

## RESEARCH PROGRAM

### LOW FREQUENCY WHOLE BODY VIBRATION OF HORSES AND EFFECTS ON SKELETAL MUSCLES, BLOOD FLOW AND BLOOD PARAMETERS

**SPECIFIC OBJECTIVES** To assess if low frequency, whole body vibration treatment could offer a safe, effective, laboursaving method for muscular adaptations in horses.

#### BACKGROUND

During the last years, several studies in human medicine have documented positive effects of low frequency whole body vibrations on the neuromuscular and neuroendocrine systems in the body (Cardinale and Wakeling, 2005). To use methods causing vibrations are proposed to be a safe and effective way to exercise musculoskeletal structures. People that have participated in vibration studies have not experienced any unpleasant feeling from vibration treatment (Scherzer, 2002). However, with long-term occupational exposure to intense whole body vibration in people it can have negative effects and be associated with an increased risk for disorders of the lumbar spine and the connected nervous system (Cardinale and Pope 2003, Bovenzi, 2006). In patients suffering from hand-arm vibration syndrome, morphological changes in muscle are observed indicating that the vibrating tools may cause direct damage to muscle fibres (Necking et al, 2004). This shows that there are both positive and negative effects when the body is exposed to vibration. The type and intensity of vibration and the duration for exposure are thus important factors that have to be considered when the body is exposed to vibrations. Active treatments in humans with so called low frequency whole body vibration (25 – 45 Hz) have proved to increase strength and power of leg muscles (Issurin et al 1994; Issurin and Teenenbaum 1999; Bosco et al 1999a; Torvinen et al 2002a,b; Delecluse et al 2003). Vibration-treatment has during the last years been introduced in athletic medicine, both to prevent and treat injuries in programs for rehabilitation as well as to increase capability of the athletes (Jordan et al, 2005, Mester et al, 2006). A number of machines are now available on the market for use in humans ([www.power-plate.com](http://www.power-plate.com)). Recently, it has also been shown that people suffering from type 2 diabetes receiving low frequency vibration during 12 weeks, improved their glycemic control (Baum et al 2007). It has also been suggested that vibration exercise could be a therapeutic approach for sarcopenia and osteoporosis (Cardinale and Wakeling, 2005). Whole-body-vibration training increases knee-extension strength and speed of movement in older women (Roelants et al, 2004). Experimental studies have shown that vibration counteract osteoporosis in ovariectomized rats (Flieger et al 1998). Chronic lumbal pain has been successfully treated with vibration (Rittweger et al 2002a). Positive effects on paretic muscles have also been shown as they regain their ability to move (Hagbarth and Eklund 1966). The effects reflect an increased efficacy of the neuromuscular transmission (Bosco et al 1999b) and adaptive changes in the skeleton muscles (Bosco et al 1998). Earlier studies have shown that vibration treatment of quadriceps muscles lowers the stimulation threshold for the patella reflex (Burke et al 1996). Vibration of agonistic muscles also increases activity of antagonistic muscles (Rothmuller and Cafarelli 1995). Also the spindles are activated (Kasai et al 1992). The increased metabolic power observed in humans in association with whole body vibration is due to muscular activity (Rittweger et al, 2002b).

Vibration also affects endocrine systems in humans resulting in increased levels of testosterone and growth hormone and lowered cortisol in plasma of the blood (Bosco et al 2000). Whole body vibration exercise transiently reduces plasma glucose, possibly by increasing glucose utilization by contracting muscles (DiLoreto et al, 2004). Low frequency vibration increases the blood flow in the muscles and does not have the negative effects on peripheral circulation known from occupational high-frequency vibration (Kerschman-Schindl et al 2001). Muscle tissues of rats are affected after vibration for 5 hours/day for 2 days at 80 Hz and especially slow-twitch fibers get increased diameters and increased centrally positioned muscle fibre nuclei (Necking et al 1992, 1996). There are no data on horses or other species concerning muscular adaptations to low frequency vibration. The gluteus muscle of horses contain different proportions of fibres and Type I (slow twitch) fibres have a higher capillary supply, a higher lipid content and a higher oxidative capacity compared to type II A and IIB (fast twitch) fibres (Essen-Gustavsson et al, 1984, Karlström et al, 1994). Type I fibres are those that are recruited when exercise is performed at lower intensities while type IIA and IIB get recruited as the intensity increase (Valberg 1989). During low frequency vibration it is likely that type I fibres are activated causing an increased demand for aerobic energy supply in the mitochondria. Reactive oxygen species may be produced during increased aerobic metabolism and cause oxidative damage to membranes. Phospholipids in the membranes of the cell contain fatty acids that can vary in their susceptibility to reactive oxygen species. High levels of polyunsaturated fatty acids can cause peroxidation whereas monounsaturated and saturated fatty acids are more resistant to oxidation (Hulbert, 2005). Thus the fatty acid composition of phospholipids plays an important role for membrane function. Susceptibility of membranes to lipid peroxidation is said to be an important factor that determines longevity of animals (Hulbert 2005). When rats perform regular exercise on a treadmill or long-term wheel running the fatty acid profile of membrane phospholipids are modified (Helge et al, 1999, Nikolaidis et al, 2004). In humans, regular exercise training also exerts an effect on the muscle membrane phospholipid fatty acid composition (Helge et al 2001). Well-known effects of regular training in horses are increased mitochondrial volume, capillary supply and oxidative capacity in the muscle fibers (Essen-Gustavsson et al, 1989). However, nothing is known about the effect of exercise or low frequency vibration on the fatty acid profile of membrane phospholipids in horse muscle. An important question to answer is how skeletal muscle adapts in horses when they are exposed to repeated low frequency vibration as several trainers already are using vibration methods as an aid in training. For horses, there are systems for vibration such as vibrating floors. Therefore it is of great importance to perform well controlled studies to evaluate if low frequency whole body vibrating exercise has any effect on muscle properties of horses. To get a true picture of the membrane function in muscle exposed to vibration, measurements need to be done of fatty acid composition in phospholipids of the membranes. The metabolic response after one acute treatment of low frequency exposure to vibration also needs to be investigated by analyses of heart rate, rectal temperature and blood parameters (glucose, lactate, free fatty acids, electrolytes, cortisol, creatin kinase). This should be studied both before and after a training period using vibration treatment. In one preliminary study using VITAFLOOR™ vibration system, we have shown that low frequency whole body vibration for 45 minutes is well tolerated by horses (Tingbö 2005). Whole body vibration may therefore comprise a safe, easy and time saving method that can be used as a complement in training of healthy horses as well as being a beneficial method in training of convalescent horses. The aim of this study is to investigate both acute effects of a low frequency whole body vibration treatment and muscular adaptations to long-term effects of vibration treatments.

## QUESTIONS

1. Does low frequency vibration have an effect on blood flow in muscles and blood parameters?
2. Does long term low frequency vibration have an effect on muscle fiber types/areas and metabolic profiles?
3. Does long term low frequency vibration have any effect on muscle phospholipid fatty acid composition?

## HYPOTHESIS

Low frequency whole body vibration will have an effect on blood flow in muscles. Especially type I fibres will adapt to low frequency whole body vibration by increasing, the oxidative capacity and capillary supply and increase membrane function by altering fatty acid composition of membrane phospholipids. This could be beneficial for performance capacity.

## EXPERIMENTAL DESIGN.

One part of the study will use 12 thoroughbred yearlings that is just about to start their training career at the training camp of Bendik Bö, Täby. Besides regular training, half of the group will be subjected to low frequency vibration for 45 min/day, 5 days a week, during 3 months. Before start of the study, a blood sample and a muscle biopsy from m. gluteus will be taken to evaluate muscle properties of each horse (Essen-Gustavsson et al, 1984, Karlström and Essen-Gustavsson, 2002). After 3 months of training,, including vibration for half of the horses, blood samples and biopsies will be taken from both groups of horses again.

The other part of the study will be a crossed study, using 6 six adult and untrained horses (research horses at our department). Before start of the study, blood samples will be collected from all these horses. The horses will then be divided into 2 equal groups. One group will be subjected to low frequency vibration for 45 min per day 5 days a week during 2 weeks by using VITAFLOOR™ while the other group will stay in the vibrating box without vibration during 45 min. Blood samples will be collected directly after the first vibration and also after 24 hours. This will be repeated at the end of the 2 weeks period. The six horses will then have a resting period for 1 – 2 months. Thereafter, the former 3 control horses will now go into the vibrating group, whereas the former vibrated horses will now become control horses. The horses of both groups will be fed and handled in a similar way. Measurements will be made of heart rate, rectal temperature, blood flow using a wireless blood flow monitor (WBFM) based on PPG (photoplethysmography, Lars-Göran Lindberg, Dept Biomedical Engineering in Linköping) and skin/hoof temperature using thermography (Meditherm vet 2000) before and after each 2 week vibration period. **Vibration method:** VITAFLOOR™ is a patented Norwegian construction (Bö Horse & Invention as, Tveitenveien 94, 3265 Larvik, Norge. The floor of VITAFLOOR™ is installed at our department in a standard horse box with all regulatory devices outside the box. The floor vibrates vertically and the speed can be variable (speed 0 – 10). We will use speed 6 which corresponds to 1500 vibrations/min (around 30 Hz). **Blood samples:** Analyses of creatinkinase (CK), cortisol, glucose, free fatty acids and electrolytes will be made. The methods are those used at the muscle metabolic laboratory using FL600 fluorometric apparatus for glucose, free fatty acids, creatinkinase and Analoxapparatus for lactate. Electrolytes will be analysed using an I-stat machine. Cortisol will be analysed at the clinical chemistry laboratory at SLU.

**Muscle samples:** Muscle biopsies from m.gluteus will be obtained as described by Lindholm and Piehl, 1974. One piece of muscle for biochemical analyses is immediately frozen in liquid nitrogen while another piece for histochemical analyses is rolled in talcum powder before being frozen in liquid nitrogen. Fibre types, fibre areas and oxidative capacity of the fibres will be identified with immunohistochemical and histochemical methods as described in Karlström and Essen-Gustavsson,2002.. Lipids and fatty acids in the membrane phospholipids will be analysed using both gas and thin layer chromatography. Extraction is performed as described by Olsen and Hendersen (1989).

## **PRELIMINARY RESULTS**

Preliminary results have been presented to Norwegian veterinarians and horse trainers at a meeting arranged by the Norwegian Veterinary Association at Bjerke Horse hospital north of Oslo 4<sup>th</sup> November 2004. Moreover, results has been published in an examination essay by Marianne Tingbö 2005, SLU, ISSN 1652-8697, that can be down loaded SLU's library. Here, clinical examination, blood sampling and thermographic analyses were made before and after whole body, low frequency vibration for 45 min in 9 healthy horses using the system VITAFLOOR™ (Tingbö 2005). During this short period of vibration no changes occurred in rectal temperature and heart rate or in blood parameters such as S-CK, S-phosphate, S-Na, SK, S-Cl, B-Hb, B-LPK, B-Neut, B-Eosin, B-Basoph, B-Lymph and B-Mono. On the other hand, thermography revealed a significant decrease in temperature of the hoof walls and fetlock joints of the front legs after vibration.

## **ETHICAL PERMISSION**

Ethical permission is granted for vibration and blood sampling (C151/3) and will be renewed and extended to sampling of muscle biopsies.

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