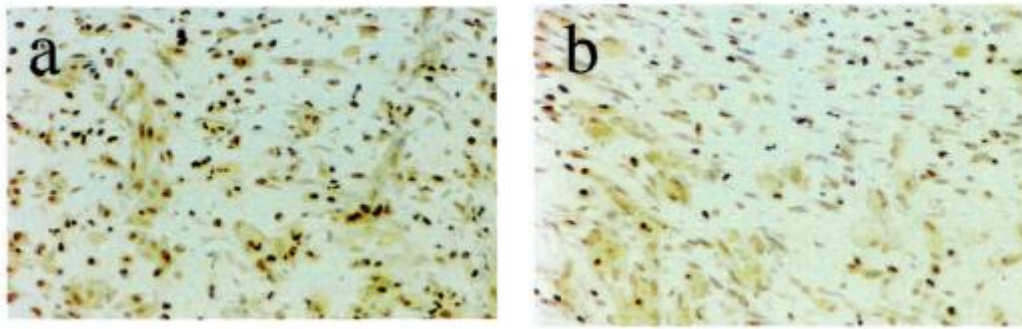
Figure 3 shows a comparison of the number of infiltrating fibroblast expressing TGF-β1 between Group A, and Group B which was not exposed to infrared (Toyokawa et al, 2003). These images were taken under 400 x magnifications on day 7.

Figure 3 shows an increased occurrence of fibroblasts expressing TGF-β1 when exposed to IR (figure a) and can be summarised in figure 4:
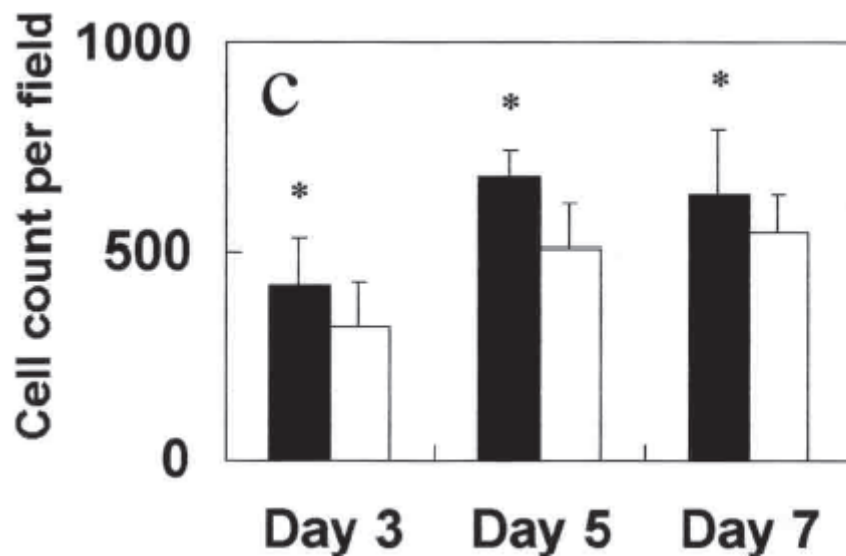
Figure 4 shows the mean number of fibroblasts expressing TGF-β1 on specific points in time (Group A in black, Group B in white) there is a significant difference between Group A and B with P=0.001 (Toyokawa et al, 2003).

The explanation of the effect that infrared has upon rates of healing and recovery comes under the very broad term of 'bio stimulation'. It has been demonstrated that infrared radiation increases respiratory metabolism of certain cells (Whelan, 2001) along with increases in fibroblast proliferation, synthesis of collagen and procollagen, growth factor production and extracellular matrix production (Whelan, 2001) during infrared laser exposure. Studies have shown positive results for human skin graft healing and epithelial wound closure healing (Conlan, 1996) with an increase of 155 – 171% in the growth of human epithelial cells (Whelan, 2001) when exposed to LED emitted infrared radiation. Other in vitro study results have also demonstrated an increase in mouse skeletal muscle cell growth of 140 – 200% (Whelan, 2001) from the same source of infrared radiation.

There are no known studies on the effects of FIR in relation to the effects on healing rate in horses, although this is a possible area for further research.

2.3.0   FAR INFRARED EMITTING FABRICS

FIR emitting fabrics such as those used in the *"Back on Track"* rugs, emit IR depending on the temperature that they are heated to; the chemical composition of the ceramic and the particle size also have an effect (Vatansever and Hambin, 2012). The first law of thermodynamics states that energy can neither be created, nor destroyed, as described by Vatansever and Hambin (2012). In this case this means that the IR emitted by the ceramic nanoparticles in the fabric must be transferred from something else. The energy in this case comes primarily from the heat (molecular vibrational) energy radiated from the wearer (Vatansever and Hambin, 2012), in addition any IR emitted by the wearer will also be absorbed and re-emitted. The result of this is a net gain in FIR because the ceramic particles

act as "perfect absorbers", maintaining their temperature and emitting FIR back towards the body of the wearer (Vatansever and Hambin, 2012).

2.4.0   SIMILAR STUDIES

This investigation intends to measure the improvement in locomotion of horses wearing "*Back on Track*" rugs by analysing the stride length over a prolonged period of infrared exposure. When originally designed it was not based on any previous studies, however further research has shown that there has been one similar study conducted in the past. A double-blind study was conducted by Sara Grundström and Stina Burströmusing (Date unknown) using 10 horses at the Strömsholms Riding School, Sweden. They also used Back on Track fabrics as a therapeutic infrared source, however the analysis technique for this study was very subjective as it was done by what could be seen by a vet and what a rider could feel. This makes it open to differing interpretations and opinions on movement and also the placebo effect. Although the vet and rider where not using the products themselves, if they expected an improvement in movement then they may have seen improvements that weren't there.

On the next page is a graph that summarises the results of the study conducted by Grundström & Burströmusing (date unkown). Although only 10 horses were used, it is reported that there was a significant improvement in movement when using the Back on Track rugs.
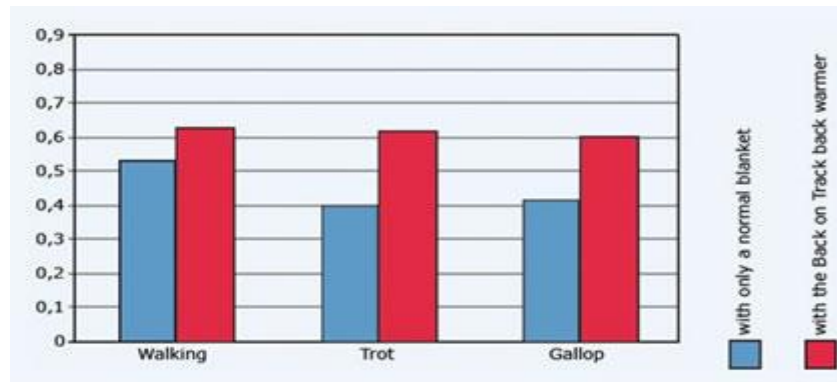
Figure 5 showing the condition scores for the horses back movement on the y-axis (0 being poor, 1,0 being perfect) and the pace on the x-axis.

The methodology used by Grundström and Burströmusing (date unknown) will be improved upon in this study because the investigation is not likely to have a placebo effect. This is because the need for human interpretation will be taken out of the analysis process by using digital analysis to generate empirical measurements instead. Horses are not likely to be subject to placebo effects because they are not likely to be aware of what is causing the change.

2.5.0 EQUINE BIOMECHANICS

There are 4 basic paces of a horse:

- Walk: is defined as a symmetrical gait (Clayton, 2001) with a 4 time rhythm because the leg movements work one leg at a time. The horses near side (left) hind starts the movement off, followed by the near side fore leg, then off side (right) hind and then finally the off side fore leg (Equestrian and Horse, 2012).

- Trot: is also a symmetrical gait (Clayton, 2001) but has a 2 time movement. This means that the horse moves it's legs in diagonal pairs, both at the same time. The

near side hind leg and off side fore leg will start the movement off, then the off side hind and near side fore legs will follow.

- Canter: is defined as an asymmetrical gait (Clayton, 2001) with a three time rhythm. The order of the legs will depend on which rein/leg/direction the rider is trying to achieve (Equestrian and Horse, 2012). For example if the rider is asking for a right rein/leg canter, which means traveling in a clockwise direction, then the horses near side hind limb starts the movement, followed by the off side hind and near side fore legs and finally the off side fore leg is the last leg to touch down again (Equestrian and Horse, 2012).

- Gallop: is the fastest pace of the horse and is also an asymmetrical gait (Clayton, 2001), although gallop has a 4 time rhythm. This is because in a gallop all 4 legs strike the ground separately with a moment of suspension in between each stride.

Speed is a determining factor of performance in many equestrian sporting disciplines such as racing, eventing, and to some degree show jumping (Clayton, 2001). Horses change their speed by altering the spatial and temporal relationship between their limbs (Clayton, 2001), or in other words their stride length and stride frequency is altered. It can be expressed with the following equation:

Speed = stride length x stride frequency

This produces different gaits and variations within the gait, a gait in this instance can be thought of as the pace (for example walk, trot and canter). Every gait has an optimum speed at which the metabolic energy cost is minimised, it has been suggested that changes in gait can often be attributed to energetic cost (Hoyt and Taylor, 1981). Much like humans who

naturally walk at speeds up to 2.4m/s before jogging to reduce energy costs, it is thought that horses will naturally change their gait or stride in order to minimise energetic expenditure too (Hoyt and Taylor, 1981). In general stride length increases linearly with speed (Leach and Cymbulk, 1986), whereas stride frequency increases more slowly and in a nonlinear manner as speed increases (Leach and Cymbulk, 1986).

Like many quadrupeds the horse has a greater loading on its forelimbs, locating approximately 60% of its weight on to them at rest which increases during locomotion (Schamhardt, 1998). Consequently the forelimbs of the horse have evolved to a primarily support role, providing little propulsive force. The hind limbs, however, have evolved to support less weight but provide much greater amounts of propulsive force (Wilson et al., 2000). This is achieved by the forelimbs acting as energy efficient springs, which store and release energy to reduce the energy cost of locomotion (Wilson et al., 2000).

Stride length will also increase with limb length (Clayton, 2001) because this increases the theoretical reach of each limb. Generally the fore limbs rotate around the point of the tuber spinae scapular (Clayton, 2001). Whereas in a symmetric gait (walk and trot) the hind limbs will rotate around the hip joint, but in an asymmetrical gait (canter and gallop), the effective limb length is longer when the hind limbs rotate around the lumbosacral joint. In the symmetrical gaits of walk and trot, lateral flexion (side to side) of the vertebral column may enhance stride length. However in the asymmetrical gaits of canter and gallop it is dorsal flexion (up and down) of the vertebral column that can enhance stride length (Clayton, 2001). It is for these reasons that the IR emission of the *"Back on Track"* rugs may improve performance, because if the back and gluteus muscles are tight or in spasm then it will impede these forms of flexion (Geoff and Stubbs, 2008).
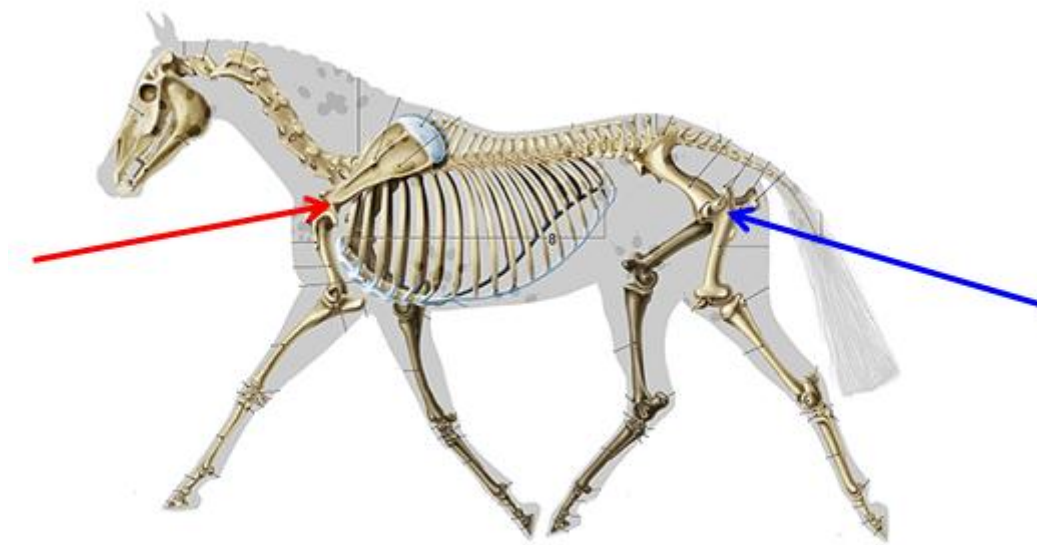
Figure 6 diagram of a horse's skeletal system with, the red arrow indicating the point of the tuber spinae scapular and the blue arrow indicating the hip joint (Inky Mouse Studios, 2013)

Some studies on equine biomechanics indicate that a correlation between quality of movement and performance. For example a study was presented at the 6th international conference on equine locomotion, Normandy, France, that demonstrated a correlation between forelimb action (movement) and sales price of the individual horse (Lawson, 2008). Sale's price can be a good indicator of a horse's performance or at very least a good predictor of performance in a young or unproven horse. These findings suggest that if the infrared radiation exposure does lead to an improvement in locomotion, then logically that may lead to an improvement in performances seen in the participating individuals.

2.6.0   HISTORY OF GAIT ANALYSIS

The first scientific comments on the equestrian gait are found in teachings during the antiquity of the Greek and Roman civilisations (René van Weeren, 2001). The first extensive work on equine confirmation was conducted by Xenophon (430 – 354 BC) and the first

documented study on animal locomotion was titled "De motu animalium" or "On the motion of animals" (Aristotle, 350 BC). Following this there was no real progression in scientific understanding or research until the 18th century when the modern approach to making observations and conducting experiments was first developed (René van Weeren, 2001). During the 18th century veterinary colleges were first founded and, as the horse was the primary form of transportation and was also still being used heavily for warfare (René van Weeren, 2001) they were focused on almost exclusively. The first published paper on equine gait analysis was published by scientists in France during the 18th century and they retained the lead in such research until the end of the 19th century (René van Weeren, 2001), when the Germans started to experiment with new techniques such as using early cine film cameras to capture footage in order to conduct equestrian gait analysis.

With the outbreak of the two world wars in the 20th century, and the mechanical revolution the horse had become obsolete as a war machine and source of transport so research into equestrian locomotion and gait analysis came to a halt (René van Weeren, 2001). However during the late 1960s and 1970s this field of research became popular once more in parallel with the increasing popularity of equestrian performance sports such as racing and three day eventing, sports that are still growing in popularity today.

2.6.1 GAIT AND LOCOMOTION ANALYSIS TODAY

Videographic analysis using varying qualities of video cameras and commercial software packages is the most popular method of locomotion or gait analysis (also known as kinematic analysis). More advanced systems use skin markers for use as reference points for analysis and auto digitisation (Clayton and Schamhardt, 2001). Markers are generally white and or made of a reflective material and 2 – 3cm in diameter (Clayton and Schamhardt,

2001). When conducting a 2D analysis circular markers can be used, however if the analysis is to be done in 3D then spherical markers are needed (Clayton and Schamhardt, 2001). If analysis of joint mobility is to be conducted then there must be a minimum of 3 markers, one on the joint and one either side of joint (Clayton and Schamhardt, 2001). During recording the video camera must be perpendicular to the plane of interest (see method), the standard video camera records at 30 frames/second, which is adequate for most software packages. However for more accurate footage then high speed video cameras are useful, although studies have shown that there is no great difference in cameras recording at 60 frames/second or at 200 frames/second (Linford, 1994). It should be noted however that higher frame rates are more light sensitive so if footage is being recorded in an uncontrolled environment then a 60 frames/second camera would be preferable to account for changes in natural lighting (Clayton and Schamhardt, 2001).

2.6.2 DIGITAL ANALYSIS

There are many different digital analysis products on the market that are capable of analysing equine stride length or a 'gait analysis'. In this study the software package "*On Track Equine"* was used, however similar software packages such as Quintic's "*Centaur Equine Biomechanics"* could have been used to the same effect. The advantage of using a software package to conduct the analysis rather than a visual scoring system is that it reduces human error and any placebo or bias effects that may influence the observer(s).

2.7.0 RESEARCH AIMS AND QUESTION

Although it has been shown that infrared radiation has the previously discussed benefits, there has been limited research done into the practical applications of infrared for day to day therapeutic uses. This investigation will be an analytical study that will provide clear,

objective and numerical data in support of the use of infrared radiation, through the use of infrared based therapeutic rugs. Consequently the aim is to investigate the effects of infrared radiation on equine locomotion, and provide an answer to the question: *Will equine locomotion be significantly improved by the regular use of "Back on Track" infrared based therapeutic horse rugs?*

The theory behind the research and the hypothesis is that the use of the rugs will improve equine locomotion and consequently performance, which will be improved because all forms of equine sport, in which performance can be measured, are greatly affected by locomotion. For short distance sprinting sports such as some racing categories and show jumping, the requirements are for rapid acceleration and the ability to generate maximum speed or power (Clayton, 2001); this is achieved through a high stride frequency. Whereas in cross country, mid distance and endurance racing events the requirement is on stamina, which is achieved by the horse taking longer strides (Deuel and Park, 1990). As was described previously, stride length can be enhanced by either lateral or dorsal flexion of the vertebral column (Clayton, 2001) depending on whether the mode of locomotion is through a symmetric gait or an asymmetric one. Because back flexibility is essential to the accomplishment of sport exercises (Denoix and Audigié, 2001) improving this through either pain alleviation or muscular relaxation will not only enhance locomotion but also improve performance. It has been long established that back pain is an important cause of poor performance in horses (Denoix, 1998), however mechanisms such as the ones proposed in this study are relatively unexplored. Soft tissue problems in the back such as tension or sensitivity will cause the horse to resist ventroflexion (Malikides et al., 2008) which is extension of the thoracolumbar spine in a vertical plain, and dorsiflexion which is the flexion

of the lumbosacral region in a horizontal plain. The *"Back on Track"* therapeutic rugs are predicted to relieve muscular tension in areas that they cover (shoulders, chest, back, flank and rear) and alleviate any pain in these areas. As shown in H_0, these effects are expected to improve the measures of locomotion in this study:

- Fore Limb Stride Length (FLSL) which is the distance between the fore hoof leaving the ground at the start of the stride and landing again at the end of the stride.

- Hind Limb Stride Length (HLSL) which is the distance between the hind hoof leaving the ground at the start of the stride and landing again at the end of the stride.

- Hind Limb Protraction (HLP) which is measured by the under or over track of the horse. An under track is when the hind hoof lands behind the fore hoof on the same side. An over track is when the hind hoof lands ahead of where the fore hoof landed on the same side.

This will be achieved by allowing a greater freedom of movement.

2.7.1 HYPOTHESIS (H_0)

There will be a significant improvement in fore limb stride length (FLSL), hind limb stride length (HLSL) and hind limb protraction (HLP) when exposed to the therapeutic effects of *"Back on Track"* rugs.

2.7.2 NULL HYPOTHESIS (H_1)

There will be no significant improvement in fore limb stride length (FLSL), hind limb stride length (HLSL) and hind limb protraction (HLP) when exposed to the therapeutic effects of *"Back on Track"* rugs.

3.0.0 METHOD, EQUIPMENT AND EXPERIMENTAL DESIGN

3.1.0 EQUIPMENT

In order to conduct the research involved in this investigation, the following equipment is required:

- Video camera (30 frames/second)

- Tripod

- White board and whiteboard pen

- Distance markers: in this case two, 2m lengths of guttering with a 1m mark on them that is clearly visible on film

- Laptop, PC, Macbook or Mac

- Stride analysis software: in this case 'On Track Equine'

- Data base software: in this case Excel

- Data analysis software: in this case Minitab

- Word Processing software: in this case Word

3.1.1 Reasons:

A video camera was needed to record footage of the participants being trotted up twice a week for later analysis.

A tripod was needed to stabilise the camera in order to get high quality footage.

White boards were used to record the participant's name, date, temperature and weather conditions and make note of any extenuating circumstances such as 'just ridden' or 'lame'.

Distance markers were used to ensure enough strides were filmed as horses had to be trotted from one end of the guttering to the other. The guttering was set 4 meters apart making the length 8m in total. The other purpose for using the distance markers is that the stride analysis software needs something in the same horizontal plain as the subject to calibrate from.

The Laptop is needed to run the stride and data analysis software and store all of the data and footage. An external hard drive has also been useful here, as the recorded footage occupies a lot of space; however is not a necessity.

Stride analysis software is used to calculate the stride length for both the fore and hind legs, in addition to calculating the hind limb protraction which in this case is being analysed by calculating the over or under track of the horse. The *"On Track Equine"* works by allowing you to over lay an adjustable horizontal line measure to the footage. One end of the line measure is placed at the tip of a chosen hoof when it is flat on the ground, then footage is moved forward to where that hoof next lands and the other end of the line measure is moved to the tip of the same hoof in its new position. This calculates the stride length for that chosen limb. Similarly the hind limb protraction is calculated by placing one end of the horizontal line measure at the tip of a front hoof, then the footage is moved forward until the hind hoof on the same side lands and the other end of the line measure is moved to the tip of the hind hoof. See figure 7 for a screen shot of the *"On Track Equine"* software being used.
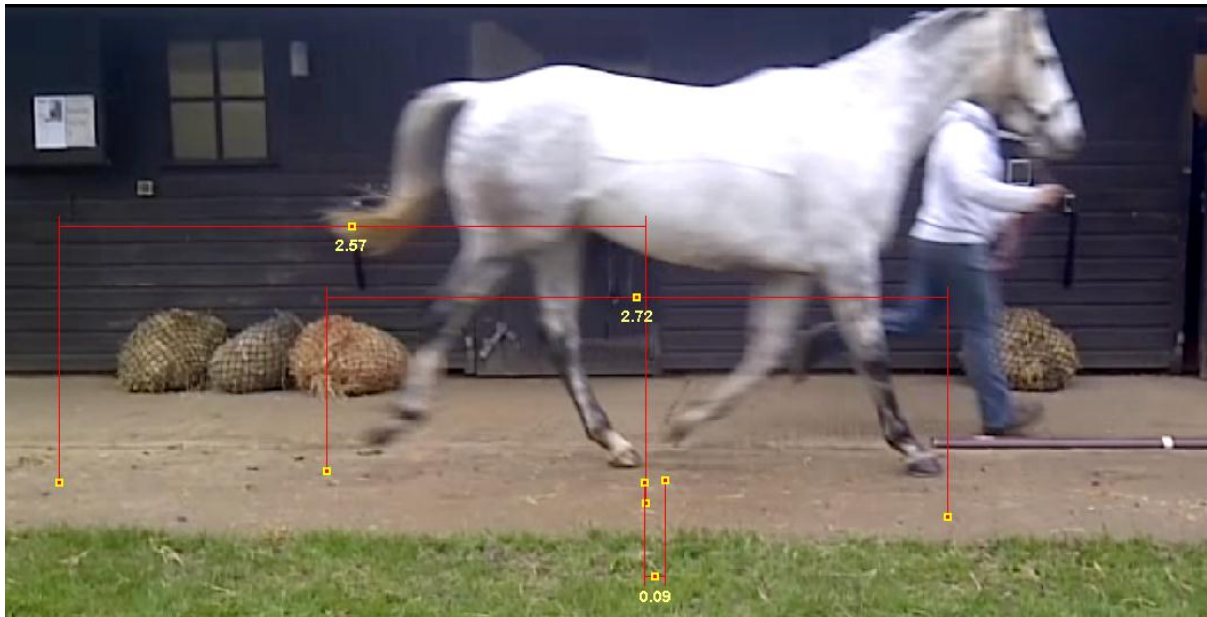
Figure 7 a screen shot of the "On Track Equine" being used, showing the line measurements for a hind limb stride, a forelimb stride and a measurement for hind limb protraction.

Data base software such as Excel is used to compile raw data.

Data analysis software is needed to test the null hypothesis (that there will be no improvement) through analysis of the raw data; minitab version 16.0.0 was used in this case.

3.2.0 EXPERIMENTAL DESIGN:

- 44 horses were randomly allocated to 4 different groups, originally 45 horses were allocated to groups however one horse had to leave the study and results were removed.

   This number was chosen partly because of the availability of volunteered participants and also because of the time constraints in which the research had to be undertaken.

- Each group of horses was exposed to both the experimental treatment, when "*Back on Track*" rugs were worn as under rugs/stable rugs, and a control treatment, when normal (non-therapeutic) under and stable rugs were used.

    o Each horse acts as its own control making the results more valid than comparing it to another individual.

    o The order of the periods (experimental or control first) was then randomly allocated.

- Each treatment period lasted 4 weeks, meaning that every group wore the *"Back on Track"* rugs for 4 weeks during the experimental period, and normal ones for 4 weeks during the control period.

    o 4 week long treatment periods were chosen, rather than shorter periods and more participants because it allowed for changes in the horses locomotion to be fully examined and monitored as it was expected that there would be an improvement over time.

- Treatment periods were split into 8 observations (twice a week).

    o Observations were taken twice a week because it provided a more detailed data set than what would have been obtained with only 4 observations (once a week).

- In each observation the participating horses were trotted past the camera 3 times in the same direction (left to right as it appears in the footage) and an average result for each measurement of the three trot-ups was calculated.

    o The reason for this was to try and account for any variations within each observation (e.g. speed).

- o Every stride of every trot-up was analysed and the best measurement was used. The best measurement is the measurement that shows the most improvement or the least deterioration.

3.4.0 METHOD:

- Collect basic, non-personal (to owners) back ground data on the horses.

- Mark 1m from the end of each length of guttering, a white label was used in this case. The mark must be clearly visible on the footage.

- Set guttering in line with one another and 4m apart.

- Set up the tripod and camera 1m in from the inner edge of the right length of guttering (right of frame) and 7m away. 7 meters will ensure that you are far enough away to get footage of a full stride cycle and fit the whole horse in, but are not too far away to make analysis hard.

See Figure 8 below for a visual representation:
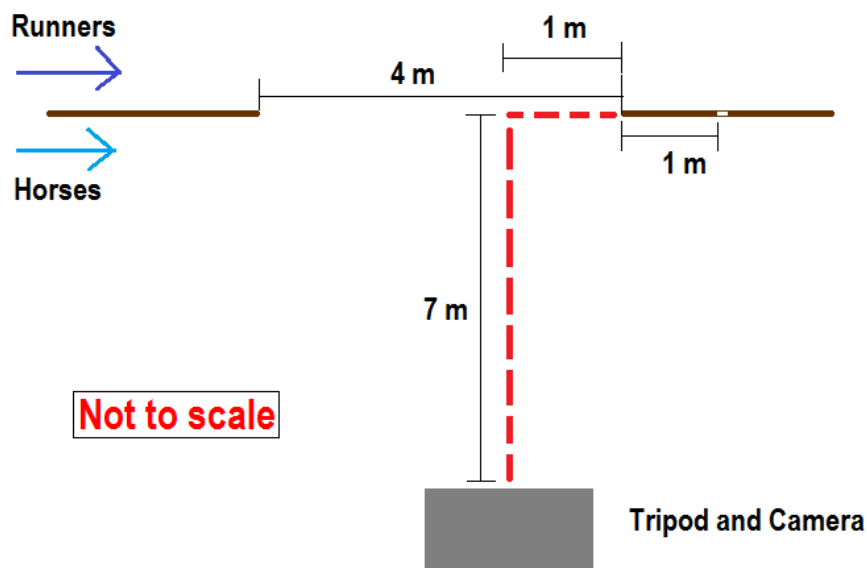
Figure 8 a visual representation of the set up for the recording of footage.

- Prepare white board with name of horse, the date, the air temperature, the general weather conditions and any extenuating circumstances.

- Prepare horses by removing all rugs and ensuring nothing on their person will impede their movement.

- If a runner is available, return to camera in order to film while the runner trots the horse up in the direction depicted by the blue arrows in the diagram. The runner should be on the left of the guttering/distance markers and the horse as close to them as possible on the right. Repeat twice more, or until 3 good trot ups have been recorded.

  o Ensure that the camera is recording before the horse enters the shot to get the best footage, but do not leave the camera running once each trot up is done to save time and battery life.

- If a runner is not available then press record, return to the horse and carry out the trot past as described above.

  o Ensure the horse is trotting before reaching the first distance marker/length of guttering.

  o A good trot up must include a full stride cycle at an even pace

    ▪ The runner should aim to go at the same speed for every trot up

- Once the three trot ups have been completed, return the horse to its original state (rugs, stable etc) and repeat with the next horse.

- Once footage has been taken of all the horses for the day, upload the footage onto a laptop, PC, Mac etc.

- Name files according to the date, time, location and horse name for easy future reference.

- Load footage into 'On Track Equestrian' software (or similar) and analyse the stride length, and the hind limb protraction.

  o Load file

  o Calibrate software using the inside edge of the white stripe and the inside edge of the guttering which are 1m apart.

  o Measure stride lengths.

  o Measure hind limb protraction.

    ▪ The hind limb protraction is measured by measuring the under or over track. An over or under track is the distance between where the fore hoof and hind hoof on the same size land, as shown by the measurement 0.09 in figure 7.

- Record the data from the analysis software on a data-base, e.g. Excel.

- Analyse data.

- Draw conclusions.

- Present results in written form.

3.5.0   STATISTICAL ANALYSIS

The statistical analysis used in this study was a General Linear Module (GLM) Analysis of Variance (ANOVA). Within the model was treatment, age, observation and a comparison between observation and treatment to see whether the changes occurred over time or at once. The statistical outputs can be found in appendix 2 (FLSL), 3 (HLSL) and 4 (HLP). The values used for the results in section 4.0.0 are highlighted in red, followed by the

comparisons between the observations which show if and when the results differ

significantly from one another, as indicated by an adjusted P value of less than 0.05.

4.0.0 RESULTS

To obtain the final set of results, the footage from every observation (2 observations per week), for all 44 horses was analysed and the average measurements for hind limb protraction, fore limb and hind limb stride lengths. The averages were the mean average of the measurements of each of the three runs that were recorded in every observation. These averages were then formatted for statistical analysis in minitab and the least square means from the general linear model ANOVA output are presented for all three of the measurements of locomotion used in this study (FLSL, HLSL, HLP). The observation number in the figures and tables that follow refers to when the footage was collected; observation 1 is the first set of footage in week 1, observation 3 is the first set of footage in week 2.

4.1.0 FORE LIMB STRIDE LENGTH

There was a significant difference (P = < 0.001) between the fore limb stride lengths (FLSLs) of the experimental treatment when compared to the control treatment. Meaning there is less than a 0.1% chance that the null hypothesis is correct.
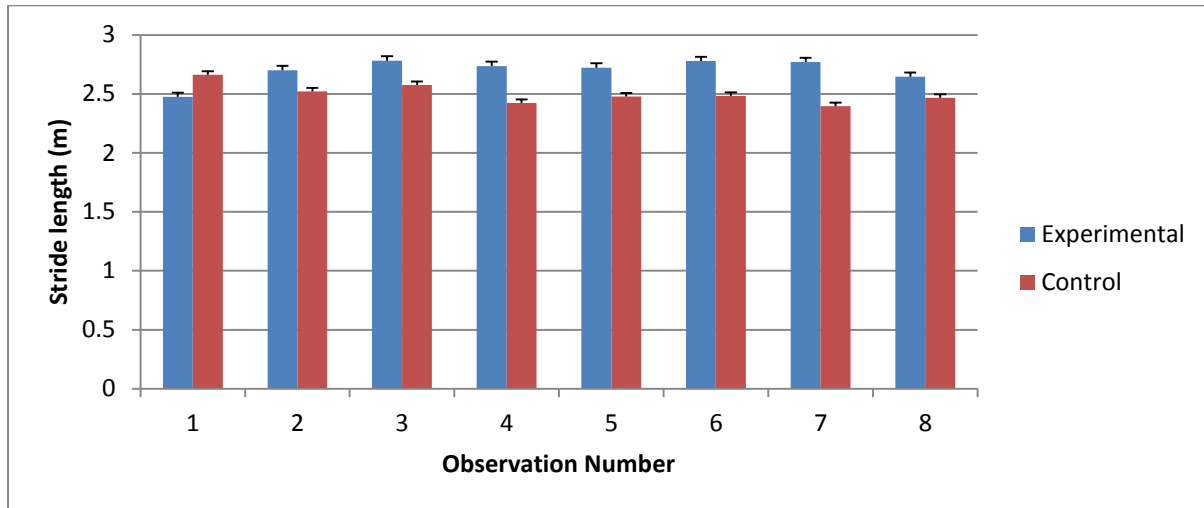
Figure 9 The average fore limb stride length of all 44 horses in meters across the 4 week observation period, with Observation number on the x-axis and Stride Length (m) on the y-axis.

Table 2 The average numerical values of the fore limb stride length of all 44 horses across the 4 week period.

	Observation								S.E.M
	1	2	3	4	5	6	7	8	
Experimental (m)	2.474_a	2.701_b	2.782_b	2.736_b	2.723_b	2.778_b	2.770_b	2.645_a	0.051
Control (m)	2.662_a	2.521_a	2.574_a	2.424_a	2.477_a	2.483_a	2.397_a	2.467_a	0.060

The different subscripts indicate a significant difference with in the row (P = <0.05)

Furthermore there was a significant difference within the experimental treatment between the first observations and all subsequent observations other than observation 8, although there was not a significant difference between observations 2 – 7, whereas there were no significant differences between observations across the control period.

There was a significant difference (P = < 0.001) between the changes in the fore limb stride lengths of the experimental treatment when compared to the control treatment.
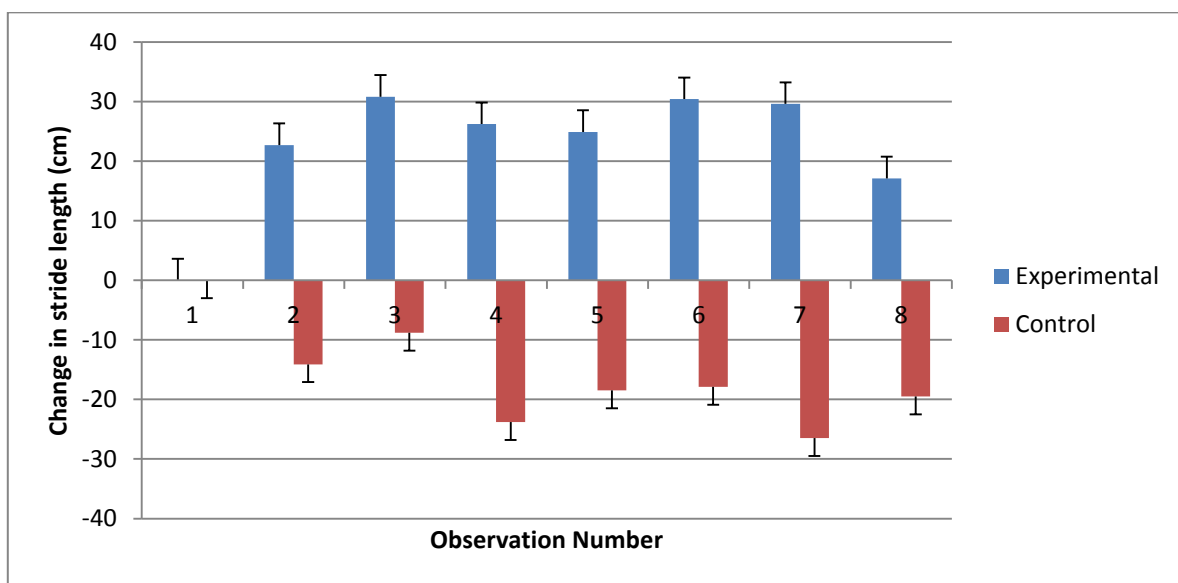
Figure 10 The average changes to fore limb stride length of all 44 horses in cm across the week observation period, with Observation number on the x-axis and Stride Length (m) on the y-axis.

All values for Figure 10 and Table 3 are compared to the base reading on week 1, observation one. This observation was before any experimental or control rugs were introduced. The positive results or bars represent an improvement and the negitive bars or results are deteriorations in the fore limb stride length.

Table 3 The average numerical values of the change in fore limb stride length of all 44 horses across the 4 week period compared to observation 1.

	Observation							
	1	2	3	4	5	6	7	8
Experimental (cm)	0	22.7	30.8	26.2	24.9	30.4	29.6	17.1
Control (cm)	0	-14.1	-8.8	-23.8	-18.5	-17.9	-26.5	-19.5
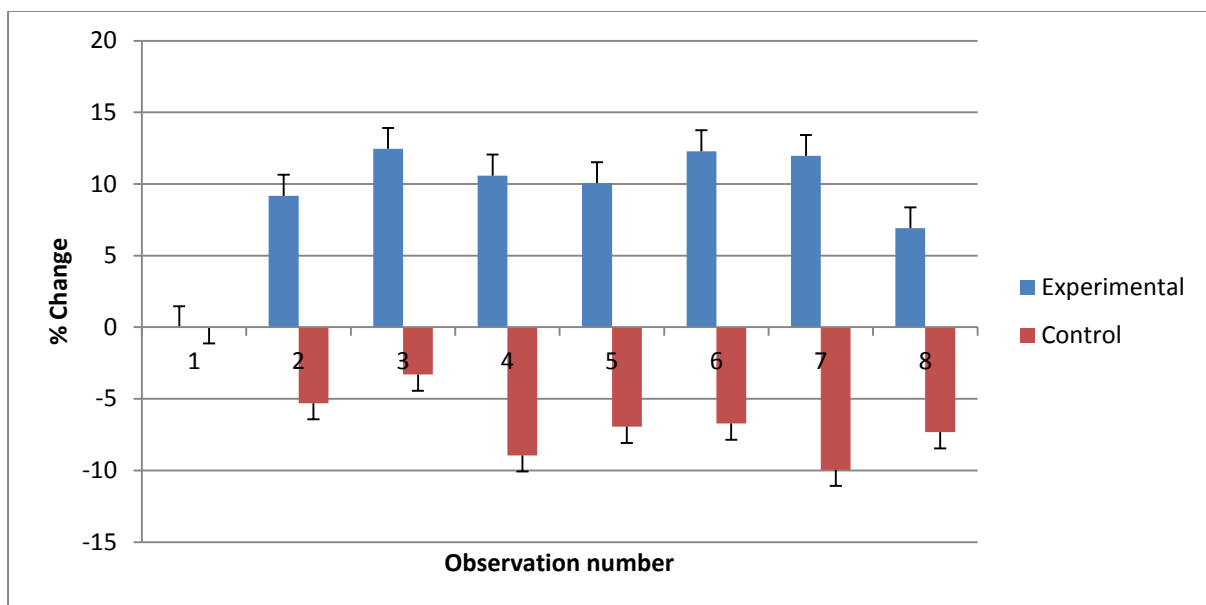
Figure 11 Percentage change in fore limb stride length

The percentages displayed in figure 11 and table 4 are percentage changes in fore limb stride length compared to the base measurement on observation 1. Observation one is the first set of footage before any rugs were put on the horses.

Table 4 the Percentage change in fore limb stride length

	Observation							
	1	2	3	4	5	6	7	8
Experimental	0	9.16	12.45	10.59	10.06	12.29	11.96	6.91
Control	0	-5.30	-3.31	-8.94	-6.95	-6.72	-9.95	-7.33

4.2.0 HIND LIMB STRIDE LENGTH

There was a significant difference (P = < 0.001) between the hind limb stride lengths (HLSLs)

of the experimental treatment when compared to the control treatment.
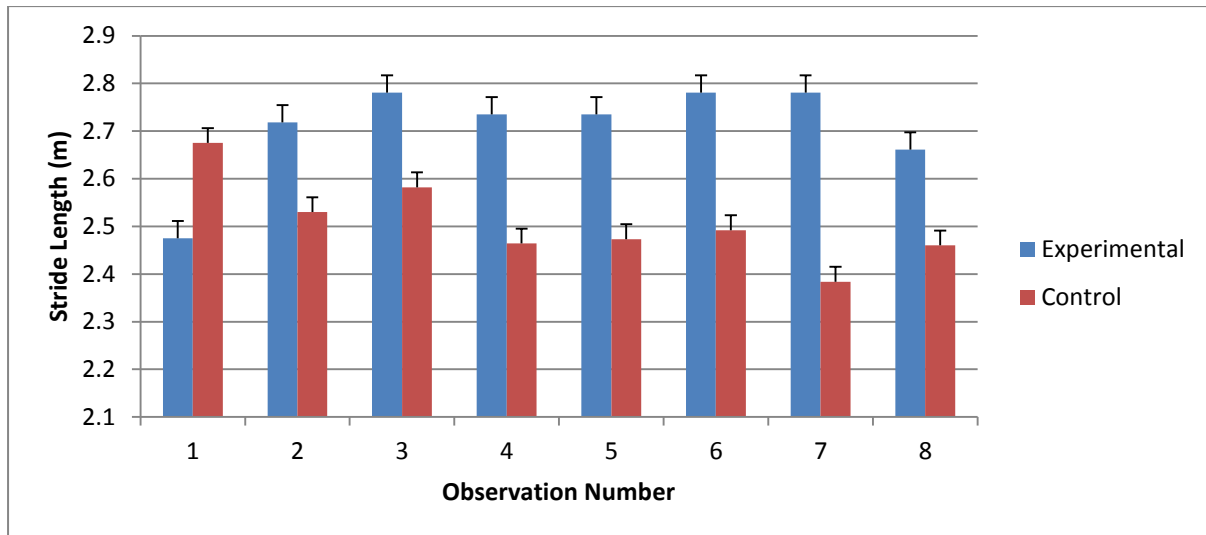
Figure 12 The average hind limb stride length of all 44 horses in meters across the 4 week

observation period, with Observation number on the x-axis and Stride Length (m) on the y-axis.

Table 5 The average numerical values of the hind limb stride length of all 44 horses across the 4

week period.

	Observation								S.E.M
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	
Experimental (m)	2.475_a	2.718_b	2.781_b	2.735_b	2.735_b	2.781_b	2.781_b	2.661_a	0.052
Control (m)	2.675_a	2.530_a	2.582_a	2.464_a	2.473_a	2.492_a	2.384_a	2.460_a	0.062

The different subscripts indicate a significant difference with in the row (P = <0.05)

There was a significant difference within the experimental treatment between the first

observations and all subsequent observations other than observation 8, although there was

no significant difference between observations 2 – 7, whereas there were no significant differences between observations across the control period.

There was a significant difference (P = < 0.001) between the changes in the hind limb stride lengths of the experimental treatment when compared to the control treatment.
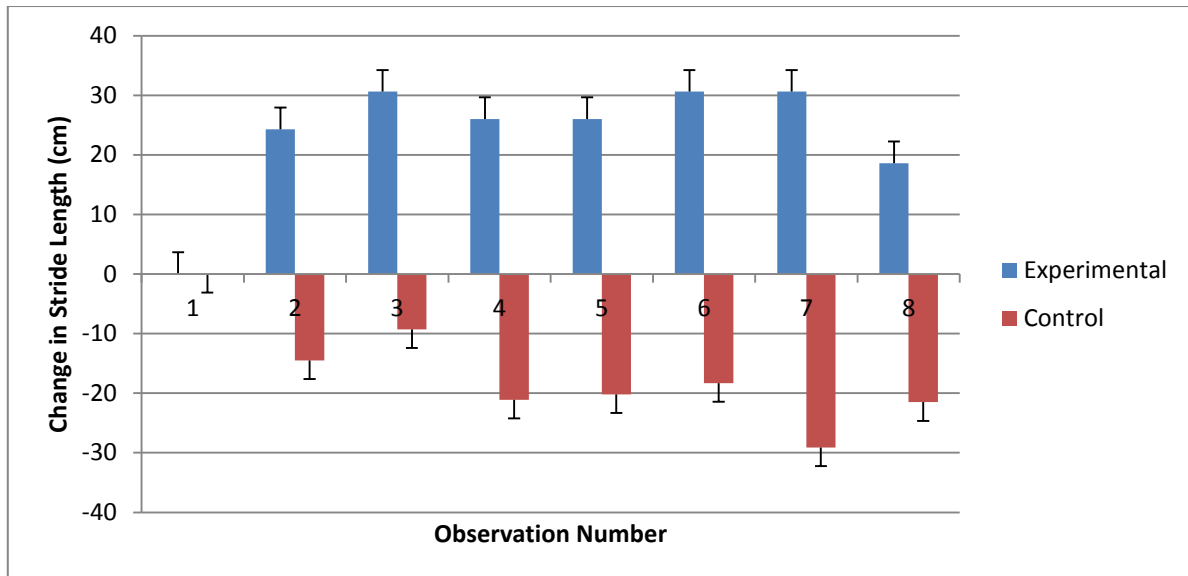
Figure 13 The average changes to hind limb stride length of all 44 horses in cm across the 4 week observation period, with Observation number on the x-axis and Stride Length (m) on the y-axis.

All values for Figure 13 and Table 6 are compared to the base reading on week 1, observation one. This observation was before any experimental or control rugs were introduced. The positive values or bars represent an improvement and the negitive bars or results are deteriorations in the fore limb stride length.

Table 6 The average numerical values of the change in hind limb stride length of all 44 horses across the 4 week period compared to observation 1.

	Observation							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Experimental (cm)	0	24.3	30.6	26.0	26.0	30.6	30.6	18.6
Control (cm)	0	-14.5	-9.3	-21.1	-20.2	-18.3	-29.1	-21.5
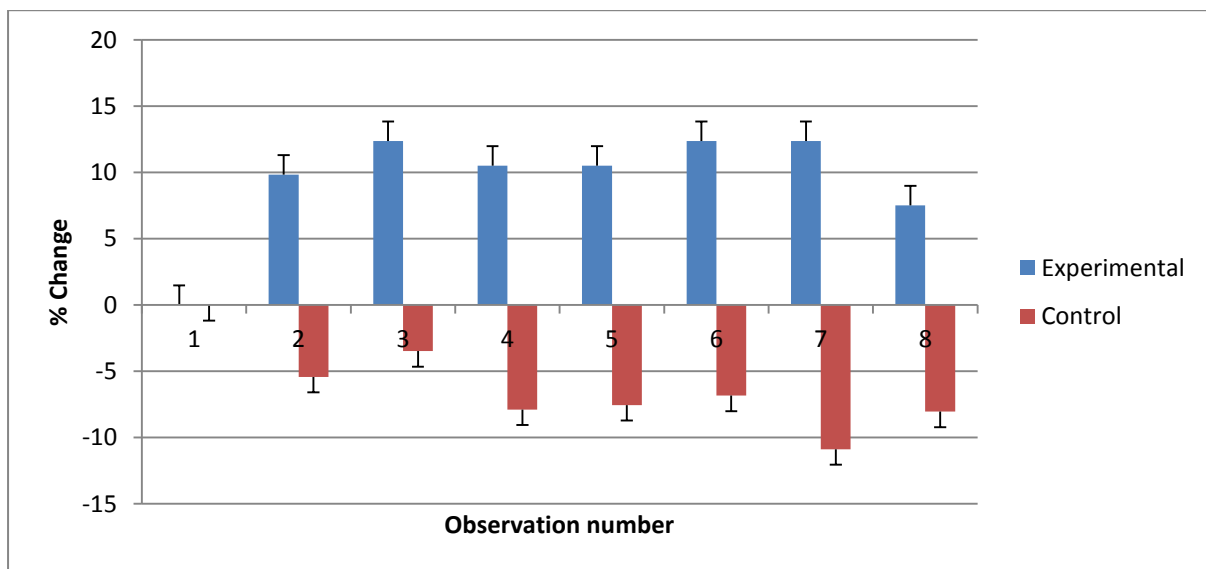
Figure 14 Percentage change in Hind Limb Stride Length

The percentages displayed in figure 14 and table 7 are percentage changes in fore limb stride length compared to the base measurement on observation 1.

Table 7 Percentage change in Hind Limb Stride Length

	Observation							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Experimental	0	9.82	12.36	10.51	10.51	12.36	12.36	7.52
Control	0	-5.42	-3.48	-7.89	-7.55	-6.84	-10.88	-8.04

4.3.0   HIND LIMB PROTRACTION

There was a significant difference (P = < 0.001) between the hind limb protraction lengths

(HLPs) of the experimental treatment when compared to the control treatment.
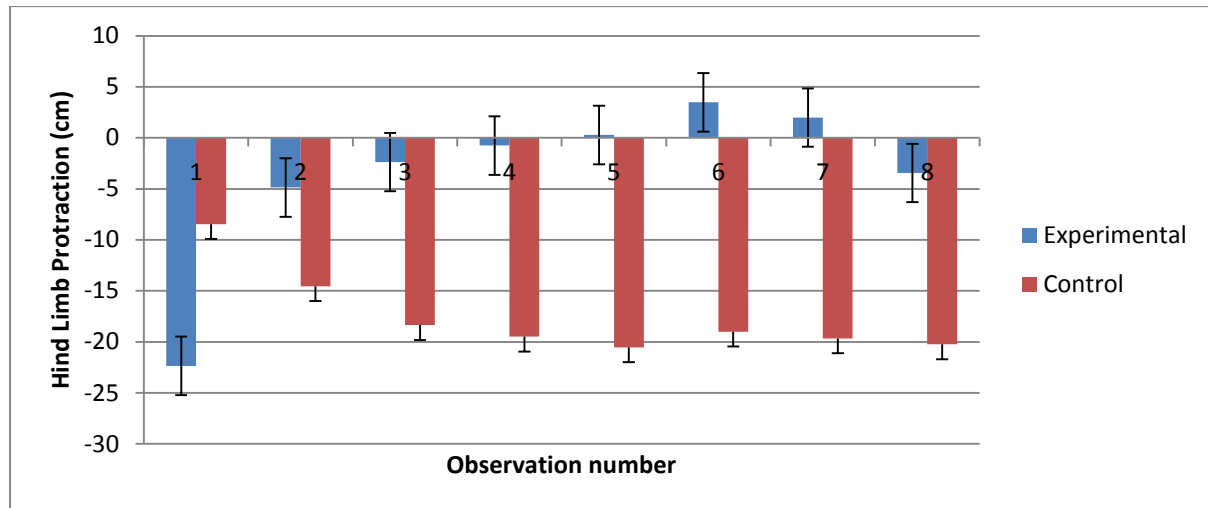
Figure 15 The average hind limb protraction of all 44 horses in meters across the 4 week observation

period, with Observation number on the x-axis and Hind Limb Protraction (cm) on the y-axis.

The negative values of figure 12 and table 6 depict an under track (the hind hoof landing behind the

fore hoof of the same side), and positive values represent an over track (the hind hoof landing ahead

of the fore hoof on the same side).

Table 8 The average numerical values of the hind limb protraction length of all 44 horses across the

4 week period.

	Observation								S.E.M
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	
Experimental (cm)	-22.35_a	-4.87_b	-2.37_b	-0.76_b	0.28_b	3.48_b	1.98_b	-3.44_b	2.137
Control (cm)	-8.46_a	-14.55_a	-18.36_a	-19.48_b	-20.53_b	-19.00_a	-19.65_a	-20.24_a	2.551

The different subscripts indicate a significant difference with in the row (P = <0.05)

For hind limb protraction there was a significant difference within the experimental treatment between the first observations and all subsequent observations other than observation 8, but there were no significant differences within observations 2 – 7, whereas there were significant differences between observations 1 and 4 and 5 across the control period. However these significant differences in the control were significant depreciations rather than improvements.

There was a significant difference ($P = < 0.001$) between the changes in the hind limb protraction lengths of the experimental treatment when compared to the control treatment.
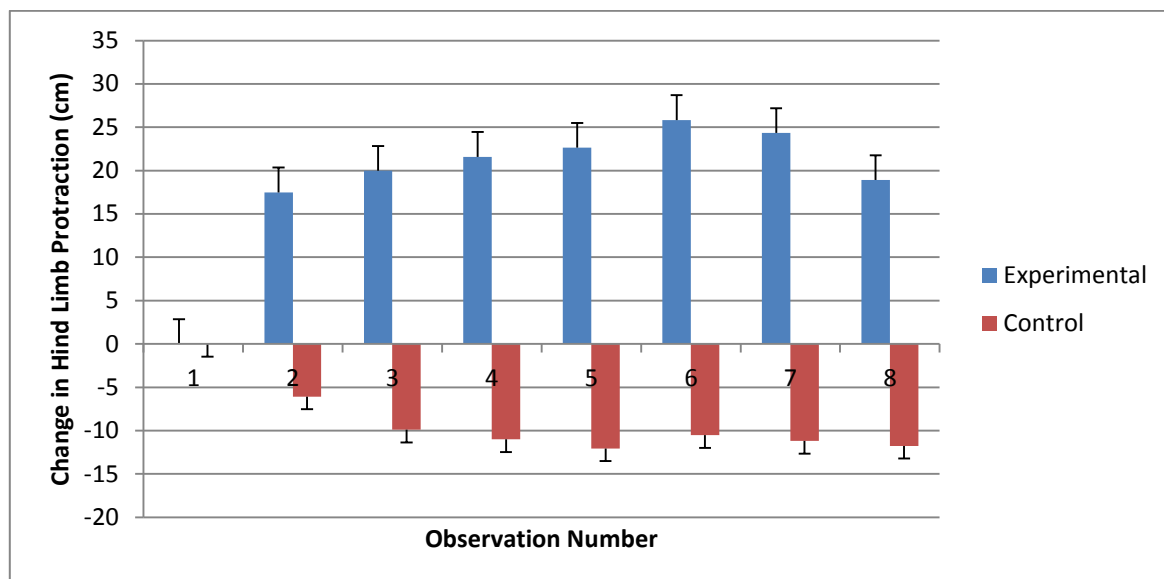
Figure 16 The average changes to hind limb protraction length of all 44 horses in cm across the 4 week observation period, with Observation number on the x-axis and Hind Limb Protraction (cm) on the y-axis.

All values for Figure 16 and Table 9 are compared to the base reading on week 1, observation one. This observation was before any experimental or control rugs were

introduced. The positive values or bars represent an improvement and the negitive bars or results are deteriorations in the fore limb stride length.

Table 9 The average numerical values of the change in hind limb stride length of all 44 horses across the 4 week period compared to observation 1.

	Observation							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Experimental (cm)	0	17.48	19.98	21.59	22.63	25.83	24.33	18.91
Control (cm)	0	-6.09	-9.9	-11.02	-12.07	-10.54	-11.19	-11.78
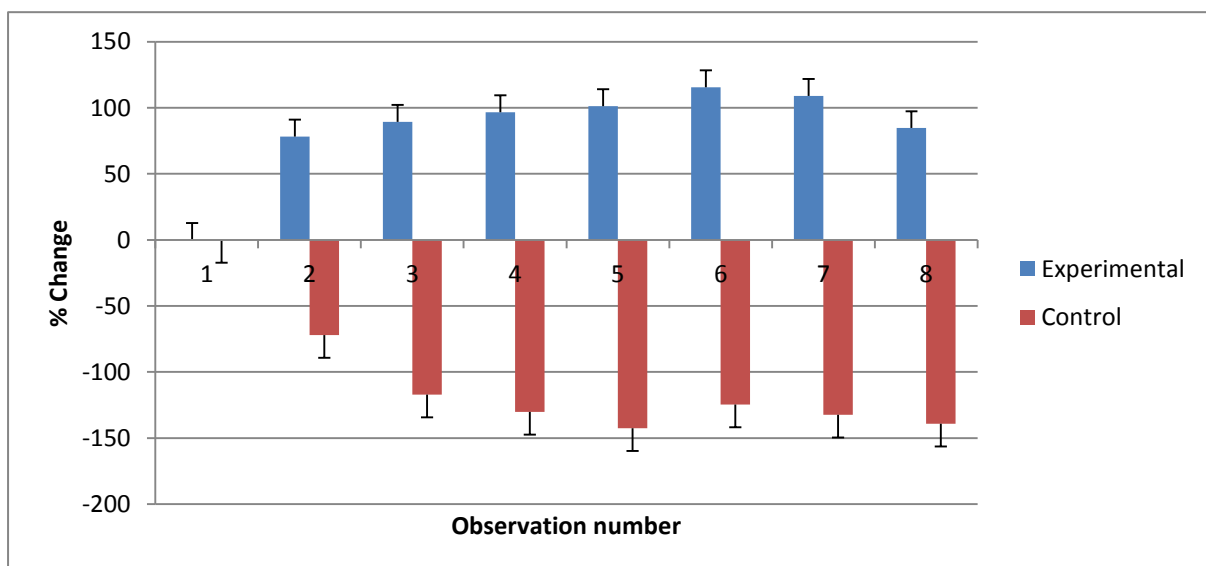
Figure 17 Percentage change in Hind Limb Protraction

The percentages displayed in figure 17 and table 10 are percentage changes in fore limb stride length compared to the base measurement on observation 1.

Table 10 Percentage change in Hind Limb Protraction

	Observation							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Experimental	0	78.21	89.40	96.60	101.25	115.57	108.96	84.61
Control	0	-71.99	-117.02	-130.26	-142.67	-124.59	-132.27	-139.24

4.4.0 SUMMARY OF RESULTS:

Table 11 A summary of results

	Treatment		S.E.M	P – Value
	Control	**Experimental**		
FLSL (m)	2.473	2.696	0.021	<0.001
HLSL (m)	2.507	2.708	0.021	<0.001
HLP (cm)	-17.53	-3.51	0.873	<0.001

The results show that highly significant differences were observed in all three measurements, when using *"Back on Track"* rugs. Meaning that there is less than a 0.1% chance of the null hypothesis (H_1) being correct.

5.0.0 DISCUSSION

5.1.0   OBSERVATIONS

There were multiple observations made in relation to the analysed results such as the ones described below, a full table of raw results can be found in appendix 1. In observation 1 of all three measurements the experimental result was worse than the control one, as shown in figures 8, 10 and 12. This is likely to have been because some groups had to go straight from the experimental treatment to the control one with only a week off. This likely resulted in an improvement in the results for observation 1 of the control period because of latent improvements that had not fully worn off from exposure to the "*Back on Track"* rugs. In addition there was deterioration in all three measurements during observation 8. This may have been as a result of poor weather conditions and the surface used as described in section 5.3.0. However this also lends support to the unofficial theory that "*Back on Track"* rugs are best used intervals so that their efficacy does not depreciate (Back on Track, 2013).

5.1.1   Fore limb stride length

The results showed that the average peak improvement in fore limb stride length was 30.8cm, and came on observation 3, and then there was a small drop in stride length before it rose back to an improvement of 30.4cm in observation 6. The fall in fore limb stride length may be as a result of the wet weather in observations 4, 5 and 6 which is described in section 5.3.0, it could also be due to a change in the runner decreasing the speed at which the horse trots at, also described in section 5.3.0. There was a significant difference seen between the experimental base observation (observation 1) which was before the rugs were used, and all subsequent observations (P<0.05) other than observation 8 for which although there was an improvement, it was not significant. This was determined by conducting a

pairwise comparison between the effects of treatment on observation. The comparison showed that when comparing observation 1 to all other observations, that observations 2 – 7 were significantly different (P<0.05) however when comparing observations 2 – 7 to one another they were not significantly different (P>0.05).

5.1.2 Hind limb stride length

The results showed that the average peak improvement in hind limb stride length was 30.6cm, and was seen in observations 3, 6 and 7. The dip in experimental hind limb stride length during observations 4 and 5 coincides with the same fall in fore limb stride length, this may also have been due to the wet weather and the horses not wanting to move as forward, as before, in it, or the runner. Another possible reason for this deterioration is that during the first group's experimental period, several horses had their rugs removed and had to have their subsequent results discarded, this may have skewed the results for these observations. There was a significant difference seen between the experimental base observation (observation 1) which was before the rugs were used, and all subsequent observations (P<0.05) other than observation 8 for which, although there was an improvement, it was not significant. This was determined by conducting a pairwise comparison between the effects of treatment on observation. The comparison showed that when comparing observation 1 to all other observations, that observations 2 – 7 were significantly different (P<0.05) however when comparing observations 2 – 7 to one another they were not significantly different (P>0.05).

5.1.3 Hind limb protraction

The results showed that the peak improvement in hind limb protraction was 25.83cm on average which was seen in observation 6. There was a highly significant difference seen

between the experimental base observation (observation 1) which was before the rugs were used, and all subsequent observations (P<0.001). This was determined by conducting a pairwise comparison between the effects of treatment on observation. The comparison showed that when comparing observation 1 to all other observations, that observations 2 – 7 were significantly different (P<0.05) however when comparing observations 2 – 7 to one another they were not significantly different (P>0.05). The percentage change of the hind limb protraction is far larger than the other measurements of locomotion, this is likely to be because the base measurement is a far smaller value (cm rather than m).

All of the above observations are likely to be due to the FIR emission of the *"Back on Track"* therapeutic rugs because the other factors were kept as constant as possible and, the improvements were highly significant for all measurements. The FIR will have helped to relax back and gluteus muscles of the horses that participated in the study, through the stimulation of NO synthesis (Stamler, 1994). This will allow for a greater freedom of movement and an improvement in the locomotion of the participating horses. In addition to relaxation of muscles, the FIR exposure will allow any existing injuries to heal quicker (Toyokawa et al, 2003) improving locomotion further still. Finally the relationship between FIR exposure and NO synthesis can also alleviate pain in the exposed areas (Ferreira, 1992). The NO activates cGMP in the same way that it does when it's synthesis is stimulated by an opiate such as morphine (Burke, 2009). This results in an alleviation of pain that may be caused by an injury such as trauma resulting in skeletal muscles being in spasm, muscle ache from exercise or an injury induced by some form of trauma. This pain alleviation may further the relaxation of skeletal muscles and will improve locomotion.

5.2.0   RELEVANCE OF RESEARCH

Of the horses in the UK, approximately 20% are performance horses that take part in some form of equestrian sport (BETA, 2011). Clear links have been made between the wellbeing and performance (Baptiste, 2008), furthermore links between performance of horses and their value are well established (Lawson, 2008). A study conducted by Lawson (2008) found that the locomotion score of horses being sold can significantly influence the sales price of a horse because a good locomotion score is seen as an indicator of future performance.

The results of this study show that *"Back on Track"* products both improve performance of equine athletes and the wellbeing of all that are able to use FIR therapy to their benefit. This has an economic impact on both the potential value of performance horses due to improvements in performance, but also on the expenses of professionals that treat horses; such as vets and physiotherapists.

Finally the results of this study can be used as a model for the effects that similar products and therapies may have on humans, however this would require further research before gaining mainstream acceptance.

5.3.0   LIMITATIONS

Although every effort was made to make this study as accurate and valid as possible through the development of a simple experimental design and an effort to control or account for as many variables as possible, there were still limitations of the research.

The easiest way to effect the stride length of any animal is to increase or decrease the speed that it is travelling at (Vanhooydonck et al., 2002). Although every effort was made to standardise the speed that horses were trotted up, sometimes this was not possible due to

unwillingness of the participating horses. In addition, the person trotting the horses up was supposed to be the same for every horse throughout both the experimental and control period. However due to injuries and unavailability this was not always the case; for example if one runner had to stop running due to injury then their replacement may achieve a different speed which skews the results slightly. This is seen in observations 3, 4, 5 and 6 for some groups in the raw data shown in appendix 1. Biomechanically speaking, speed is calculated using the following equation:

Speed = Stride length x stride frequency

This equation demonstrates that with an increase in speed, there will likely be an accompanying increase in stride length (Magness, 2010). A study that analysed the biomechanics of two Olympic sprinters compared the sprinting velocity with the stride length of the individual human runners and found that there was a highly significant positive correlation ($P<0.001$) between their stride length and sprinting velocity (Ito, 2007). If this research was to be repeated then either the same runner will be used for every single horse, or the speed at which the horse is trotted at will be measured and factored into the results.

There were also different surfaces used in this study. Ideally every surface would have been a firm, even and level surface. Unfortunately because the study was conducted at several different locations, this was an uncontrollable variable. At one location a loose, gravel surface was the only suitable area to trot the horses up. During the wetter weather in December the surface became wet and heavy, which had a negative effect on the stride lengths on the two horses at this location, meaning that their stride length was shorter. See appendix 1, Harley and Stubble, observations 5 and 7 for a representation of this effect. A

study conducted by Orlande *et al.* (2012) on the effect of two different surfaces had a significant effect (P<0.001) on equestrian hoof slip. Different surfaces have different properties such as hardness and traction (Orlande et al., 2012) which can influence the risk of a horse obtaining injury and make a horse more hesitant to move forward and therefore take shorter strides. However despite the effects of this deterioration the two horses still showed an improvement and this had a negligible effect on the averages as a whole.

The final limitation in this study is that the control rugs were not regulated closely enough. Although no other therapeutic rugs were used during the control period, it was left to the owners discretion as to how heavily the horses were rugged up, which meant that the temperatures that the rugs maintained may have varied (Gibbs, 2013). This could have had an effect on the stride lengths of the horses during the control periods (Gibbs, 2013), however the differences between treatments were still highly significant (P<0.001).

5.4.0 FUTURE RESEARCH

The potential for further research into this area is vast, while remaining in the investigation of IR based therapeutic horse rugs, there is scope to conduct research into the effects of the rugs on the equine condition known as shivers. The University of Minnesota Equine Centre (UMEC) defines shivers as chronic neuromuscular disease caused by a multitude of potential factors:

- Neurological causes: neurotransmitter defects are a possibility (UMEC, 2012) in particular at the Neuromuscular Junction or NMJ (Bishop, 2011). A condition known as Myathenia Gravis (MG) is condition where the NMJs are blocked by antibodies

which block neuromuscular transition of acetylcholine (Bishop, 2011). The result is a delayed seizure like muscular contraction that in horses is a symptom of shivers.

- Myopathic causes: which are diseases of the muscles could also be responsible (UMEC, 2012). If there are deficient amounts of stored glycogen within muscle cells then glycogen levels are depleted quicker and can result in localised muscle cramping. This problem is more problematic in horses than other mammals because they do not have any accelerated rate of glycogen replenishment after exercise like the mechanisms found in humans (Lacombe, 2004).

- Genetic causes: Shivers is most common in draft horse breeds and crosses which would suggest that there may be a genetic predisposition to shivers, however there is not any definitive evidence to support this (UMEC, 2012).

- Infectious disease: shivers may be caused as an after effect of an infectious disease such as influenza, strangles or other systemic diseases (UMEC, 2012). It is suggested that shivers can be caused by neuropathic lesions that are produced as a result of infections or toxins derived from an antecedent disease (a disease that occurs before the legions are developed).

- Trauma: the last suggested cause of shivers is as a response to some form of accidental trauma such as a severe fall (UMEC, 2012).

During the investigation it transpired that one of the horses was a sufferer of shivers. The horse was a control for the first 4 weeks, and was then in the second experimental group. The vet that regularly visits the yard and the yard manager noticed a vast improvement in the horse's shivers symptoms and mood. After the rug was taken away the horse then regressed back to his previous state and started suffering from his shivers symptoms again,

the centre purchased the rug after the 4 months of research were completed and the horse continues to use it to treat his shivers. It is hard to definitively say why the rugs helped alleviate the shivers symptoms, because the causes are still being debated. However the increased circulatory benefits that the rugs give (Lavery, 2003), and the muscular relaxation effects (Stamler, 1994) are highly likely to have helped in this instance.

The results from this study can be used as models to predict how other mammals will respond to use of similar technologies. Humans in particular are of great interest as the performance enhancing effects of the IR exposure could lead to the next advancement in performance enhancing sporting apparel. In the case of human apparel it can be worn during exercise, unlike the rugs that were used in this study. This means that in addition to the muscle relaxing effects (Stamler, 1994) and increased rate of recovery from training or injury (Toyokawa et al, 2003), muscle fatigue during exercise can be reduced which will enhance performance (McClue, 2005). Muscle fatigue is reduced because of the effects of FIR on oxygen perfusion as energy produced at a cellular level is increased (McClue, 2005). To perform during high intensity exercise sports men and women need to generate and maintain a high power output (Korioukhina, 2003). This requires both a high anaerobic capacity and the ability to generate the necessary force and velocity for a given power requirement (Johnston et al, 1998).

The inability to maintain the desired power output defines fatigue (Korioukhina, 2003), and can generally be caused by the accumulation of undesirable substances such as lactic acid which causes a decrease in pH (Nashner and Berthoz, 1978) or the lack of important substances (Lepers et al, 1997) such as glycogen (Vuillerme et al, 2001). Following glycolysis, there is a further step in aerobic (oxygen dependant) cellular respiration; the Kreb/Citric

acid cycle. The citric acid cycle produces adenosine triphosphate (ATP) and oxygen plays a crucial role in the process. Increasing the amount of oxygen in muscular tissue through FIR exposure (Lavery, 2003) will mean that there is more oxygen available for ATP production, thus increasing the available energy, power and strength and, delaying fatigue.

The FIR exposure can also be beneficial for post exercise use due to the beneficial effects of an increased rate of recovery (Whelan, 2001). This can aid with recovering from training, or other forms of intense physical exertions such as competitions. In addition this, along with the relaxing effects on skeletal muscles (Stamler, 1994) may reduce the chances of injury occurring as a result of exercise (Whelan, 2001). One study conducted by Whelan (2001) on Navy Seals showed an improvement of over 40% in the injury rates, giving support to this hypothesis.

Beneficial effects of FIR have been observed in numerous studies such as; Ishibashi et al. (2008), Udagawa et al. (1999) and Nagasawa et al. (1999). There are two different mechanisms described in the literature on the effects of FIR on cancerous cells; one is NO dependant (Leung et al, 2008), and the other mechanism is dependent on the amount of Heat shock protein (HSP), in particular HSP_{70A} (Ishibashi et al, 2008). The effects of FIR induced NO on human breast cancer cells (MCF-7 cells) were studied by Leung et al (2008). They found that in vitro NO acts as an inhibitory factor of carcinogenesis in human breast cancer cells. During their study they observed the effects of non-heating FIR (from a ceramic source rather than electric) and found that there were higher levels of NOS activity in FIR irradiated MCF-7 human breast cancer cells than in the un-irradiated controls. It had been observed previously that NO is a secretory product of normal healthy breast tissue (Nathan, 1992) and that normal healthy breast cells show 100% staining for NOS (Lahari and Martin,

2004). These previous studies also found that there was a reduced expression of NOS in cancerous cells with higher malignancy (Martin et al, 2000) furthermore that NO plays a role in supressing the proliferation of human breast cancer cells (Reveneau et al, 1999). The second mechanism in relation to treatment of cancerous cells with FIR is that of the effect of HSP_{70A}. Ishibashi et al (2008) studied the effects of FIR exposure on 5 different human cancer cell lines; A431 cells found in female vulva tumours, HSC3 found in tumours of the tongue, Sa3 cells found in tumours of the gums or gingiva, A549 found in tumours in the lungs, and MCF-7 cells of female breast tumours. The results of their study showed that cell types HSC3, Sa3, and A549 are FIR sensitive due to low basal expression of HSP_{70A}. However the A431 and MCF-7 cell types were less sensitive to FIR because of high basal expression of HSP_{70A} which protects them against the inhibitory effects on cancerous cell proliferation of FIR exposure.

There is potential to conduct further research into the combined effects of NO on various human cancerous cell types, and on the inhibitory effects of HSP_{70A}. Through gene knockdown techniques using small interfering RNA (siRNA) to inhibit the activity of $HSP_{70A\&C}$. Ishibashi et al (2008) were able to increase the susceptibility of A431 and MCF-7 cell types to FIR exposure and significantly reduce the proliferation of these cancerous cell types. The proliferation of the cancerous cells was measured using Bromodeoxyuridine (BrdU) which can be incorporated into newly synthesised DNA to detect proliferation of living tissue. The results of Ishsibasi et al's (2008) study shows that if a low cost method of accurately detecting HSP_{70} expression can be developed then it can be used to assess the efficacy of FIR treatment of cancer on an individual basis.

Within the field of photobiology there has been a lot of research conducted on the beneficial effects of exposure to infrared radiation. One effect of infrared exposure causes an increase in nitric oxide (NO) levels in exposed tissues (Leung et al, 2008). The NO subsequently causes vasodilation (Hassid, 1989), and an increase in blood flow in the exposed tissues and surrounding areas (Klabunde, 2010). It is my belief that these effects can not only be utilised for the benefits of athletic performance and general wellbeing, it can also be used for production purposes. For example, it takes at least 400 litres of blood to pass through the udders of a lactating dairy cow to produce 1 litre of milk (Dairy Australia, 2011). The average Holstein has roughly 45 litres of blood in her body (Dairy Australia, 2011), in theory, so long as the limiting factors such as nutritional requirements are met, exposing the udders or whole cow to infrared radiation would increase milk yield by increasing blood flow to the udders. This theory has already been investigated in humans (Ogita et al., 1990), in a study designed to aid lactation in women who struggle to produce adequate amounts of milk to breast feed. Ceramic disks were attached to the breast skin of 27 peurperal women who previously had poor lactation, and 36 women with currently poor weaning (Ogita et al., 1990). The study found that ¾ of the women in the study had significantly increased rates of milk production one month after the disks were attached (Ogita et al., 1990) and half were able to breast feed right the way through to weaning.

I would suggest that the infrared radiation could be delivered in one of two ways:

- While the cows are in the parlour then their underside could be exposed to infrared radiation through the use of some infrared emitting hardware embedded in the parlour floor.

- Alternatively a preferential method could be developed through an understanding of optically responsive minerals and their ability to reflect and re-emit heat radiation back as infrared radiation. In the 1990s scientist David Horinek spent most of the decade developing a revolutionary new textile that is now known as *"Celliant"*. The textile is embedded with minerals specifically chosen for their infrared reflective properties (Schnurer et al, 2006). It is written within the corresponding patent that titanium dioxide, quartz and aluminium oxide have the highest infrared reflectance (Schnurer et al, 2006). If a mineral composite of the three listed minerals could be created within a resin and ground to a similar consistency as the sand bedding then it could be put within the sand beds of the dairy housing and will not cause abrasions.

If the proposed mineral composite was included with in the sand beds, then like the sand it would be inert, as it is in-organic it would not add to any disease risk. When the cows lay on the mineral/sand beds, their body heat would be absorbed by the minerals and then reflected back at them in the form of far infrared radiation (FIR). The FIR would stimulate vasodilation and cause an increase in blood flow to exposed areas; if the cows lay on their underside the udders, venous and arterial vessels would be affected.

In addition to increasing blood circulation the infrared radiation will have other beneficial effects such as:

- Pain alleviation through activation of the same metabolic pathways as use of an opiate would activate (Burke, 2009). When the exposed to infrared radiation the NO

activates cyclic guanine monophosphate (cGMP) which mediates the pain relief response.

- Muscle relaxation (Stamler, 1994) is caused by the combined effect of NO and cGMP.

- Increased rate of recovery and wound healing (Toyokawa et al, 2003).

Thus improving the general state of wellbeing which has further potential to increase milk yield in lactating dairy cows (Dairy Australia, 2011).

5.5.0   CONCLUSION

To conclude, the results of this study support those of the study conducted by Grundström and Burströmusing. All three measurements of locomotion were significantly improved (P<0.001) by the use of *"Back on Track"* therapeutic garments over the four week experimental period, whereas there was no significant difference (P>0.05) in the four week control period. This shows that the *"Back on Track"* rugs do improve locomotion, and these affect are highly likely to be due to the effects of the infrared radiation that they emit after absorbing heat energy.

ACKNOWLEDGEMENTS

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APPENDIX 1

Horse	Age	Treatment	Period	Observation	FLSL	HLSL	HLP
Tan	19	Experimental	1	1	2.15	2.216	-0.303
Tan	19	Experimental	1	2	2.533	2.533	-0.27
Tan	19	Experimental	1	3	2.506	2.49	-0.22
Tan	19	Experimental	1	4	2.576	2.556	-0.16
Tan	19	Experimental	1	5	2.49	2.51	-0.196
Tan	19	Experimental	1	6	2.556	2.543	-0.18
Tan	19	Experimental	1	7	2.446	2.446	-0.113
Tan	19	Experimental	1	8	*	*	*
Tan	19	Control	2	1	2.446	2.446	-0.113
Tan	19	Control	2	2	2.366	2.34	-0.346
Tan	19	Control	2	3	2.416	2.43	-0.313
Tan	19	Control	2	4	*	*	*
Tan	19	Control	2	5	2.36	2.346	-0.253
Tan	19	Control	2	6	*	*	*
Tan	19	Control	2	7	2.293	2.283	-0.24
Tan	19	Control	2	8	*	*	*
Ben	15	Experimental	1	1	2.193	2.203	-0.386
Ben	15	Experimental	1	2	2.453	2.456	-0.303
Ben	15	Experimental	1	3	2.55	2.563	-0.206
Ben	15	Experimental	1	4	2.616	2.603	-0.206
Ben	15	Experimental	1	5	2.78	2.773	-0.2
Ben	15	Experimental	1	6	2.613	2.576	-0.15
Ben	15	Experimental	1	7	2.466	2.423	-0.17
Ben	15	Experimental	1	8	*	*	*
Ben	15	Control	2	1	2.466	2.423	-0.17
Ben	15	Control	2	2	2.343	2.356	-0.263
Ben	15	Control	2	3	2.372	2.4	-0.36
Ben	15	Control	2	4	2.263	2.303	-0.376
Ben	15	Control	2	5	*	*	*
Ben	15	Control	2	6	2.26	2.273	-0.363
Ben	15	Control	2	7	*	*	*
Ben	15	Control	2	8	2.343	2.336	-0.393
Bruce	16	Experimental	1	1	2.196	2.24	-0.273
Bruce	16	Experimental	1	2	2.466	2.49	-0.23
Bruce	16	Experimental	1	3	2.783	2.75	-0.106
Bruce	16	Experimental	1	4	2.77	2.773	-0.003
Bruce	16	Experimental	1	5	*	*	*
Bruce	16	Experimental	1	6	*	*	*
Bruce	16	Experimental	1	7	*	*	*
Bruce	16	Experimental	1	8	*	*	*
Bruce	16	Control	2	1	2.77	2.773	-0.003
Bruce	16	Control	2	2	2.45	2.436	-0.2

Bruce	16	Control	2	3	2.383	2.403	-0.223
Bruce	16	Control	2	4	2.273	2.296	-0.253
Bruce	16	Control	2	5	2.083	2.093	-0.223
Bruce	16	Control	2	6	2.443	2.483	-0.286
Bruce	16	Control	2	7	*	*	*
Bruce	16	Control	2	8	2.226	2.203	-0.286
Polly	16	Experimental	1	1	2.473	2.373	-0.23
Polly	16	Experimental	1	2	2.586	2.573	-0.086
Polly	16	Experimental	1	3	3	3.01	0.013
Polly	16	Experimental	1	4	3.136	3.17	0.05
Polly	16	Experimental	1	5	3.183	3.186	0.03
Polly	16	Experimental	1	6	2.856	2.816	-0.083
Polly	16	Experimental	1	7	2.673	2.653	0
Polly	16	Experimental	1	8	*	*	*
Polly	16	Control	2	1	2.673	2.653	0
Polly	16	Control	2	2	2.336	2.326	-0.113
Polly	16	Control	2	3	*	*	*
Polly	16	Control	2	4	2.37	2.346	-0.23
Polly	16	Control	2	5	*	*	*
Polly	16	Control	2	6	2.456	2.466	-0.14
Polly	16	Control	2	7	*	*	*
Polly	16	Control	2	8	*	*	*
Beau	15	Experimental	1	1	2.946	2.943	-0.14
Beau	15	Experimental	1	2	3.193	3.23	-0.003
Beau	15	Experimental	1	3	3.286	3.37	0.096
Beau	15	Experimental	1	4	*	*	*
Beau	15	Experimental	1	5	3.17	3.18	0.023
Beau	15	Experimental	1	6	3.203	3.213	0.273
Beau	15	Experimental	1	7	3.346	3.35	0.136
Beau	15	Experimental	1	8	*	*	*
Beau	15	Control	2	1	3.346	3.35	0.136
Beau	15	Control	2	2	*	*	*
Beau	15	Control	2	3	2.5	2.596	-0.136
Beau	15	Control	2	4	*	*	*
Beau	15	Control	2	5	*	*	*
Beau	15	Control	2	6	2.536	2.553	-0.163
Beau	15	Control	2	7	*	*	*
Beau	15	Control	2	8	2.736	2.766	-0.113
Rossa	7	Experimental	1	1	2.923	2.973	-0.086
Rossa	7	Experimental	1	2	3.096	3.09	-0.023
Rossa	7	Experimental	1	3	3.34	3.31	0.103
Rossa	7	Experimental	1	4	3.343	3.373	0.18
Rossa	7	Experimental	1	5	3.22	3.223	0.07
Rossa	7	Experimental	1	6	3.256	3.296	0.223
Rossa	7	Experimental	1	7	3.24	3.273	0.103

Rossa	7	Experimental	1	8	*	*	*
Rossa	7	Control	2	1	3.24	3.273	0.103
Rossa	7	Control	2	2	2.913	2.91	0.056
Rossa	7	Control	2	3	*	*	*
Rossa	7	Control	2	4	3.053	3.11	-0.006
Rossa	7	Control	2	5	*	*	*
Rossa	7	Control	2	6	2.7	2.683	-0.123
Rossa	7	Control	2	7	*	*	*
Rossa	7	Control	2	8	2.995	2.965	-0.06
AK	10	Experimental	1	1	2.873	2.96	-0.173
AK	10	Experimental	1	2	3.016	3.05	-0.093
AK	10	Experimental	1	3	3.456	3.49	0.13
AK	10	Experimental	1	4	3.53	3.526	0.15
AK	10	Experimental	1	5	3.076	3.113	0.153
AK	10	Experimental	1	6	3.99	4.15	0.543
AK	10	Experimental	1	7	3.62	3.65	0.196
AK	10	Experimental	1	8	*	*	*
AK	10	Control	2	1	3.62	3.65	0.196
AK	10	Control	2	2	3.303	3.313	0.003
AK	10	Control	2	3	*	*	*
AK	10	Control	2	4	*	*	*
AK	10	Control	2	5	*	*	*
AK	10	Control	2	6	2.846	2.89	-0.146
AK	10	Control	2	7	*	*	*
AK	10	Control	2	8	2.873	2.87	-0.17
Patch	5	Experimental	1	1	2.666	2.69	-0.213
Patch	5	Experimental	1	2	3.063	3.086	-0.066
Patch	5	Experimental	1	3	3.083	3.093	0.05
Patch	5	Experimental	1	4	*	*	*
Patch	5	Experimental	1	5	*	*	*
Patch	5	Experimental	1	6	*	*	*
Patch	5	Experimental	1	7	*	*	*
Patch	5	Experimental	1	8	*	*	*
Patch	5	Control	2	1	3.083	3.093	0.05
Patch	5	Control	2	2	*	*	*
Patch	5	Control	2	3	*	*	*
Patch	5	Control	2	4	2.48	2.483	-0.173
Patch	5	Control	2	5	*	*	*
Patch	5	Control	2	6	*	*	*
Patch	5	Control	2	7	*	*	*
Patch	5	Control	2	8	*	*	*
Domino	9	Experimental	1	1	2.823	2.723	0.023
Domino	9	Experimental	1	2	3.26	3.246	0.156
Domino	9	Experimental	1	3	3.313	3.29	0.213
Domino	9	Experimental	1	4	*	*	*

Domino	9	Experimental	1	5	3.226	3.22	0.196
Domino	9	Experimental	1	6	3.053	3.073	0.21
Domino	9	Experimental	1	7	3.04	3.016	0.166
Domino	9	Experimental	1	8	*	*	*
Domino	9	Control	2	1	*	*	*
Domino	9	Control	2	2	*	*	*
Domino	9	Control	2	3	*	*	*
Domino	9	Control	2	4	*	*	*
Domino	9	Control	2	5	*	*	*
Domino	9	Control	2	6	*	*	*
Domino	9	Control	2	7	*	*	*
Domino	9	Control	2	8	*	*	*
Paddy	7	Experimental	1	1	2.553	2.566	-0.26
Paddy	7	Experimental	1	2	2.723	2.633	-0.116
Paddy	7	Experimental	1	3	2.93	2.883	-0.036
Paddy	7	Experimental	1	4	*	*	*
Paddy	7	Experimental	1	5	2.683	2.686	-0.076
Paddy	7	Experimental	1	6	2.59	2.626	-0.023
Paddy	7	Experimental	1	7	2.775	2.765	-0.08
Paddy	7	Experimental	1	8	*	*	*
Paddy	7	Control	2	1	2.775	2.765	-0.08
Paddy	7	Control	2	2	*	*	*
Paddy	7	Control	2	3	*	*	*
Paddy	7	Control	2	4	2.563	2.533	-0.143
Paddy	7	Control	2	5	*	*	*
Paddy	7	Control	2	6	2.146	2.123	-0.296
Paddy	7	Control	2	7	*	*	*
Paddy	7	Control	2	8	2.003	1.973	-0.446
Ellie		Experimental	1	1	2.633	2.66	-0.096
Ellie	16	Experimental	1	2	2.87	2.973	0.03
Ellie	16	Experimental	1	3	3.37	3.41	0.193
Ellie	16	Experimental	1	4	3.55	3.523	0.41
Ellie	16	Experimental	1	5	3.14	3.126	0.24
Ellie	16	Experimental	1	6	3.233	3.19	0.273
Ellie	16	Experimental	1	7	3.33	3.306	0.28
Ellie	16	Experimental	1	8	*	*	*
Ellie	16	Control	2	1	3.33	3.306	0.28
Ellie	16	Control	2	2	2.993	2.993	0.003
Ellie	16	Control	2	3	2.95	3.03	0.01
Ellie	16	Control	2	4	*	*	*
Ellie	16	Control	2	5	*	*	*
Ellie	16	Control	2	6	2.516	2.51	-0.106
Ellie	16	Control	2	7	*	*	*
Ellie	16	Control	2	8	2.65	2.62	-0.126
Ash	16	Control	1	1	2.683	2.666	-0.073

Ash	16	Control	1	2	2.673	2.676	-0.08
Ash	16	Control	1	3	2.766	2.713	-0.05
Ash	16	Control	1	4	2.723	2.723	-0.016
Ash	16	Control	1	5	2.576	2.563	-0.13
Ash	16	Control	1	6	2.693	2.716	-0.073
Ash	16	Control	1	7	2.376	2.336	-0.186
Ash	16	Control	1	8	*	*	*
Ash	16	Experimental	2	1	2.376	2.336	-0.186
Ash	16	Experimental	2	2	2.88	2.93	0.133
Ash	16	Experimental	2	3	2.74	2.733	0.03
Ash	16	Experimental	2	4	2.76	2.79	0.086
Ash	16	Experimental	2	5	2.933	2.936	0.106
Ash	16	Experimental	2	6	2.9	2.906	0.096
Ash	16	Experimental	2	7	3.006	3.016	0.143
Ash	16	Experimental	2	8	*	*	*
Stan	20	Control	1	1	2.256	2.286	-0.153
Stan	20	Control	1	2	2.713	2.663	-0.153
Stan	20	Control	1	3	2.43	2.393	-0.15
Stan	20	Control	1	4	2.06	2.046	-0.213
Stan	20	Control	1	5	2.053	2.13	-0.393
Stan	20	Control	1	6	2.536	2.503	-0.183
Stan	20	Control	1	7	2.146	2.16	-0.323
Stan	20	Control	1	8	*	*	*
Stan	20	Experimental	2	1	2.146	2.16	-0.323
Stan	20	Experimental	2	2	2.406	2.426	-0.086
Stan	20	Experimental	2	3	2.603	2.58	-0.046
Stan	20	Experimental	2	4	2.373	2.333	-0.06
Stan	20	Experimental	2	5	2.466	2.506	-0.016
Stan	20	Experimental	2	6	2.43	2.416	-0.006
Stan	20	Experimental	2	7	2.563	2.57	0.046
Stan	20	Experimental	2	8	*	*	*
Bob	11	Control	1	1	2.756	2.816	-0.07
Bob	11	Control	1	2	2.786	2.84	-0.076
Bob	11	Control	1	3	2.886	2.86	-0.033
Bob	11	Control	1	4	2.58	2.583	-0.08
Bob	11	Control	1	5	2.623	2.596	-0.09
Bob	11	Control	1	6	2.77	2.73	-0.066
Bob	11	Control	1	7	2.83	2.803	-0.106
Bob	11	Control	1	8	*	*	*
Bob	11	Experimental	2	1	2.83	2.803	-0.106
Bob	11	Experimental	2	2	3.073	3.106	0.163
Bob	11	Experimental	2	3	3.183	3.2	0.156
Bob	11	Experimental	2	4	2.936	2.916	0.21
Bob	11	Experimental	2	5	3.305	3.36	0.3
Bob	11	Experimental	2	6	3.2	3.21	0.24

Bob	11	Experimental	2	7	3.163	3.16	0.283
Bob	11	Experimental	2	8	*	*	*
Woody	9	Control	1	1	2.573	2.65	-0.096
Woody	9	Control	1	2	2.606	2.65	-0.08
Woody	9	Control	1	3	2.613	2.64	-0.08
Woody	9	Control	1	4	2.486	2.433	-0.126
Woody	9	Control	1	5	2.786	2.763	-0.126
Woody	9	Control	1	6	2.55	2.566	-0.1
Woody	9	Control	1	7	2.186	2.16	-0.223
Woody	9	Control	1	8	*	*	*
Woody	9	Experimental	2	1	2.186	2.16	-0.223
Woody	9	Experimental	2	2	2.766	2.843	0.04
Woody	9	Experimental	2	3	2.9	2.953	0.206
Woody	9	Experimental	2	4	3.063	3.143	0.266
Woody	9	Experimental	2	5	3.076	3.096	0.27
Woody	9	Experimental	2	6	3.053	3.056	0.276
Woody	9	Experimental	2	7	*	*	*
Woody	9	Experimental	2	8	*	*	*
GV	17	Control	1	1	2.436	2.47	-0.21
GV	17	Control	1	2	2.383	2.396	-0.183
GV	17	Control	1	3	2.386	2.4	-0.18
GV	17	Control	1	4	2.376	2.383	-0.21
GV	17	Control	1	5	2.043	2.08	-0.283
GV	17	Control	1	6	*	*	*
GV	17	Control	1	7	2.023	2.01	-0.32
GV	17	Control	1	8	*	*	*
GV	17	Experimental	2	1	2.023	2.01	-0.32
GV	17	Experimental	2	2	2.57	2.583	-0.033
GV	17	Experimental	2	3	2.116	2.103	-0.096
GV	17	Experimental	2	4	2.433	2.46	-0.013
GV	17	Experimental	2	5	2.37	2.36	-0.01
GV	17	Experimental	2	6	2.446	2.493	0.083
GV	17	Experimental	2	7	*	*	*
GV	17	Experimental	2	8	*	*	*
George	4	Control	1	1	2.246	2.3	-0.136
George	4	Control	1	2	2.236	2.24	-0.136
George	4	Control	1	3	2.24	2.273	-0.14
George	4	Control	1	4	2.09	2.11	-0.173
George	4	Control	1	5	2.303	2.29	-0.203
George	4	Control	1	6	2.01	2.063	-0.22
George	4	Control	1	7	2.283	2.286	-0.186
George	4	Control	1	8	*	*	*
George	4	Experimental	2	1	2.283	2.286	-0.186
George	4	Experimental	2	2	2.543	2.65	0.04
George	4	Experimental	2	3	2.62	2.69	0.073

George	4	Experimental	2	4	2.156	2.216	-0.003
George	4	Experimental	2	5	2.373	2.436	0.006
George	4	Experimental	2	6	2.51	2.52	0.056
George	4	Experimental	2	7	2.603	2.633	0.03
George	4	Experimental	2	8	*	*	*
Enid	17	Control	1	1	2.573	2.623	-0.253
Enid	17	Control	1	2	2.583	2.596	-0.233
Enid	17	Control	1	3	2.606	2.646	-0.183
Enid	17	Control	1	4	2.266	2.296	-0.183
Enid	17	Control	1	5	2.673	2.596	-0.286
Enid	17	Control	1	6	*	*	*
Enid	17	Control	1	7	2.423	2.47	-0.336
Enid	17	Control	1	8	*	*	*
Enid	17	Experimental	2	1	2.423	2.47	-0.336
Enid	17	Experimental	2	2	2.593	2.576	-0.106
Enid	17	Experimental	2	3	2.596	2.59	-0.183
Enid	17	Experimental	2	4	2.36	2.346	-0.136
Enid	17	Experimental	2	5	2.336	2.343	-0.18
Enid	17	Experimental	2	6	2.64	2.646	-0.043
Enid	17	Experimental	2	7	2.56	2.55	-0.07
Enid	17	Experimental	2	8	*	*	*
Kizzy	22	Control	1	1	2.29	2.276	-0.356
Kizzy	22	Control	1	2	2.163	2.213	-0.326
Kizzy	22	Control	1	3	2.146	2.15	-0.346
Kizzy	22	Control	1	4	2.18	2.186	-0.343
Kizzy	22	Control	1	5	2.106	2.116	-0.353
Kizzy	22	Control	1	6	2.086	2.12	-0.356
Kizzy	22	Control	1	7	1.98	1.98	-0.37
Kizzy	22	Control	1	8	2.04	2.05	-0.363
Kizzy	22	Experimental	2	1	2.04	2.05	-0.363
Kizzy	22	Experimental	2	2	2.23	2.236	-0.246
Kizzy	22	Experimental	2	3	2.063	2.103	-0.276
Kizzy	22	Experimental	2	4	2.226	2.25	-0.23
Kizzy	22	Experimental	2	5	2.29	2.19	-0.263
Kizzy	22	Experimental	2	6	2.463	2.44	-0.166
Kizzy	22	Experimental	2	7	2.12	2.125	-0.23
Kizzy	22	Experimental	2	8	2.12	2.14	-0.263
Dan	23	Control	1	1	2.86	2.896	-0.12
Dan	23	Control	1	2	2.676	2.716	-0.176
Dan	23	Control	1	3	2.446	2.473	-0.236
Dan	23	Control	1	4	2.426	2.403	-0.26
Dan	23	Control	1	5	2.336	2.35	-0.316
Dan	23	Control	1	6	2.54	2.576	-0.243
Dan	23	Control	1	7	2.29	2.27	-0.276
Dan	23	Control	1	8	2.48	2.476	-0.253

Dan	23	Experimental	2	1	2.48	2.476	-0.253
Dan	23	Experimental	2	2	2.525	2.545	-0.176
Dan	23	Experimental	2	3	2.5	2.556	-0.166
Dan	23	Experimental	2	4	2.436	2.463	-0.13
Dan	23	Experimental	2	5	2.483	2.503	-0.156
Dan	23	Experimental	2	6	2.88	2.943	0
Dan	23	Experimental	2	7	2.73	2.713	-0.106
Dan	23	Experimental	2	8	2.24	2.46	-0.1
Jack	17	Control	1	1	2.213	2.23	-0.33
Jack	17	Control	1	2	1.98	2.003	-0.326
Jack	17	Control	1	3	2.106	2.103	-0.296
Jack	17	Control	1	4	1.96	1.946	-0.35
Jack	17	Control	1	5	2.183	2.156	-0.33
Jack	17	Control	1	6	2.023	2.016	-0.36
Jack	17	Control	1	7	2.003	1.97	-0.366
Jack	17	Control	1	8	1.84	1.826	-0.376
Jack	17	Experimental	2	1	1.84	1.826	-0.376
Jack	17	Experimental	2	2	2.063	2.046	-0.243
Jack	17	Experimental	2	3	2.186	2.173	-0.236
Jack	17	Experimental	2	4	2.136	2.13	-0.216
Jack	17	Experimental	2	5	2.296	2.296	-0.173
Jack	17	Experimental	2	6	2.37	2.346	-0.183
Jack	17	Experimental	2	7	2.273	2.616	-0.196
Jack	17	Experimental	2	8	2.09	2.07	-0.18
Louby	12	Control	1	1	2.916	2.88	0.006
Louby	12	Control	1	2	2.9	3.016	-0.06
Louby	12	Control	1	3	2.68	2.623	-0.1
Louby	12	Control	1	4	2.396	2.443	-0.203
Louby	12	Control	1	5	2.51	2.476	-0.213
Louby	12	Control	1	6	2.533	2.523	-0.143
Louby	12	Control	1	7	*	*	*
Louby	12	Control	1	8	2.393	2.423	-0.12
Louby	12	Experimental	2	1	2.393	2.423	-0.12
Louby	12	Experimental	2	2	3.3	3.236	0.06
Louby	12	Experimental	2	3	3.29	3.256	0.103
Louby	12	Experimental	2	4	3.046	3.023	0.06
Louby	12	Experimental	2	5	*	*	*
Louby	12	Experimental	2	6	*	*	*
Louby	12	Experimental	2	7	3.06	3.036	0.11
Louby	12	Experimental	2	8	3.11	3.053	0.083
Bailey	17	Control	1	1	2.343	2.376	-0.053
Bailey	17	Control	1	2	2.643	2.62	-0.08
Bailey	17	Control	1	3	2.613	2.61	-0.076
Bailey	17	Control	1	4	*	*	*
Bailey	17	Control	1	5	2.52	2.473	-0.106

Bailey	17	Control	1	6	2.653	2.643	-0.063
Bailey	17	Control	1	7	2.646	2.596	-0.096
Bailey	17	Control	1	8	2.46	2.473	-0.11
Bailey	17	Experimental	2	1	2.46	2.473	-0.11
Bailey	17	Experimental	2	2	2.713	2.746	-0.01
Bailey	17	Experimental	2	3	2.57	2.606	-0.006
Bailey	17	Experimental	2	4	2.613	2.63	-0.006
Bailey	17	Experimental	2	5	2.636	2.67	-0.006
Bailey	17	Experimental	2	6	2.59	2.585	0.02
Bailey	17	Experimental	2	7	2.52	2.57	-0.006
Bailey	17	Experimental	2	8	2.946	2.96	0.146
Jay	16	Control	1	1	3.113	3.21	0.233
Jay	16	Control	1	2	2.536	2.55	0.06
Jay	16	Control	1	3	3.02	3.05	0.15
Jay	16	Control	1	4	*	*	*
Jay	16	Control	1	5	2.663	2.636	0.06
Jay	16	Control	1	6	2.836	2.85	0.15
Jay	16	Control	1	7	2.723	2.726	0.103
Jay	16	Control	1	8	2.573	2.543	0.043
Jay	16	Experimental	2	1	2.573	2.543	0.043
Jay	16	Experimental	2	2	2.866	2.856	0.146
Jay	16	Experimental	2	3	2.756	2.783	0.153
Jay	16	Experimental	2	4	2.786	2.74	0.163
Jay	16	Experimental	2	5	2.796	2.78	0.13
Jay	16	Experimental	2	6	2.746	2.743	0.153
Jay	16	Experimental	2	7	2.61	2.613	0.13
Jay	16	Experimental	2	8	2.803	2.826	0.23
Harley	21	Control	1	1	2.89	2.966	-0.086
Harley	21	Control	1	2	2.763	2.8	-0.096
Harley	21	Control	1	3	2.703	2.726	-0.083
Harley	21	Control	1	4	2.99	2.896	-0.13
Harley	21	Control	1	5	3.093	3.02	-0.15
Harley	21	Control	1	6	*	*	*
Harley	21	Control	1	7	*	*	*
Harley	21	Control	1	8	*	*	*
Harley	21	Experimental	2	1	3.093	3.02	-0.15
Harley	21	Experimental	2	2	2.906	2.896	-0.016
Harley	21	Experimental	2	3	2.863	2.93	0.07
Harley	21	Experimental	2	4	3.165	3.15	0.11
Harley	21	Experimental	2	5	2.77	2.74	0.035
Harley	21	Experimental	2	6	3.203	3.213	0.183
Harley	21	Experimental	2	7	2.863	2.826	0.056
Harley	21	Experimental	2	8	2.69	2.733	0.06
Stubble	7	Control	1	1	2.983	2.96	0.003
Stubble	7	Control	1	2	3.063	3.043	0.016

Stubble	7	Control	1	3	2.753	2.67	-0.06
Stubble	7	Control	1	4	2.265	3.15	0.015
Stubble	7	Control	1	5	3.156	3.193	-0.04
Stubble	7	Control	1	6	*	*	*
Stubble	7	Control	1	7	*	*	*
Stubble	7	Control	1	8	*	*	*
Stubble	7	Experimental	2	1	3.156	3.193	-0.04
Stubble	7	Experimental	2	2	3.18	3.266	0.006
Stubble	7	Experimental	2	3	3.136	3.153	0.13
Stubble	7	Experimental	2	4	3.143	3.146	0.13
Stubble	7	Experimental	2	5	2.946	3.203	0.05
Stubble	7	Experimental	2	6	3.056	3.063	0.083
Stubble	7	Experimental	2	7	2.726	2.72	0
Stubble	7	Experimental	2	8	3.23	3.24	0.17
Horace	8	Experimental	1	1	2.67	2.75	-0.113
Horace	8	Experimental	1	2	2.686	2.753	-0.023
Horace	8	Experimental	1	3	2.84	2.75	-0.003
Horace	8	Experimental	1	4	2.72	2.743	0.0166
Horace	8	Experimental	1	5	2.723	2.76	0
Horace	8	Experimental	1	6	2.68	2.696	0.06
Horace	8	Experimental	1	7	2.593	2.596	0
Horace	8	Experimental	1	8	2.656	2.666	0.023
Horace	8	Control	2	1	2.656	2.666	0.023
Horace	8	Control	2	2	2.226	2.286	-0.133
Horace	8	Control	2	3	2.713	2.733	-0.073
Horace	8	Control	2	4	*	*	*
Horace	8	Control	2	5	*	*	*
Horace	8	Control	2	6	*	*	*
Horace	8	Control	2	7	*	*	*
Horace	8	Control	2	8	*	*	*
Scotty	22	Experimental	1	1	2.48	2.476	-0.253
Scotty	22	Experimental	1	2	2.82	2.903	-0.036
Scotty	22	Experimental	1	3	2.693	2.713	-0.04
Scotty	22	Experimental	1	4	2.656	2.646	-0.016
Scotty	22	Experimental	1	5	2.43	2.43	-0.06
Scotty	22	Experimental	1	6	2.706	2.703	-0.006
Scotty	22	Experimental	1	7	2.806	2.81	-0.013
Scotty	22	Experimental	1	8	2.456	2.463	-0.043
Scotty	22	Control	2	1	2.456	2.463	-0.043
Scotty	22	Control	2	2	2.29	2.256	-0.146
Scotty	22	Control	2	3	2.373	2.396	-0.18
Scotty	22	Control	2	4	2.54	2.546	-0.1
Scotty	22	Control	2	5	*	*	*
Scotty	22	Control	2	6	*	*	*
Scotty	22	Control	2	7	*	*	*

Scotty	22	Control	2	8	*	*	*
Count	17	Experimental	1	1	2.48	2.476	-0.253
Count	17	Experimental	1	2	2.84	2.883	-0.086
Count	17	Experimental	1	3	3.17	3.17	-0.066
Count	17	Experimental	1	4	3.073	3.036	-0.02
Count	17	Experimental	1	5	2.793	2.763	-0.073
Count	17	Experimental	1	6	3.24	2.17	0.01
Count	17	Experimental	1	7	3.23	3.176	-0.023
Count	17	Experimental	1	8	*	*	*
Count	17	Control	2	1	3.23	3.176	-0.023
Count	17	Control	2	2	*	*	*
Count	17	Control	2	3	*	*	*
Count	17	Control	2	4	2.84	2.873	-0.163
Count	17	Control	2	5	*	*	*
Count	17	Control	2	6	*	*	*
Count	17	Control	2	7	*	*	*
Count	17	Control	2	8	*	*	*
Aussie	7	Experimental	1	1	2.48	2.476	-0.253
Aussie	7	Experimental	1	2	2.76	2.74	-0.02
Aussie	7	Experimental	1	3	2.986	2.996	-0.01
Aussie	7	Experimental	1	4	2.88	2.823	0.01
Aussie	7	Experimental	1	5	2.9	2.955	-0.02
Aussie	7	Experimental	1	6	2.936	2.91	0.043
Aussie	7	Experimental	1	7	2.825	2.815	0.005
Aussie	7	Experimental	1	8	2.586	2.583	0.01
Aussie	7	Control	2	1	2.586	2.583	0.01
Aussie	7	Control	2	2	2.41	2.353	-0.096
Aussie	7	Control	2	3	2.813	2.82	-1.03
Aussie	7	Control	2	4	2.653	2.656	-0.083
Aussie	7	Control	2	5	*	*	*
Aussie	7	Control	2	6	*	*	*
Aussie	7	Control	2	7	*	*	*
Aussie	7	Control	2	8	*	*	*
Missy	12	Experimental	1	1	2.48	2.476	-0.253
Missy	12	Experimental	1	2	2.91	2.906	-0.086
Missy	12	Experimental	1	3	2.9	2.933	-0.1
Missy	12	Experimental	1	4	2.846	2.86	-0.103
Missy	12	Experimental	1	5	2.6	2.613	-0.12
Missy	12	Experimental	1	6	2.876	2.913	-0.086
Missy	12	Experimental	1	7	2.836	2.816	-0.093
Missy	12	Experimental	1	8	2.556	2.606	-0.12
Missy	12	Control	2	1	2.556	2.606	-0.12
Missy	12	Control	2	2	2.29	2.293	-0.246
Missy	12	Control	2	3	2.483	2.546	-0.243
Missy	12	Control	2	4	2.47	2.523	-0.196

Missy	12	Control	2	5	*	*	*
Missy	12	Control	2	6	*	*	*
Missy	12	Control	2	7	*	*	*
Missy	12	Control	2	8	*	*	*
Alibi	16	Control	1	1	2.9	2.976	-0.193
Alibi	16	Control	1	2	2.743	2.82	-0.206
Alibi	16	Control	1	3	2.926	2.933	-0.146
Alibi	16	Control	1	4	2.706	2.77	-0.193
Alibi	16	Control	1	5	2.896	2.9	-0.086
Alibi	16	Control	1	6	2.98	3.02	-0.13
Alibi	16	Control	1	7	2.69	2.68	-0.106
Alibi	16	Control	1	8	2.813	2.796	-0.133
Alibi	16	Experimental	2	1	2.813	2.796	-0.133
Alibi	16	Experimental	2	2	2.586	2.62	0.006
Alibi	16	Experimental	2	3	2.856	2.823	-0.02
Alibi	16	Experimental	2	4	2.833	2.893	-0.03
Alibi	16	Experimental	2	5	2.776	2.753	0.006
Alibi	16	Experimental	2	6	*	*	*
Alibi	16	Experimental	2	7	*	*	*
Alibi	16	Experimental	2	8	*	*	*
Silvie	16	Control	1	1	2.32	2.306	-0.266
Silvie	16	Control	1	2	2.41	2.4	-0.263
Silvie	16	Control	1	3	2.516	2.54	-0.206
Silvie	16	Control	1	4	2.54	2.5	-0.226
Silvie	16	Control	1	5	2.383	2.403	-0.266
Silvie	16	Control	1	6	2.37	2.426	-0.296
Silvie	16	Control	1	7	*	*	*
Silvie	16	Control	1	8	*	*	*
Silvie	16	Experimental	2	1	2.37	2.426	-0.296
Silvie	16	Experimental	2	2	2.223	2.17	-0.116
Silvie	16	Experimental	2	3	2.536	2.543	-0.096
Silvie	16	Experimental	2	4	2.566	2.586	-0.09
Silvie	16	Experimental	2	5	*	*	*
Silvie	16	Experimental	2	6	2.4	2.443	-0.113
Silvie	16	Experimental	2	7	*	*	*
Silvie	16	Experimental	2	8	2.503	2.553	-0.15
Harley (BCA)	8	Control	1	1	2.123	2.13	-0.296
Harley (BCA)	8	Control	1	2	2.19	2.213	-0.25
Harley (BCA)	8	Control	1	3	2.186	2.21	-0.283
Harley (BCA)	8	Control	1	4	2.096	2.07	-0.33
Harley	8	Control	1	5	2.153	2.17	-0.3

(BCA) Harley							
(BCA) Harley	8	Control	1	6	*	*	*
(BCA) Harley	8	Control	1	7	*	*	*
(BCA) Harley	8	Control	1	8	*	*	*
(BCA) Harley	8	Experimental	2	1	2.153	2.17	-0.3
(BCA) Harley	8	Experimental	2	2	2.36	2.42	-0.023
(BCA) Harley	8	Experimental	2	3	2.406	2.373	-0.136
(BCA) Harley	8	Experimental	2	4	2.276	2.28	-0.166
(BCA) Harley	8	Experimental	2	5	*	*	*
(BCA) Harley	8	Experimental	2	6	2.266	2.286	-0.143
(BCA) Harley	8	Experimental	2	7	*	*	*
(BCA) Harley	8	Experimental	2	8	2.32	2.35	-0.133
Tegan	6	Control	1	1	2.276	2.283	-0.216
Tegan	6	Control	1	2	2.503	2.496	-0.113
Tegan	6	Control	1	3	2.46	2.433	-0.186
Tegan	6	Control	1	4	2.26	2.243	-0.236
Tegan	6	Control	1	5	2.126	2.17	-0.233
Tegan	6	Control	1	6	2.26	2.26	-0.176
Tegan	6	Control	1	7	*	*	*
Tegan	6	Control	1	8	*	*	*
Tegan	6	Experimental	2	1	2.26	2.26	-0.176
Tegan	6	Experimental	2	2	2.49	2.506	0.01
Tegan	6	Experimental	2	3	2.67	2.666	-0.05
Tegan	6	Experimental	2	4	2.446	2.46	-0.06
Tegan	6	Experimental	2	5	*	*	*
Tegan	6	Experimental	2	6	2.38	2.373	-0.036
Tegan	6	Experimental	2	7	*	*	*
Tegan	6	Experimental	2	8	2.573	2.626	-0.016
Noddy	20	Control	1	1	2.443	2.426	-0.273
Noddy	20	Control	1	2	2.473	2.47	-0.226
Noddy	20	Control	1	3	2.58	2.563	-0.256
Noddy	20	Control	1	4	2.356	2.386	-0.32
Noddy	20	Control	1	5	2.42	2.426	-0.323
Noddy	20	Control	1	6	2.343	2.303	-0.31
Noddy	20	Control	1	7	*	*	*

Noddy	20	Control	1	8	*	*	*
Noddy	20	Experimental	2	1	2.343	2.303	-0.31
Noddy	20	Experimental	2	2	2.496	2.48	-0.076
Noddy	20	Experimental	2	3	2.903	2.86	-0.04
Noddy	20	Experimental	2	4	2.616	2.573	-0.153
Noddy	20	Experimental	2	5	*	*	*
Noddy	20	Experimental	2	6	2.57	2.556	-0.126
Noddy	20	Experimental	2	7	*	*	*
Noddy	20	Experimental	2	8	2.626	2.63	-0.17
Milly	17	Control	1	1	2.543	2.55	-0.213
Milly	17	Control	1	2	2.49	2.526	-0.163
Milly	17	Control	1	3	2.58	2.586	-0.203
Milly	17	Control	1	4	2.2	2.236	-0.306
Milly	17	Control	1	5	2.346	2.373	-0.3
Milly	17	Control	1	6	2.516	2.54	-0.3
Milly	17	Control	1	7	*	*	*
Milly	17	Control	1	8	*	*	*
Milly	17	Experimental	2	1	2.516	2.54	-0.3
Milly	17	Experimental	2	2	2.536	2.556	-0.09
Milly	17	Experimental	2	3	2.743	2.676	-0.113
Milly	17	Experimental	2	4	*	*	*
Milly	17	Experimental	2	5	*	*	*
Milly	17	Experimental	2	6	*	*	*
Milly	17	Experimental	2	7	*	*	*
Milly	17	Experimental	2	8	*	*	*
Lenny	9	Control	1	1	2.466	2.456	-0.186
Lenny	9	Control	1	2	*	*	*
Lenny	9	Control	1	3	2.6	2.57	-0.183
Lenny	9	Control	1	4	*	*	*
Lenny	9	Control	1	5	*	*	*
Lenny	9	Control	1	6	*	*	*
Lenny	9	Control	1	7	*	*	*
Lenny	9	Control	1	8	*	*	*
Lenny	9	Experimental	2	1	2.466	2.456	-0.186
Lenny	9	Experimental	2	2	2.613	2.667	0.063
Lenny	9	Experimental	2	3	2.45	2.423	-0.07
Lenny	9	Experimental	2	4	2.516	2.503	-0.086
Lenny	9	Experimental	2	5	*	*	*
Lenny	9	Experimental	2	6	2.723	2.706	0.003
Lenny	9	Experimental	2	7	*	*	*
Lenny	9	Experimental	2	8	2.78	2.753	0
Acido	17	Control	1	1	2.276	2.363	-0.34
Acido	17	Control	1	2	2.363	2.31	-0.34
Acido	17	Control	1	3	*	*	*
Acido	17	Control	1	4	2.243	2.213	-0.293

Acido	17	Control	1	5	2.19	2.226	-0.323
Acido	17	Control	1	6	2.28	2.29	-0.34
Acido	17	Control	1	7	*	*	*
Acido	17	Control	1	8	*	*	*
Acido	17	Experimental	2	1	2.28	2.29	-0.34
Acido	17	Experimental	2	2	2.383	2.366	-0.063
Acido	17	Experimental	2	3	2.606	2.6	-0.116
Acido	17	Experimental	2	4	2.58	2.576	-0.093
Acido	17	Experimental	2	5	*	*	*
Acido	17	Experimental	2	6	2.396	3.36	-0.01
Acido	17	Experimental	2	7	*	*	*
Acido	17	Experimental	2	8	2.433	2.436	-0.146
Dulcie	10	Control	1	1	2.446	2.386	-0.173
Dulcie	10	Control	1	2	2.696	2.673	-0.143
Dulcie	10	Control	1	3	*	*	*
Dulcie	10	Control	1	4	2.49	2.49	-0.156
Dulcie	10	Control	1	5	2.44	2.43	-0.176
Dulcie	10	Control	1	6	2.33	2.356	-0.203
Dulcie	10	Control	1	7	*	*	*
Dulcie	10	Control	1	8	*	*	*
Dulcie	10	Experimental	2	1	2.33	2.356	-0.203
Dulcie	10	Experimental	2	2	*	*	*
Dulcie	10	Experimental	2	3	2.643	2.613	-0.033
Dulcie	10	Experimental	2	4	2.55	2.536	-0.053
Dulcie	10	Experimental	2	5	*	*	*
Dulcie	10	Experimental	2	6	2.59	2.603	-0.013
Dulcie	10	Experimental	2	7	*	*	*
Dulcie	10	Experimental	2	8	2.596	2.596	-0.036
Commet	10	Experimental	1	1	2.48	2.476	-0.253
Commet	10	Experimental	1	2	2.533	2.553	0.046
Commet	10	Experimental	1	3	2.493	2.493	0.05
Commet	10	Experimental	1	4	2.49	2.496	0.026
Commet	10	Experimental	1	5	2.416	2.41	0.08
Commet	10	Experimental	1	6	2.493	2.513	0.066
Commet	10	Experimental	1	7	2.503	2.536	0.076
Commet	10	Experimental	1	8	*	*	*
Commet	10	Control	2	1	2.503	2.536	0.076
Commet	10	Control	2	2	2.016	2.043	-0.053
Commet	10	Control	2	3	*	*	*
Commet	10	Control	2	4	*	*	*
Commet	10	Control	2	5	*	*	*
Commet	10	Control	2	6	*	*	*
Commet	10	Control	2	7	*	*	*
Commet	10	Control	2	8	*	*	*
Casey	10	Experimental	1	1	2.48	2.476	-0.253

Casey	10	Experimental	1	2	2.74	2.776	-0.043
Casey	10	Experimental	1	3	2.62	2.6	-0.04
Casey	10	Experimental	1	4	2.69	2.653	-0.023
Casey	10	Experimental	1	5	2.476	2.433	-0.026
Casey	10	Experimental	1	6	2.596	2.576	-0.043
Casey	10	Experimental	1	7	2.676	2.646	-0.006
Casey	10	Experimental	1	8	*	*	*
Casey	10	Control	2	1	2.676	2.646	-0.006
Casey	10	Control	2	2	2.303	2.306	-0.16
Casey	10	Control	2	3	*	*	*
Casey	10	Control	2	4	*	*	*
Casey	10	Control	2	5	*	*	*
Casey	10	Control	2	6	*	*	*
Casey	10	Control	2	7	*	*	*
Casey	10	Control	2	8	*	*	*
Tico	17	Experimental	1	1	2.48	2.476	-0.253
Tico	17	Experimental	1	2	2.663	2.626	-0.023
Tico	17	Experimental	1	3	2.476	2.47	-0.066
Tico	17	Experimental	1	4	2.566	2.55	0
Tico	17	Experimental	1	5	2.41	2.343	0
Tico	17	Experimental	1	6	*	*	*
Tico	17	Experimental	1	7	2.473	2.516	0.01
Tico	17	Experimental	1	8	*	*	*
Tico	17	Control	2	1	2.473	2.516	0.01
Tico	17	Control	2	2	2.256	2.276	-0.123
Tico	17	Control	2	3	*	*	*
Tico	17	Control	2	4	*	*	*
Tico	17	Control	2	5	*	*	*
Tico	17	Control	2	6	*	*	*
Tico	17	Control	2	7	*	*	*
Tico	17	Control	2	8	*	*	*
Jade	23	Experimental	1	1	2.48	2.476	-0.253
Jade	23	Experimental	1	2	2.486	2.55	-0.11
Jade	23	Experimental	1	3	2.506	2.463	-0.11
Jade	23	Experimental	1	4	2.64	2.64	-0.11
Jade	23	Experimental	1	5	2.5	2.52	-0.063
Jade	23	Experimental	1	6	2.69	2.683	-0.056
Jade	23	Experimental	1	7	2.6	2.61	-0.076
Jade	23	Experimental	1	8	*	*	*
Jade	23	Control	2	1	2.6	2.61	-0.076
Jade	23	Control	2	2	2.393	2.39	-0.196
Jade	23	Control	2	3	*	*	*
Jade	23	Control	2	4	*	*	*
Jade	23	Control	2	5	*	*	*
Jade	23	Control	2	6	*	*	*

| Jade | 23 | Control | 2 | 7 | * | * | * |
| Jade | 23 | Control | 2 | 8 | * | * | * |

APPENDIX 2
FLSL

General Linear Model: FLSL versus Treatment, Period, Observation

```
Factor       Type   Levels  Values
Treatment    fixed       2  Control, Experimental
Period       fixed       2  1, 2
Observation  fixed       8  1, 2, 3, 4, 5, 6, 7, 8


Analysis of Variance for FLSL, using Adjusted SS for Tests

Source                 DF    Seq SS    Adj SS    Adj MS      F      P
Age                     1   2.43248   1.55082   1.55082  17.60  0.000
Treatment               1   4.12941   4.01961   4.01961  45.62  0.000
Period                  1   0.59387   0.29926   0.29926   3.40  0.066
Treatment*Period        1   2.14139   1.54369   1.54369  17.52  0.000
Observation             7   0.74022   0.67175   0.09596   1.09  0.369
Treatment*Observation   7   3.74050   3.74050   0.53436   6.06  0.000
Error                 493  43.43856  43.43856   0.08811
Total                 511  57.21643


S = 0.296834   R-Sq = 24.08%   R-Sq(adj) = 21.31%


Term          Coef   SE Coef       T      P
Constant   2.75265   0.03880   70.94  0.000
Age       -0.010632  0.002534   -4.20  0.000


Unusual Observations for FLSL

Obs     FLSL      Fit    SE Fit   Residual   St Resid
 73   3.34600  2.68727  0.05181    0.65873     2.25 R
100   3.53000  2.86735  0.05262    0.66265     2.27 R
102   3.99000  2.90924  0.05254    1.08076     3.70 R
103   3.62000  2.90097  0.05611    0.71903     2.47 R
105   3.62000  2.74043  0.05225    0.87957     3.01 R
106   3.30300  2.59973  0.05590    0.70327     2.41 R
160   2.00300  2.57701  0.08442   -0.57401    -2.02 R
164   3.55000  2.80356  0.05237    0.74644     2.55 R
169   3.33000  2.67664  0.05209    0.65336     2.24 R
221   3.30500  2.67075  0.05619    0.63425     2.18 R
268   2.15600  2.75876  0.05687   -0.60276    -2.07 R
346   3.30000  2.63797  0.04865    0.66203     2.26 R
388   2.99000  2.32802  0.05776    0.66198     2.27 R
389   3.09300  2.37122  0.06134    0.72178     2.49 R
393   3.09300  2.31553  0.05068    0.77747     2.66 R
396   3.16500  2.57802  0.05206    0.58698     2.01 R
405   3.15600  2.52006  0.06289    0.63594     2.19 R
409   3.15600  2.46438  0.05152    0.69162     2.37 R
416   3.23000  2.63584  0.07112    0.59416     2.06 R
650   2.01600  2.59973  0.05590   -0.58373    -2.00 R

R denotes an observation with a large standardized residual.


Means for Covariates

Covariate  Mean   StDev
Age       14.21   5.247
```

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Least Squares Means for FLSL

Treatment               Mean  SE Mean
Control                2.502  0.02324
Experimental           2.701  0.01816
Period
1                      2.628  0.01841
2                      2.575  0.02236
Treatment*Period
Control      1         2.468  0.02539
Control      2         2.536  0.03778
Experimental 1         2.787  0.02685
Experimental 2         2.615  0.02401
Treatment*Observation
Control        1       2.662  0.04535
Control        2       2.521  0.04838
Control        3       2.574  0.05412
Control        4       2.434  0.05391
Control        5       2.477  0.06223
Control        6       2.483  0.05884
Control        7       2.397  0.08164
Control        8       2.467  0.07935
Experimental   1       2.474  0.04533
Experimental   2       2.701  0.04529
Experimental   3       2.782  0.04479
Experimental   4       2.736  0.04764
Experimental   5       2.723  0.05170
Experimental   6       2.778  0.04820
Experimental   7       2.770  0.05339
Experimental   8       2.645  0.06888


Tukey Simultaneous Tests
Response Variable FLSL
All Pairwise Comparisons among Levels of Treatment*Observation
Treatment = Control
Observation = 1  subtracted from:

                          Difference      SE of              Adjusted
Treatment     Observation  of Means    Difference  T-Value   P-Value
Control        2           -0.1407      0.06613     -2.128    0.7475
Control        3           -0.0878      0.07027     -1.250    0.9974
Control        4           -0.2279      0.07014     -3.249    0.0859
Control        5           -0.1847      0.07638     -2.418    0.5338
Control        6           -0.1786      0.07398     -2.413    0.5372
Control        7           -0.2645      0.09289     -2.847    0.2425
Control        8           -0.1953      0.09141     -2.137    0.7415
Experimental   1           -0.1880      0.06410     -2.932    0.1991
Experimental   2            0.0388      0.06408      0.605    1.0000
Experimental   3            0.1201      0.06373      1.885    0.8832
Experimental   4            0.0745      0.06577      1.133    0.9991
Experimental   5            0.0609      0.06878      0.886    1.0000
Experimental   6            0.1164      0.06617      1.759    0.9304
Experimental   7            0.1081      0.07006      1.544    0.9774
Experimental   8           -0.0165      0.08245     -0.200    1.0000


Treatment = Control
Observation = 2  subtracted from:

                          Difference      SE of              Adjusted
Treatment     Observation  of Means    Difference  T-Value   P-Value
Control        3            0.0529      0.07199      0.734    1.0000
Control        4           -0.0872      0.07193     -1.212    0.9981
Control        5           -0.0440      0.07771     -0.566    1.0000
Control        6           -0.0379      0.07565     -0.501    1.0000
```

```
Control         7                   -0.1238    0.09395   -1.317    0.9954
Control         8                   -0.0546    0.09294   -0.588    1.0000
Experimental    1                   -0.0473    0.06630   -0.713    1.0000
Experimental    2                    0.1795    0.06627    2.708    0.3249
Experimental    3                    0.2608    0.06593    3.956    0.0076
Experimental    4                    0.2152    0.06790    3.170    0.1076
Experimental    5                    0.2016    0.07080    2.848    0.2421
Experimental    6                    0.2571    0.06830    3.765    0.0157
Experimental    7                    0.2488    0.07205    3.454    0.0458
Experimental    8                    0.1242    0.08417    1.475    0.9853
```

```
Treatment = Control
Observation = 3  subtracted from:

                            Difference     SE of              Adjusted
Treatment       Observation of Means    Difference  T-Value  P-Value
Control         4            -0.1401     0.07543     -1.857   0.8952
Control         5            -0.0969     0.08034     -1.206   0.9982
Control         6            -0.0907     0.07894     -1.149   0.9990
Control         7            -0.1766     0.09612     -1.838   0.9029
Control         8            -0.1075     0.09605     -1.119   0.9993
Experimental    1            -0.1001     0.07060     -1.418   0.9900
Experimental    2             0.1266     0.07057      1.794   0.9190
Experimental    3             0.2079     0.07025      2.960   0.1862
Experimental    4             0.1623     0.07210      2.252   0.6600
Experimental    5             0.1488     0.07484      1.987   0.8327
Experimental    6             0.2042     0.07248      2.818   0.2585
Experimental    7             0.1960     0.07602      2.578   0.4144
Experimental    8             0.0713     0.08760      0.814   1.0000
```

```
Treatment = Control
Observation = 4  subtracted from:

                            Difference     SE of              Adjusted
Treatment       Observation of Means    Difference  T-Value  P-Value
Control         5             0.04320    0.08054      0.5364   1.0000
Control         6             0.04932    0.07895      0.6247   1.0000
Control         7            -0.03658    0.09634     -0.3797   1.0000
Control         8             0.03257    0.09596      0.3394   1.0000
Experimental    1             0.03991    0.07041      0.5669   1.0000
Experimental    2             0.26667    0.07039      3.7882   0.0144
Experimental    3             0.34800    0.07007      4.9666   0.0001
Experimental    4             0.30240    0.07194      4.2035   0.0028
Experimental    5             0.28881    0.07470      3.8660   0.0108
Experimental    6             0.34429    0.07230      4.7623   0.0002
Experimental    7             0.33602    0.07589      4.4279   0.0011
Experimental    8             0.21138    0.08745      2.4172   0.5344
```

```
Treatment = Control
Observation = 5  subtracted from:

                            Difference     SE of              Adjusted
Treatment       Observation of Means    Difference  T-Value  P-Value
Control         6             0.00612    0.08376      0.0731   1.0000
Control         7            -0.07978    0.09912     -0.8048   1.0000
Control         8            -0.01063    0.10084     -0.1054   1.0000
Experimental    1            -0.00329    0.07700     -0.0427   1.0000
Experimental    2             0.22347    0.07697      2.9032   0.2132
Experimental    3             0.30480    0.07668      3.9749   0.0071
Experimental    4             0.25920    0.07837      3.3072   0.0723
Experimental    5             0.24561    0.08090      3.0359   0.1540
Experimental    6             0.30109    0.07872      3.8247   0.0126
Experimental    7             0.29282    0.08199      3.5712   0.0310
Experimental    8             0.16818    0.09283      1.8116   0.9128
```

```
Treatment = Control
Observation = 6  subtracted from:

                          Difference      SE of              Adjusted
Treatment      Observation  of Means   Difference  T-Value   P-Value
Control        7           -0.08590     0.09902    -0.8674    1.0000
Control        8           -0.01675     0.09880    -0.1695    1.0000
Experimental   1           -0.00941     0.07427    -0.1266    1.0000
Experimental   2            0.21735     0.07425     2.9274    0.2014
Experimental   3            0.29868     0.07394     4.0395    0.0055
Experimental   4            0.25308     0.07571     3.3430    0.0649
Experimental   5            0.23949     0.07833     3.0575    0.1456
Experimental   6            0.29497     0.07606     3.8784    0.0103
Experimental   7            0.28670     0.07946     3.6082    0.0274
Experimental   8            0.16206     0.09058     1.7891    0.9207


Treatment = Control
Observation = 7  subtracted from:

                          Difference      SE of              Adjusted
Treatment      Observation  of Means   Difference  T-Value   P-Value
Control        8            0.06914     0.11380     0.6076    1.0000
Experimental   1            0.07649     0.09343     0.8187    1.0000
Experimental   2            0.30324     0.09339     3.2470    0.0864
Experimental   3            0.38458     0.09316     4.1281    0.0038
Experimental   4            0.33898     0.09453     3.5858    0.0296
Experimental   5            0.32539     0.09662     3.3677    0.0601
Experimental   6            0.38087     0.09485     4.0156    0.0060
Experimental   7            0.37259     0.09754     3.8201    0.0128
Experimental   8            0.24796     0.10685     2.3206    0.6082


Treatment = Control
Observation = 8  subtracted from:

                          Difference      SE of              Adjusted
Treatment      Observation  of Means   Difference  T-Value   P-Value
Experimental   1            0.007345    0.09141     0.08035   1.0000
Experimental   2            0.234099    0.09139     2.56165   0.4259
Experimental   3            0.315434    0.09114     3.46081   0.0448
Experimental   4            0.269831    0.09256     2.91522   0.2073
Experimental   5            0.256241    0.09470     2.70583   0.3265
Experimental   6            0.311725    0.09287     3.35662   0.0622
Experimental   7            0.303449    0.09563     3.17303   0.1066
Experimental   8            0.178811    0.10510     1.70139   0.9470


Treatment = Experimental
Observation = 1  subtracted from:

                          Difference      SE of              Adjusted
Treatment      Observation  of Means   Difference  T-Value   P-Value
Experimental   2            0.2268      0.06402     3.542     0.0343
Experimental   3            0.3081      0.06365     4.840     0.0002
Experimental   4            0.2625      0.06566     3.998     0.0065
Experimental   5            0.2489      0.06882     3.617     0.0266
Experimental   6            0.3044      0.06609     4.606     0.0005
Experimental   7            0.2961      0.07014     4.222     0.0026
Experimental   8            0.1715      0.08218     2.086     0.7742


Treatment = Experimental
Observation = 2  subtracted from:
```

Treatment	Observation	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Experimental	3	0.08134	0.06365	1.2778	0.9967
Experimental	4	0.03573	0.06567	0.5441	1.0000
Experimental	5	0.02214	0.06877	0.3220	1.0000
Experimental	6	0.07763	0.06609	1.1745	0.9987
Experimental	7	0.06935	0.07007	0.9897	0.9998
Experimental	8	-0.05529	0.08227	-0.6720	1.0000

Treatment = Experimental
Observation = 3   subtracted from:

Treatment	Observation	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Experimental	4	-0.0456	0.06531	-0.698	1.0000
Experimental	5	-0.0592	0.06845	-0.865	1.0000
Experimental	6	-0.0037	0.06574	-0.056	1.0000
Experimental	7	-0.0120	0.06977	-0.172	1.0000
Experimental	8	-0.1366	0.08194	-1.667	0.9552

Treatment = Experimental
Observation = 4   subtracted from:

Treatment	Observation	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Experimental	5	-0.01359	0.07038	-0.193	1.0000
Experimental	6	0.04189	0.06768	0.619	1.0000
Experimental	7	0.03362	0.07168	0.469	1.0000
Experimental	8	-0.09102	0.08335	-1.092	0.9994

Treatment = Experimental
Observation = 5   subtracted from:

Treatment	Observation	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Experimental	6	0.05548	0.07074	0.7844	1.000
Experimental	7	0.04721	0.07426	0.6357	1.000
Experimental	8	-0.07743	0.08632	-0.8970	1.000

Treatment = Experimental
Observation = 6   subtracted from:

Treatment	Observation	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Experimental	7	-0.0083	0.07201	-0.115	1.0000
Experimental	8	-0.1329	0.08383	-1.586	0.9712

Treatment = Experimental
Observation = 7   subtracted from:

Treatment	Observation	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Experimental	8	-0.1246	0.08749	-1.425	0.9896

APPENDIX 3
HLSL

General Linear Model: HLSL versus Treatment, Period, Observation

```
Factor        Type   Levels  Values
Treatment     fixed      2   Control, Experimental
Period        fixed      2   1, 2
Observation   fixed      8   1, 2, 3, 4, 5, 6, 7, 8


Analysis of Variance for HLSL, using Adjusted SS for Tests

Source                 DF     Seq SS     Adj SS    Adj MS      F      P
Age                     1    2.75991    1.85890   1.85890  20.30  0.000
Treatment               1    4.00809    4.08416   4.08416  44.60  0.000
Period                  1    0.41082    0.21758   0.21758   2.38  0.124
Treatment*Period        1    1.72333    1.16841   1.16841  12.76  0.000
Observation             7    0.67728    0.64453   0.09208   1.01  0.426
Treatment*Observation   7    3.91325    3.91325   0.55904   6.11  0.000
Error                 493   45.14262   45.14262   0.09157
Total                 511   58.63530


S = 0.302601   R-Sq = 23.01%   R-Sq(adj) = 20.20%


Term            Coef   SE Coef        T      P
Constant     2.77326   0.03956    70.11  0.000
Age        -0.011640  0.002583    -4.51  0.000


Unusual Observations for HLSL

Obs     HLSL      Fit   SE Fit   Residual   St Resid
 73   3.35000  2.69591  0.05282    0.65409      2.20 R
100   3.52600  2.85833  0.05364    0.66767      2.24 R
102   4.15000  2.90484  0.05356    1.24516      4.18 R
103   3.65000  2.90442  0.05720    0.74558      2.51 R
105   3.65000  2.75411  0.05327    0.89589      3.01 R
106   3.31300  2.60853  0.05698    0.70447      2.37 R
160   1.97300  2.57357  0.08605   -0.60057     -2.07 R
164   3.52300  2.78849  0.05339    0.73451      2.47 R
169   3.30600  2.68427  0.05311    0.62173      2.09 R
221   3.36000  2.69737  0.05728    0.66263      2.23 R
389   3.02000  2.36392  0.06254    0.65608      2.22 R
393   3.02000  2.32164  0.05166    0.69836      2.34 R
404   3.15000  2.51798  0.05935    0.63202      2.13 R
405   3.19300  2.52688  0.06411    0.66612      2.25 R
409   3.19300  2.48460  0.05252    0.70840      2.38 R
454   2.17000  2.82336  0.05401   -0.65336     -2.19 R
622   3.36000  2.67429  0.05208    0.68571      2.30 R

R denotes an observation with a large standardized residual.


Means for Covariates

Covariate   Mean   StDev
Age        14.21   5.247


Least Squares Means for HLSL
```

```
Treatment              Mean  SE Mean
Control                2.507  0.02369
Experimental           2.708  0.01851
Period
1                      2.630  0.01876
2                      2.586  0.02280
Treatment*Period
Control      1         2.478  0.02589
Control      2         2.537  0.03852
Experimental 1         2.783  0.02737
Experimental 2         2.634  0.02447
Treatment*Observation
Control      1         2.675  0.04623
Control      2         2.530  0.04932
Control      3         2.582  0.05517
Control      4         2.464  0.05496
Control      5         2.473  0.06344
Control      6         2.492  0.05998
Control      7         2.384  0.08323
Control      8         2.460  0.08089
Experimental 1         2.475  0.04621
Experimental 2         2.718  0.04617
Experimental 3         2.781  0.04566
Experimental 4         2.735  0.04856
Experimental 5         2.735  0.05270
Experimental 6         2.781  0.04914
Experimental 7         2.781  0.05443
Experimental 8         2.661  0.07022


Tukey Simultaneous Tests
Response Variable HLSL
All Pairwise Comparisons among Levels of Treatment*Observation
Treatment = Control
Observation = 1  subtracted from:

                       Difference    SE of              Adjusted
Treatment    Observation  of Means  Difference  T-Value   P-Value
Control      2            -0.1456    0.06741     -2.160    0.7258
Control      3            -0.0937    0.07163     -1.308    0.9957
Control      4            -0.2113    0.07150     -2.956    0.1881
Control      5            -0.2024    0.07786     -2.600    0.3983
Control      6            -0.1830    0.07542     -2.426    0.5273
Control      7            -0.2910    0.09470     -3.073    0.1400
Control      8            -0.2155    0.09319     -2.312    0.6147
Experimental 1           -0.2000    0.06535     -3.061    0.1442
Experimental 2            0.0424    0.06533      0.649    1.0000
Experimental 3            0.1052    0.06496      1.620    0.9651
Experimental 4            0.0595    0.06705      0.888    1.0000
Experimental 5            0.0593    0.07011      0.846    1.0000
Experimental 6            0.1060    0.06745      1.572    0.9733
Experimental 7            0.1056    0.07142      1.479    0.9850
Experimental 8           -0.0140    0.08406     -0.166    1.0000


Treatment = Control
Observation = 2  subtracted from:

                       Difference    SE of              Adjusted
Treatment    Observation  of Means  Difference  T-Value   P-Value
Control      3            0.0519     0.07339      0.707    1.0000
Control      4           -0.0658     0.07333     -0.897    1.0000
Control      5           -0.0569     0.07922     -0.718    1.0000
Control      6           -0.0374     0.07712     -0.485    1.0000
Control      7           -0.1454     0.09578     -1.518    0.9807
Control      8           -0.0699     0.09474     -0.738    1.0000
Experimental 1           -0.0545     0.06759     -0.806    1.0000
```

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```
Experimental  2                  0.1880    0.06756    2.783   0.2789
Experimental  3                  0.2508    0.06721    3.732   0.0177
Experimental  4                  0.2051    0.06922    2.963   0.1846
Experimental  5                  0.2049    0.07218    2.838   0.2472
Experimental  6                  0.2516    0.06962    3.614   0.0268
Experimental  7                  0.2512    0.07345    3.420   0.0510
Experimental  8                  0.1316    0.08581    1.534   0.9788


Treatment = Control
Observation = 3  subtracted from:

                            Difference    SE of            Adjusted
Treatment      Observation   of Means   Difference  T-Value  P-Value
Control        4             -0.1177     0.07689    -1.530   0.9792
Control        5             -0.1088     0.08190    -1.328   0.9949
Control        6             -0.0893     0.08048    -1.110   0.9993
Control        7             -0.1973     0.09799    -2.014   0.8181
Control        8             -0.1218     0.09791    -1.244   0.9975
Experimental   1             -0.1064     0.07197    -1.478   0.9850
Experimental   2              0.1361     0.07194     1.891   0.8804
Experimental   3              0.1989     0.07162     2.777   0.2820
Experimental   4              0.1532     0.07350     2.084   0.7755
Experimental   5              0.1530     0.07630     2.005   0.8232
Experimental   6              0.1997     0.07388     2.703   0.3283
Experimental   7              0.1993     0.07750     2.572   0.4188
Experimental   8              0.0797     0.08930     0.892   1.0000


Treatment = Control
Observation = 4  subtracted from:

                            Difference    SE of            Adjusted
Treatment      Observation   of Means   Difference  T-Value  P-Value
Control        5              0.00890    0.08210     0.1084   1.0000
Control        6              0.02834    0.08048     0.3522   1.0000
Control        7             -0.07962    0.09821    -0.8108   1.0000
Control        8             -0.00411    0.09783    -0.0420   1.0000
Experimental   1              0.01130    0.07178     0.1574   1.0000
Experimental   2              0.25376    0.07176     3.5361   0.0349
Experimental   3              0.31660    0.07143     4.4323   0.0010
Experimental   4              0.27089    0.07334     3.6937   0.0203
Experimental   5              0.27063    0.07616     3.5537   0.0330
Experimental   6              0.31740    0.07370     4.3066   0.0018
Experimental   7              0.31698    0.07736     4.0974   0.0043
Experimental   8              0.19736    0.08915     2.2138   0.6875


Treatment = Control
Observation = 5  subtracted from:

                            Difference    SE of            Adjusted
Treatment      Observation   of Means   Difference  T-Value  P-Value
Control        6              0.01944    0.08539     0.2277   1.0000
Control        7             -0.08853    0.10105    -0.8761   1.0000
Control        8             -0.01301    0.10279    -0.1266   1.0000
Experimental   1              0.00240    0.07850     0.0306   1.0000
Experimental   2              0.24486    0.07847     3.1205   0.1232
Experimental   3              0.30770    0.07817     3.9361   0.0082
Experimental   4              0.26198    0.07990     3.2791   0.0786
Experimental   5              0.26173    0.08247     3.1735   0.1065
Experimental   6              0.30849    0.08025     3.8440   0.0117
Experimental   7              0.30808    0.08359     3.6857   0.0209
Experimental   8              0.18846    0.09464     1.9913   0.8306


Treatment = Control
```

```
Observation = 6   subtracted from:

                            Difference    SE of              Adjusted
Treatment      Observation  of Means    Difference  T-Value  P-Value
Control        7            -0.1080      0.10095     -1.070   0.9996
Control        8            -0.0325      0.10072     -0.322   1.0000
Experimental   1            -0.0170      0.07571     -0.225   1.0000
Experimental   2             0.2254      0.07569      2.978   0.1781
Experimental   3             0.2883      0.07538      3.824   0.0126
Experimental   4             0.2425      0.07718      3.143   0.1160
Experimental   5             0.2423      0.07985      3.034   0.1546
Experimental   6             0.2891      0.07753      3.728   0.0179
Experimental   7             0.2886      0.08100      3.563   0.0319
Experimental   8             0.1690      0.09234      1.830   0.9058


Treatment = Control
Observation = 7   subtracted from:

                            Difference    SE of              Adjusted
Treatment      Observation  of Means    Difference  T-Value  P-Value
Control        8            0.07551      0.11601     0.6509   1.0000
Experimental   1            0.09092      0.09524     0.9547   0.9999
Experimental   2            0.33338      0.09521     3.5017   0.0392
Experimental   3            0.39622      0.09497     4.1721   0.0032
Experimental   4            0.35051      0.09637     3.6371   0.0247
Experimental   5            0.35026      0.09850     3.5560   0.0327
Experimental   6            0.39702      0.09669     4.1061   0.0042
Experimental   7            0.39660      0.09943     3.9887   0.0067
Experimental   8            0.27698      0.10893     2.5428   0.4397


Treatment = Control
Observation = 8   subtracted from:

                            Difference    SE of              Adjusted
Treatment      Observation  of Means    Difference  T-Value  P-Value
Experimental   1            0.01541      0.09319     0.1654   1.0000
Experimental   2            0.25787      0.09316     2.7680   0.2876
Experimental   3            0.32071      0.09292     3.4516   0.0461
Experimental   4            0.27500      0.09436     2.9144   0.2077
Experimental   5            0.27475      0.09654     2.8460   0.2430
Experimental   6            0.32151      0.09467     3.3960   0.0550
Experimental   7            0.32109      0.09749     3.2935   0.0753
Experimental   8            0.20147      0.10714     1.8805   0.8852


Treatment = Experimental
Observation = 1   subtracted from:

                            Difference    SE of              Adjusted
Treatment      Observation  of Means    Difference  T-Value  P-Value
Experimental   2            0.2425       0.06527     3.715    0.0188
Experimental   3            0.3053       0.06489     4.705    0.0003
Experimental   4            0.2596       0.06693     3.878    0.0103
Experimental   5            0.2593       0.07015     3.697    0.0201
Experimental   6            0.3061       0.06737     4.543    0.0006
Experimental   7            0.3057       0.07150     4.275    0.0021
Experimental   8            0.1861       0.08378     2.221    0.6825


Treatment = Experimental
Observation = 2   subtracted from:

                            Difference    SE of              Adjusted
Treatment      Observation  of Means    Difference  T-Value  P-Value
Experimental   3            0.06284      0.06489     0.9684   0.9999
```

```
Experimental  4                    0.01713    0.06695    0.2558    1.0000
Experimental  5                    0.01687    0.07010    0.2407    1.0000
Experimental  6                    0.06364    0.06738    0.9445    0.9999
Experimental  7                    0.06322    0.07143    0.8850    1.0000
Experimental  8                   -0.05640    0.08387   -0.6725    1.0000


Treatment = Experimental
Observation = 3  subtracted from:

                            Difference      SE of            Adjusted
Treatment      Observation   of Means   Difference  T-Value   P-Value
Experimental  4              -0.0457      0.06658     -0.687    1.0000
Experimental  5              -0.0460      0.06978     -0.659    1.0000
Experimental  6               0.0008      0.06701      0.012    1.0000
Experimental  7               0.0004      0.07112      0.005    1.0000
Experimental  8              -0.1192      0.08354     -1.427    0.9894


Treatment = Experimental
Observation = 4  subtracted from:

                            Difference      SE of            Adjusted
Treatment      Observation   of Means   Difference  T-Value   P-Value
Experimental  5              -0.00025     0.07174     -0.0035   1.000
Experimental  6               0.04651     0.06899      0.6741   1.000
Experimental  7               0.04609     0.07308      0.6307   1.000
Experimental  8              -0.07353     0.08497     -0.8653   1.000


Treatment = Experimental
Observation = 5  subtracted from:

                            Difference      SE of            Adjusted
Treatment      Observation   of Means   Difference  T-Value   P-Value
Experimental  6               0.04676     0.07211      0.6485   1.000
Experimental  7               0.04634     0.07570      0.6122   1.000
Experimental  8              -0.07328     0.08800     -0.8327   1.000


Treatment = Experimental
Observation = 6  subtracted from:

                            Difference      SE of            Adjusted
Treatment      Observation   of Means   Difference  T-Value   P-Value
Experimental  7              -0.0004      0.07341     -0.006    1.0000
Experimental  8              -0.1200      0.08545     -1.405    0.9910


Treatment = Experimental
Observation = 7  subtracted from:

                            Difference      SE of            Adjusted
Treatment      Observation   of Means   Difference  T-Value   P-Value
Experimental  8              -0.1196      0.08919     -1.341    0.9944
```

APPENDIX 4

HLP

General Linear Model: HLP versus Treatment, Period, Observation

```
Factor       Type   Levels  Values
Treatment    fixed       2  Control, Experimental
Period       fixed       2  1, 2
Observation  fixed       8  1, 2, 3, 4, 5, 6, 7, 8
```

Analysis of Variance for HLP, using Adjusted SS for Tests

```
Source                 DF    Seq SS    Adj SS    Adj MS       F      P
Age                     1   0.85084   0.71978   0.71978   45.91  0.000
Treatment               1   2.04184   1.99296   1.99296  127.11  0.000
Period                  1   0.01711   0.01062   0.01062    0.68  0.411
Treatment*Period        1   0.03310   0.00469   0.00469    0.30  0.585
Observation             7   0.38000   0.27045   0.03864    2.46  0.017
Treatment*Observation   7   1.91487   1.91487   0.27355   17.45  0.000
Error                 493   7.72961   7.72961   0.01568
Total                 511  12.96737
```

```
S = 0.125215   R-Sq = 40.39%   R-Sq(adj) = 38.22%
```

```
Term          Coef   SE Coef       T      P
Constant   -0.00228   0.01637   -0.14  0.889
Age       -0.007243  0.001069   -6.78  0.000
```

Unusual Observations for HLP

```
Obs       HLP       Fit    SE Fit  Residual  St Resid
 18  -0.30300  -0.05605   0.02081  -0.24695     -2.00 R
102   0.54300   0.06361   0.02216   0.47939      3.89 R
160  -0.44600  -0.14190   0.03561  -0.30410     -2.53 R
164   0.41000  -0.02220   0.02209   0.43220      3.51 R
165   0.24000  -0.01183   0.02298   0.25183      2.05 R
166   0.27300   0.02015   0.02217   0.25285      2.05 R
167   0.28000   0.00522   0.02349   0.27478      2.23 R
169   0.28000  -0.08935   0.02197   0.36935      3.00 R
221   0.30000   0.02765   0.02370   0.27235      2.22 R
369   0.23300  -0.10583   0.02087   0.33883      2.74 R
371   0.15000  -0.20478   0.02317   0.35478      2.88 R
373   0.06000  -0.22653   0.02511   0.28653      2.34 R
374   0.15000  -0.21126   0.02526   0.36126      2.95 R
375   0.10300  -0.21768   0.03349   0.32068      2.66 R
376   0.04300  -0.22357   0.03475   0.26657      2.22 R
377   0.04300  -0.23482   0.02027   0.27782      2.25 R
384   0.23000  -0.04570   0.02894   0.27570      2.26 R
475  -1.03000  -0.12312   0.02706  -0.90688     -7.42 R
529  -0.29600  -0.04788   0.02196  -0.24812     -2.01 R
```

R denotes an observation with a large standardized residual.

Means for Covariates

```
Covariate   Mean   StDev
Age        14.21   5.247
```

```
Least Squares Means for HLP

Treatment                  Mean    SE Mean
Control                 -0.1753   0.009802
Experimental            -0.0351   0.007659
Period
1                       -0.1101   0.007764
2                       -0.1003   0.009433
Treatment*Period
Control      1          -0.1836   0.010712
Control      2          -0.1671   0.015937
Experimental 1          -0.0367   0.011326
Experimental 2          -0.0334   0.010126
Treatment*Observation
Control      1          -0.0846   0.019129
Control      2          -0.1455   0.020408
Control      3          -0.1836   0.022830
Control      4          -0.1948   0.022742
Control      5          -0.2053   0.026251
Control      6          -0.1900   0.024820
Control      7          -0.1965   0.034440
Control      8          -0.2024   0.033474
Experimental 1          -0.2235   0.019123
Experimental 2          -0.0487   0.019106
Experimental 3          -0.0237   0.018895
Experimental 4          -0.0076   0.020096
Experimental 5           0.0028   0.021809
Experimental 6           0.0348   0.020333
Experimental 7           0.0198   0.022523
Experimental 8          -0.0344   0.029055


Tukey Simultaneous Tests
Response Variable HLP
All Pairwise Comparisons among Levels of Treatment*Observation
Treatment = Control
Observation = 1  subtracted from:

                         Difference      SE of              Adjusted
Treatment     Observation of Means   Difference  T-Value    P-Value
Control       2            -0.0609     0.02790    -2.183     0.7097
Control       3            -0.0990     0.02964    -3.338     0.0658
Control       4            -0.1102     0.02959    -3.726     0.0181
Control       5            -0.1207     0.03222    -3.746     0.0168
Control       6            -0.1054     0.03121    -3.378     0.0581
Control       7            -0.1119     0.03919    -2.854     0.2384
Control       8            -0.1177     0.03856    -3.053     0.1472
Experimental  1            -0.1389     0.02704    -5.135     0.0001
Experimental  2             0.0359     0.02703     1.329     0.9949
Experimental  3             0.0610     0.02688     2.268     0.6480
Experimental  4             0.0770     0.02774     2.776     0.2829
Experimental  5             0.0874     0.02901     3.012     0.1636
Experimental  6             0.1194     0.02791     4.277     0.0021
Experimental  7             0.1044     0.02955     3.534     0.0352
Experimental  8             0.0503     0.03478     1.445     0.9880


Treatment = Control
Observation = 2  subtracted from:

                         Difference      SE of              Adjusted
Treatment     Observation of Means   Difference  T-Value    P-Value
Control       3           -0.03807     0.03037    -1.254     0.9973
Control       4           -0.04935     0.03034    -1.626     0.9639
Control       5           -0.05982     0.03278    -1.825     0.9078
Control       6           -0.04455     0.03191    -1.396     0.9915
Control       7           -0.05097     0.03963    -1.286     0.9964
```

```
Control        8             -0.05686    0.03920   -1.450    0.9876
Experimental   1             -0.07798    0.02797   -2.788    0.2757
Experimental   2              0.09681    0.02796    3.463    0.0445
Experimental   3              0.12184    0.02781    4.381    0.0013
Experimental   4              0.13790    0.02864    4.815    0.0002
Experimental   5              0.14827    0.02987    4.964    0.0001
Experimental   6              0.18025    0.02881    6.257    0.0000
Experimental   7              0.16532    0.03039    5.439    0.0000
Experimental   8              0.11115    0.03551    3.130    0.1200


Treatment = Control
Observation = 3  subtracted from:

                         Difference     SE of            Adjusted
Treatment     Observation  of Means   Difference  T-Value  P-Value
Control        4             -0.01128    0.03182   -0.354    1.0000
Control        5             -0.02175    0.03389   -0.642    1.0000
Control        6             -0.00648    0.03330   -0.195    1.0000
Control        7             -0.01289    0.04055   -0.318    1.0000
Control        8             -0.01879    0.04052   -0.464    1.0000
Experimental   1             -0.03990    0.02978   -1.340    0.9945
Experimental   2              0.13488    0.02977    4.531    0.0007
Experimental   3              0.15992    0.02964    5.396    0.0000
Experimental   4              0.17597    0.03041    5.786    0.0000
Experimental   5              0.18635    0.03157    5.902    0.0000
Experimental   6              0.21832    0.03057    7.141    0.0000
Experimental   7              0.20339    0.03207    6.342    0.0000
Experimental   8              0.14922    0.03695    4.038    0.0055


Treatment = Control
Observation = 4  subtracted from:

                         Difference     SE of            Adjusted
Treatment     Observation  of Means   Difference  T-Value  P-Value
Control        5             -0.01047    0.03397   -0.3082   1.0000
Control        6              0.00480    0.03330    0.1441   1.0000
Control        7             -0.00162    0.04064   -0.0398   1.0000
Control        8             -0.00751    0.04048   -0.1856   1.0000
Experimental   1             -0.02863    0.02970   -0.9638   0.9999
Experimental   2              0.14616    0.02969    4.9220   0.0001
Experimental   3              0.17119    0.02956    5.7919   0.0000
Experimental   4              0.18725    0.03035    6.1703   0.0000
Experimental   5              0.19762    0.03151    6.2712   0.0000
Experimental   6              0.22960    0.03050    7.5286   0.0000
Experimental   7              0.21467    0.03201    6.7059   0.0000
Experimental   8              0.16049    0.03689    4.3507   0.0015


Treatment = Control
Observation = 5  subtracted from:

                         Difference     SE of            Adjusted
Treatment     Observation  of Means   Difference  T-Value  P-Value
Control        6              0.01527    0.03533    0.4322   1.0000
Control        7              0.00885    0.04181    0.2118   1.0000
Control        8              0.00296    0.04254    0.0696   1.0000
Experimental   1             -0.01816    0.03248   -0.5590   1.0000
Experimental   2              0.15663    0.03247    4.8238   0.0002
Experimental   3              0.18166    0.03235    5.6161   0.0000
Experimental   4              0.19772    0.03306    5.9805   0.0000
Experimental   5              0.20809    0.03413    6.0976   0.0000
Experimental   6              0.24007    0.03321    7.2292   0.0000
Experimental   7              0.22514    0.03459    6.5092   0.0000
Experimental   8              0.17097    0.03916    4.3658   0.0014
```

```
Treatment = Control
Observation = 6  subtracted from:

                              Difference      SE of              Adjusted
Treatment       Observation   of Means     Difference   T-Value   P-Value
Control         7             -0.00642      0.04177      -0.154    1.0000
Control         8             -0.01231      0.04168      -0.295    1.0000
Experimental    1             -0.03343      0.03133      -1.067    0.9996
Experimental    2              0.14136      0.03132       4.513    0.0007
Experimental    3              0.16639      0.03119       5.335    0.0000
Experimental    4              0.18245      0.03194       5.713    0.0000
Experimental    5              0.19282      0.03304       5.836    0.0000
Experimental    6              0.22480      0.03208       7.007    0.0000
Experimental    7              0.20987      0.03352       6.261    0.0000
Experimental    8              0.15570      0.03821       4.075    0.0048


Treatment = Control
Observation = 7  subtracted from:

                              Difference      SE of              Adjusted
Treatment       Observation   of Means     Difference   T-Value   P-Value
Control         8             -0.00589      0.04801      -0.1228   1.0000
Experimental    1             -0.02701      0.03941      -0.6853   1.0000
Experimental    2              0.14777      0.03940       3.7510   0.0165
Experimental    3              0.17281      0.03930       4.3974   0.0012
Experimental    4              0.18886      0.03988       4.7361   0.0003
Experimental    5              0.19924      0.04076       4.8884   0.0002
Experimental    6              0.23121      0.04001       5.7789   0.0000
Experimental    7              0.21628      0.04114       5.2568   0.0001
Experimental    8              0.16211      0.04507       3.5966   0.0285


Treatment = Control
Observation = 8  subtracted from:

                              Difference      SE of              Adjusted
Treatment       Observation   of Means     Difference   T-Value   P-Value
Experimental    1             -0.02112      0.03856      -0.5476   1.0000
Experimental    2              0.15367      0.03855       3.9863   0.0068
Experimental    3              0.17870      0.03845       4.6480   0.0004
Experimental    4              0.19476      0.03904       4.9881   0.0001
Experimental    5              0.20514      0.03995       5.1351   0.0001
Experimental    6              0.23711      0.03918       6.0526   0.0000
Experimental    7              0.22218      0.04034       5.5075   0.0000
Experimental    8              0.16801      0.04433       3.7896   0.0143


Treatment = Experimental
Observation = 1  subtracted from:

                              Difference      SE of              Adjusted
Treatment       Observation   of Means     Difference   T-Value   P-Value
Experimental    2              0.1748       0.02701       6.472    0.0000
Experimental    3              0.1998       0.02685       7.442    0.0000
Experimental    4              0.2159       0.02770       7.794    0.0000
Experimental    5              0.2263       0.02903       7.794    0.0000
Experimental    6              0.2582       0.02788       9.262    0.0000
Experimental    7              0.2433       0.02959       8.223    0.0000
Experimental    8              0.1891       0.03467       5.455    0.0000


Treatment = Experimental
Observation = 2  subtracted from:

                              Difference         SE of              Adjusted
```

Treatment	Observation	of Means	Difference	T-Value	P-Value
Experimental	3	0.02504	0.02685	0.9324	0.9999
Experimental	4	0.04109	0.02770	1.4833	0.9845
Experimental	5	0.05147	0.02901	1.7742	0.9257
Experimental	6	0.08344	0.02788	2.9928	0.1717
Experimental	7	0.06851	0.02956	2.3178	0.6104
Experimental	8	0.01434	0.03470	0.4131	1.0000

Treatment = Experimental
Observation = 3 subtracted from:

Treatment	Observation	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Experimental	4	0.01605	0.02755	0.5828	1.0000
Experimental	5	0.02643	0.02887	0.9154	0.9999
Experimental	6	0.05841	0.02773	2.1062	0.7615
Experimental	7	0.04347	0.02943	1.4772	0.9851
Experimental	8	-0.01070	0.03457	-0.3095	1.0000

Treatment = Experimental
Observation = 4 subtracted from:

Treatment	Observation	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Experimental	5	0.01038	0.02969	0.3495	1.0000
Experimental	6	0.04235	0.02855	1.4834	0.9845
Experimental	7	0.02742	0.03024	0.9068	0.9999
Experimental	8	-0.02675	0.03516	-0.7609	1.0000

Treatment = Experimental
Observation = 5 subtracted from:

Treatment	Observation	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Experimental	6	0.03197	0.02984	1.072	0.9996
Experimental	7	0.01704	0.03132	0.544	1.0000
Experimental	8	-0.03713	0.03641	-1.020	0.9998

Treatment = Experimental
Observation = 6 subtracted from:

Treatment	Observation	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Experimental	7	-0.01493	0.03038	-0.491	1.0000
Experimental	8	-0.06910	0.03536	-1.954	0.8502

Treatment = Experimental
Observation = 7 subtracted from:

Treatment	Observation	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Experimental	8	-0.05417	0.03691	-1.468	0.9860