Stress cortisol and muscle stiffness in horses used for equine-assisted therapy

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Abstract

Equine-assisted therapy uses horseback riding to improve the patient's health and wellbeing. However, the possible stress and damage of this therapy on the back horse muscle *(longissimus dorsi)* are understudied. We studied the stress cortisol and muscle stiffness of two ponies and a horse used for equine-assisted therapy on a child with autism. Salivary cortisol levels and heart rate were used as indicators of physiologic stress and a muscle indenter was employed to estimate the back muscle stiffness. Saliva samples were collected before and after equine-assisted therapy and then were analyzed by direct enzyme immunoassay technique. Heart rate was measured by a Heart Rate Monitor at rest, during therapy at 5 minutes intervals for 30 minutes, and after therapy at 5 minutes intervals for 20 minutes. The muscle stiffness and muscle tone along both sides of the segments T14, T16, T18, and L2 of the *longissimus dorsi* muscle were obtained before and after equine-assisted therapy. Both salivary cortisol and heart rate did not have significant differences as a consequence of the therapy. Similarly, although a horse tended to have more muscle stiffness after the treatment, overall the therapy had little effect or none on the horseback. The equine-assisted therapy does not seem to create a negative impact on horses. However, further studies with a greater sample size in children and horses should be carried out to confirm whether equine-assisted therapy is completely safe for horses' health.

Keywords: Equine-assisted therapy, Horse, Salivary cortisol, Heart rate, Muscle stiffness

การตรวจวัดระดับคอร์ติซอลและแรงตึงของกล้ามเนื้อในม้าอาชาบำบัด

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บทคัดย่อ

อาชาบำบัดคือการขี่ม้าเพื่อพัฒนาสุขภาพและคุณภาพชีวิตของผู้ป่วย อย่างไรก็ตามการศึกษาผลกระทบของอาชาบำบัดที่อาจมีต่อความเครียดและการบาดเจ็บกล้ามเนื้อหลังของม้ายังมีไม่มาก ในการศึกษาครั้งนี้ม้าพันธุ์เล็กเพศเมีย 2 ตัว และม้าใหญ่เพศผู้ตอน 1 ตัวได้ถูกนำมาใช้บำบัดเด็กออทิสติกคนหนึ่งเพื่อศึกษาความเครียดและแรงตึงกล้ามเนื้อที่เกิดในม้า ความเครียดในม้าจะประเมินจากระดับของคอร์ติซอลในน้ำลาย อัตราการเต้นของหัวใจ ทั้งนี้เครื่องวัดแรงตึงกล้ามเนื้อได้ถูกนำมาใช้เพื่อประเมินแรงตึงกล้ามเนื้อหลังของม้าด้วย ตัวอย่างน้ำลายจะถูกเก็บในช่วงก่อนหลังทำอาชาบำบัดและนำไปวัดค่า คอร์ติซอลโดยวิธี direct enzyme immunoassay อัตราการเต้นของหัวใจจะถูกวัดโดยใช้เครื่องวัดชีพจร วัดค่าในระยะพัก ช่วงระหว่างบำบัดทุก 5 นาทีจนครบ 30 นาทีและช่วงหลังทำบำบัดทุก 5 นาทีจนครบ 20 นาที ทั้งนี้จะมีการวัดแรงตึงกล้ามเนื้อตามแนวกล้ามเนื้อ *longissimus dorsi* ที่ตำแหน่ง T14 T16 T18 และ L2 ทั้งด้านซ้ายและขวาในช่วงก่อนและหลังทำอาชาบำบัด ผลการศึกษาพบว่าระดับคอร์ติซอลในน้ำลายและอัตราการเต้นของหัวใจมีค่าไม่แตกต่างกันอย่างมีนัยสำคัญเมื่อเทียบกับช่วงก่อนและหลังทำอาชาบำบัด สำหรับการศึกษาแรงตึงกล้ามเนื้อพบว่า กล้ามเนื้อหลังของม้าหลังทำอาชาบำบัดจะมีแรงตึงกล้ามเนื้อมากขึ้นเพียงเล็กน้อย โดยภาพรวมอาจสรุปได้ว่าอาชาบำบัดไม่สร้างผลกระทบต่อม้าในด้านลบ อย่างไรก็ตามการศึกษาในอนาคตควรจะมีการเพิ่มปริมาณของม้าและเด็กที่เข้าร่วมในการศึกษาเพื่อยืนยันให้แน่ชัดว่าการทำอาชาบำบัดไม่ส่งผลเสียต่อสุขภาพของม้า

คำสำคัญ : อาชาบำบัด ม้า ระดับคอร์ติซอลในน้ำลาย อัตราการเต้นของหัวใจ แรงตึงกล้ามเนื้อ

Introduction

Equine-assisted therapy (EAT) is a form of treatment that uses horseback riding to improve the patient's health and wellbeing. This therapy does not imply high riding skills from the patient. The goal of this therapeutic form is to improve individual's posture, balance, mobility, and function. The amount of benefits from this therapy is dependent on a combination of factors that includes the type of disability, severity, motivation, and the form of therapy offered (All and Loving, 1999). In recent reports, EAT has provided benefits on children with disabilities. For example, it improved the self-regulation behavior in autistic children (Gabriels et al., 2012), the progression of gross motor function and postural control in children with down syndrome (Champagne and Dugas, 2010), the development of gait and balance in children with spastic cerebral palsy (Kwon et al., 2011), and the encouragement of balance and strength in children with intellectual disabilities (Giagazoglou et al., 2012). Moreover, the success of this therapy is dependent on a good interaction between horse trainers and other professionals involved in the therapy itself (e.g. physical therapists, occupational therapists, speech therapists, and psychotherapists) (Heine, 1997).

Within the EAT, the method to evaluate the stress condition of the horse is also necessary. Glucocorticoids are common physiological variables for evaluating horse stress. The circulating cortisol level increases from activities such as competition, physical exercise, transportation, and social interactions within herd (Alexander and Irvine, 1998; Mircean et al., 2007; Schmidt et al., 2010; Strzelec et al., 2013). A recent study shows that the higher salivary cortisol level occurred in horses performing with 3- days event competition (i.e. dressage, cross-country, and show jumping) compared to light exercise, show jumping, and dressage. This study revealed that the stress is likely related to the intensity and duration of exercise. Regarding the stress during equine-assisted therapy, the cortisol levels in horses during the therapy was lower than during physical exercises (5 min at walking, 5 min at trotting, and 5 min at galloping) when compared to basal values (Cravana et al., 2008b).

During stress situations, the hypothalamo-pituitary-adrenal (HPA) axis is stimulated (Alexander and Irvine, 1998; Bohák et al., 2013) releasing cortisol into plasma and excretions such as saliva, feces, urine, and tear (Möstl and Palme, 2002; Higashiyama et al., 2005; Monk et al., 2013). In a study of adrenocortico-tropic hormone (ACTH) stimulation test showed that the salivary cortisol levels were increased approximately 20 minutes after administration (Bohák et al., 2013). The total plasma cortisol is composed of protein bound and free cortisol. The plasma cortisol is mainly bound with corticosteroid binding globulin (CBG) while in the salivary cortisol is only free cortisol. As a result, the free cortisol has only 2% to 15% of unbound protein that is conjugated with specific transport proteins in blood circulation. Then cortisol is passively diffused into the salivary gland. Thus, the total level of plasma cortisol is higher than salivary cortisol (Alexander and Irvine, 1998; Groschl, 2008; Schmidt et al., 2010).

In addition, the venipuncture for blood collection can induce stress to the horse whereas the sampling of saliva or feces is a non-invasive technique to reduce stress response of the horse. However, the appropriate of collecting sample technique is also necessary. For example, the salivary cortisol could reflect small and transient change in cortisol release while cortisol metabolites in feces could reflect mainly marked or prolonged stress (Schmidt et al., 2010).

In general, the cortisol level in resting horses exhibit a daily circadian rhythm. The salivary cortisol circadian rhythm shows the maximum value at 10.00 a.m. whereas the minimum value occurs at 10.00 p.m. (Bohák et al., 2013). Due to the circadian rhythm probably affecting estimates of cortisol levels in pre-sampling periods, the comparative of basal cortisol values between different groups is necessary (Fazio et al., 2008). However, some studies show that the cortisol levels associated to the circadian rhythm could be disrupted by changing the daily routine of the horse (Fazio et al., 2008; Bohák et al., 2013).

The horse's heart rate can be also used as indicators of stress response during physical exercise, transportation, or social interactions within herd (Alexander and Irvine, 1998; Jewmongkonchai et al., 2010; Schmidt et al., 2010). The fluctuation of heart rate indicates the response to the balance of the autonomic nervous system. During stress situations, the heart rate is elevated from increased sympathetic tone and reduced parasympathetic tone (Schmidt et al., 2010).

Back pain is a common problem in horse. The causes of back pain are related to the horse, the rider, and/or the saddle (Greve and Dyson, 2013). EAT should be concerned about assessing the forces and the pressure on the horseback. Numerous techniques can be used for determining back stiffness and pain (e.g. pressure measuring pad, muscle indenter, electromyography, and ultrasonographic evaluation) (Cocq et al., 2006; Wakeling et al., 2006; Janura et al., 2009; Jewmongkonchai et al., 2010; Greve and Dyson, 2013) In particular, the pressure-measuring pad is used to assess the change in pressure magnitude and distribution applied on the horseback by the rider during hippotherapy (Janura et al., 2009). Furthermore, this pad can be also used to study the effects of poorly fitting saddles on the horse (Winkelmayr et al., 2006). The custom-made muscle indenter has been used to compare the *longissimus dorsi* muscle tone in thoroughbred racehorses before and after training. The muscle indenter is a tool that determines the muscle tone to identify the stiffness and muscles. The advantage of the muscle indenter is practical, non-invasive, and inexpensive (Wakeling et al., 2006; Jewmongkonchai et al., 2010). The muscle indenter has been also used successfully for measuring the stiffness of *longissimus dorsi* muscle at the sixteenth thoracic vertebrae, eighteenth thoracic vertebrae and second lumbar vertebrae in both before and after administrating electro-acupunctures at Bai-Hui, Yao-Qian, Yao-Zhong and Yao-Hou acupoints (Phutthachalee et al., 2015)

However, the possible stress and damage of this therapy on the back horse muscle *(longissimus dorsi)* are understudied. Thus the aims of this study were to measure horse stress by assessing the salivary cortisol level, heart rate and *longissimus dorsi* muscle tone at rest, during, and after therapeutic horse riding was used. We hypothesized that 1) the salivary cortisol and heart rate will be elevated by the stress created by the therapeutic riding period and 2) the *longissimus dorsi* muscle tone will be stiffer after the therapy than at rest.

Materials and Methods

The procedure of housing and caring was approved by the Faculty of Veterinary Science - Animal Care and Use Committee (FVS-ACUC), Mahidol University. The protocol No. is MUVS-2013-18.

Participants

A male autistic child (age 5 years old, body weight 20 kg, height 100 cm) participated in the study. Each riding session consisted of riding on the horseback at walk gait for 30 minutes. The child performed 5 riding sessions on each of the three "therapy" horses.

Subjects

Two ponies and a horse were used in the study. The ages of the ponies were 12 and 29 years old, respectively, and the horse was 22 years old horse. All animals were healthy and had no clinical signs of back pain. They were housed at the same farm and were fed with the same food and supplements. All animals had been practiced for EAT for at least a month. In this study, each horse and pony were ridden 5 times by the same child. In each riding session, the animal was led to walk around an arena for 30 minutes.

Sample and Data Collection

Salivary cortisol measurement

Saliva samples were collected by three gauzes held with a sponge forceps. The gauzes were lubricated with distilled water and were then inserted in the oral cavity of horse via the diastema area. After the gauzes were soaked with saliva for approximately a minute, the gauzes were kept at 4 ^{o}C during no more than 48 hours. Next, the saliva samples were centrifuged at 1500 rpm and 4 ^{o}C for 5 minutes. The saliva samples were stored in Eppendorf tubes at -20 ^{o}C until analysis.

Cortisol levels were analyzed by using direct enzyme immunoassay (direct EIA) technique adapt from Brown (2008). The assay validation was carried out by running serial 2-fold dilution of a pooled sample. Parallel displacement to the cortisol standard curve (0-1,000 pg/well) was demonstrated. Sample dilution of 50% binding was recorded at 1:2 and selected for subsequent EIA. The CV of intra-assay and inter-assay was 2.8% and 11.38%, respectively. The procedure of direct EIA technique was started by coating the 96-well plates (NUNC Maxisorb, Sigma) with 50 μl of 1:8,500 cortisol antibody (cortisol R4866, Sigma). Incubated overnight at 4 ^{o}C, and then the washed three times. Adding 50 μl per well of standard cortisol, control, and diluted samples, respectively, and then add 50 μl of 1:20,000 cortisol HRP dilution to all wells. Incubated the plates at room temperature for exactly an hour and washing the plates with wash solution again. Prepare the ABTS substrate (40 μl 0.5 M H_2O_2, 125 μl 40 mM ABTS and 12.5 ml substrate buffer) immediately before use and pipetted 100 μl to all wells. Incubate at room temperature for no more than an hour before reading at optical density 405 nm (reference 540 nm). Data were recorded and calculated.

Heart rate measurement

The Polar® Heart Rate Monitor was used for measuring the heart rate at rest, during therapy at 5-minute intervals (0, 5, 10, 15, 20, 25, 30 minutes) and after therapy at 5-minute intervals (0, 5, 10, 15, 20 minutes).

Muscle tone measurement

A custom-made muscle indenter (Wakeling et al., 2006) was used to measure the muscle tone at rest and after therapy. The muscle tone was measured when the standing horse was quiet bearing the same weight on all four limbs. Bilateral sites of *longissimus dorsi* adjacent to dorsal spinous process of the fourteenth thoracic vertebrae (T14), sixteenth thoracic vertebrae (T16), eighteenth thoracic vertebrae (T18) and second lumbar vertebrae (L2) were evaluated. The tone was measured at 10 cm lateral from the dorsal spinous processes on the left and the right sides of T14, T16, T18 muscle segments and 8 cm lateral from the dorsal spinous processes on the left and the right sides of L2 muscle segment. The collar of the indenter was placed on the skin surface and three repeated measurements of muscle tone were recorded. The indenter generated a voltage output that was converted to the newton m^{-1} by using calibration constants.

Muscle indenter calibration

Muscle tone was measured using a custom-made muscle indenter. A 14 mm diameter nylon bead was glued to the end of a linear variable displacement transducer (LVDT; ULSC25-10, Monitran, Penn, Bucks, UK). A spring was mounted between the bead and LVDT and metal collar (29 mm external diameter) constructed to protect these parts. The indenter was calibrated against a weighing apparatus and LVDT calibration equipment, then converted its voltage output to force and length of the bead (Force: r^2 = 0.98; Length: r^2 = 0.99; n = 6) (Fig. 1)

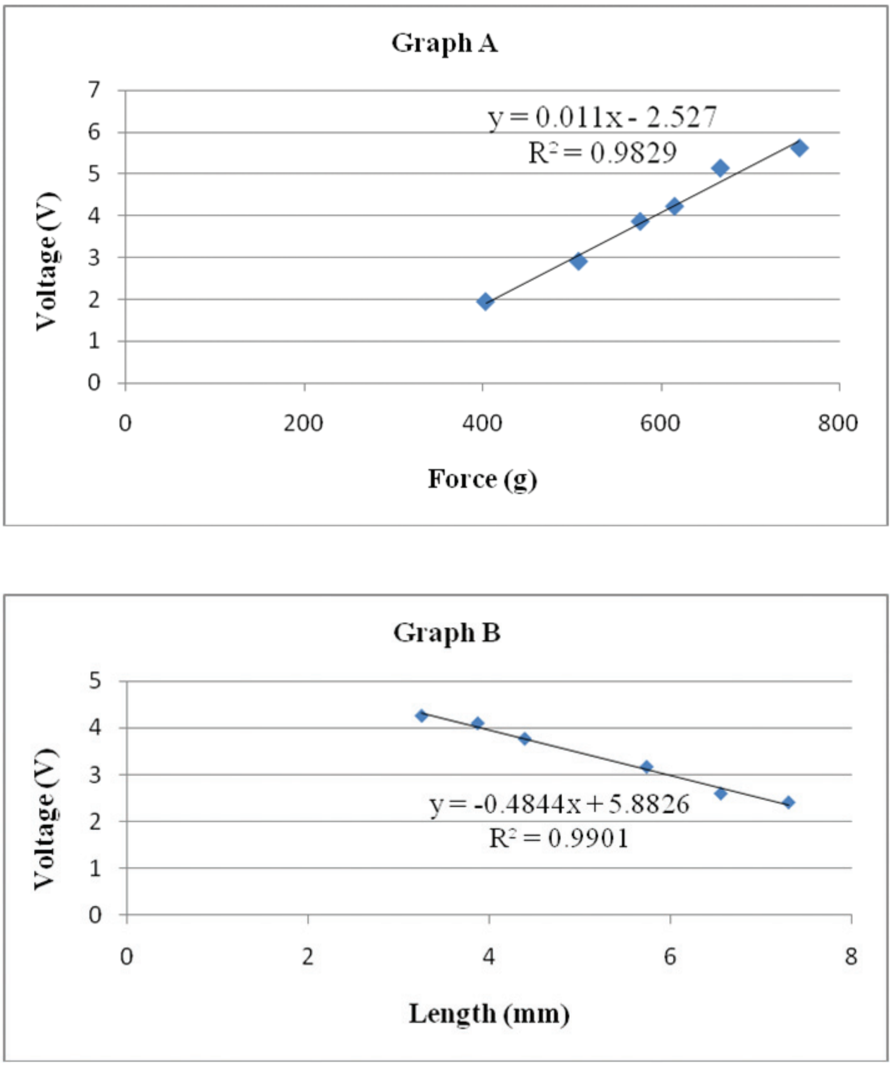

Figure 1. Graph A show voltage against force (R^2 = 0.98) and graph B show voltage against length (R^2 = 0.99).

Relating stiffness to voltage

$$\text{Stiffness} = \frac{\Delta \text{ Force}}{\Delta \text{ Length}}$$

Linear equation: $y = mx + c$

From graph A: Voltage = 0.011 x Force + 2.527

$$\text{Force (g)} = \frac{\text{Voltage} - 2.527}{0.011}$$

$$\text{Force (N)} = \frac{\text{Voltage} - 2.527}{1.1217} \quad (1\text{ N} = 101.971\text{ g}) \ldots(1)$$

From graph B: Voltage = 0.4844 x Length + 5.8826

$$\text{Length} = \frac{\text{Voltage} - 5.8826}{-0.4844} \quad \ldots(2)$$

$$\text{Stiffness (N m-1)} = \frac{(1)}{(2)} = \frac{0.4844\text{Voltage} - 1.2241}{6.5985 - 1.1217\text{Voltage}} \times 1000$$

Statistical Analyses

Statistical analyses were performed by SPSS statistics version 17.0. Data were analyzed for normal distribution by Shapiro-Wilk test and was shown as mean ± SEM. A paired-sample t-test was used for comparing data between before and after EAT. Furthermore, the statistical significance was set at $P < 0.05$ in this study.

Results

The mean salivary cortisol levels at rest and after EAT for all horses were 1.41 ± 0.17 ng/ml and 1.43 ± 0.21 ng/ml, respectively. The salivary cortisol was not significantly different between before and after EAT (Figure 2).

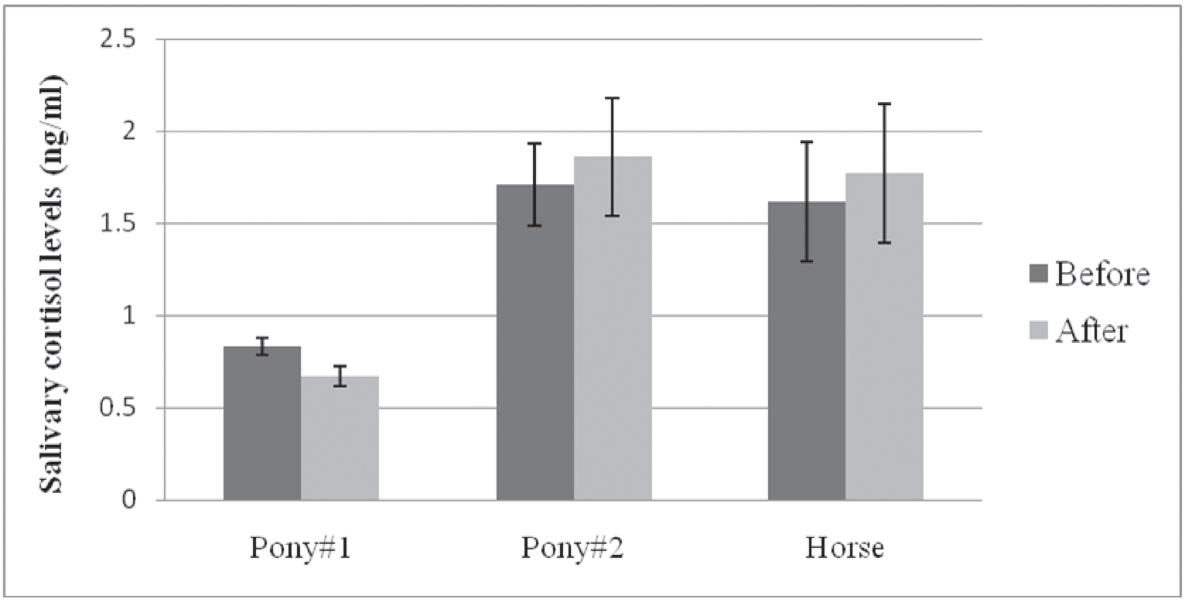

Figure 2. Salivary cortisol levels (ng/ml) of horses in resting stage (before) and after EAT (mean ± SEM).

Mean heart rate value of horses at resting stage and after EAT at 0 minute were 40.53 + 1.36 bpm and 45.20 + 5.20 bpm, respectively. The heart rate value was not significantly different between at rest and after EAT immediately. From the resting period, the mean heart rate values after EAT were increased from 39 to 42 bpm in pony no.1, 41 to 51.20 bpm in pony no.2, and 41.60 to 42.40 bpm in a horse (Figure 3).

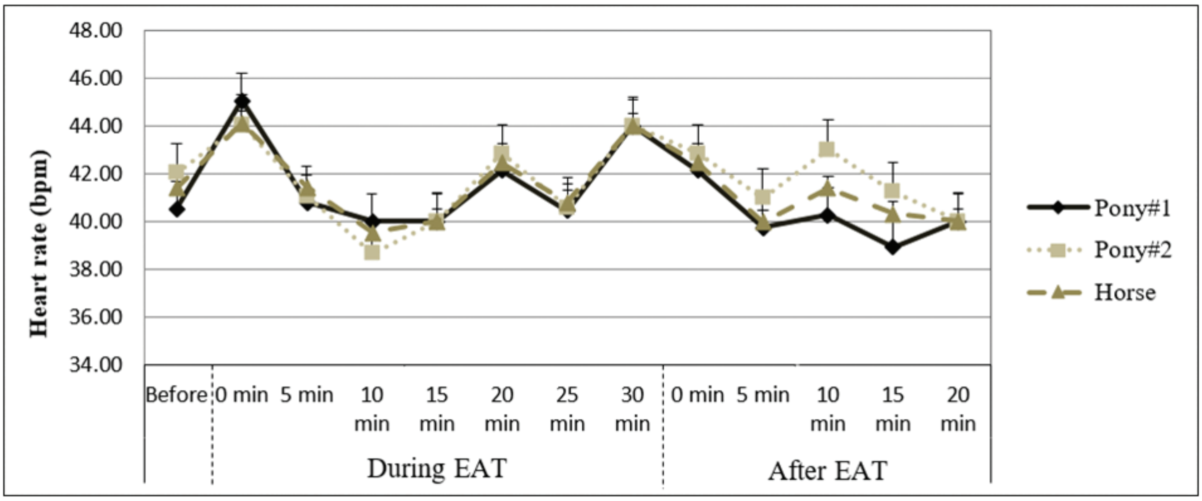

Figure 3. Heart rate (beats per minute) of horses in resting stage (before), during EAT, and after EAT (mean ± SEM).

The muscle tone on both left and right sides at T14 muscle segment after EAT revealed elevated 8.63% and 13.63% from (914.43 ± 58.09) to (993.39 ± 59.64) N.m-1 and (841.11 ± 47.45) to (955.77 ± 51.60) $N.m^{-1}$, respectively. Next, the muscle tone on both left and right sides at T16 muscle segment after EAT revealed elevated 10.86% and 14.42% from (866.47 ± 46.69) to (960.58 ± 54.85) $N.m^{-1}$ and (839.62 ± 44.44) to (960.70 ± 52.53) $N.m^{-1}$, respectively. Then the muscle tone on both left and right sides at T18 muscle segment after EAT revealed elevated 6% and 11.36% from (852.70 ± 46.56) to (903.84 ± 53.92) $N.m^{-1}$ and (830.28 ± 42.55) to (924.60 ± 48.99) $N.m^{-1}$, respectively. For the last muscle segment, the muscle tone on both left and right sides at L2 muscle segment after EAT revealed elevated 10.09% and 11.28% from (848.99 ± 39.20) to (934.62 ± 43.86) $N.m^{-1}$ and (847.17 ± 40.17) to (942.77 ± 40.18) $N.m^{-1}$, respectively. However, all of muscle segments were not significantly different between before and after EAT and were not significantly different between left and right side of the *longissimus dorsi* (Figure 4). Meanwhile, the muscle tone of pony no.2 and horse were significantly stiffer than pony no.1. Additionally, the muscle tone of the pony no.2 had no significantly different from the horse.

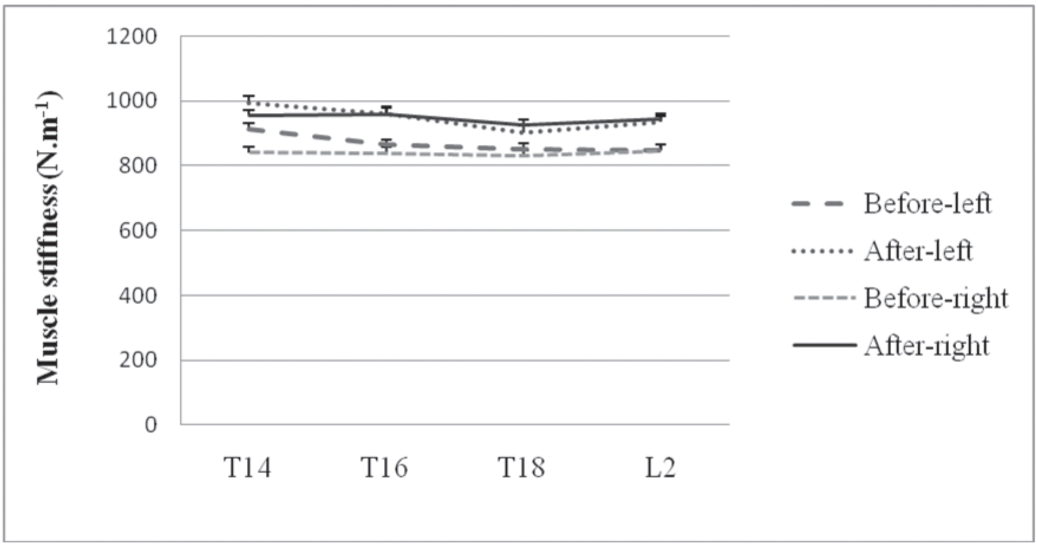

Figure 4. The comparison of muscle stiffness on both sides of T14, T16, T18, and L2 (mean ± SEM). The mean ± SEM of the muscle segments stiffness on both sides are shown as dot, solid and dash lines.

Discussion

In this study, the salivary cortisol levels were not significantly different between before and after EAT. We implied that EAT had small effects and workload on the HPA axis response that could be indicated low stress levels in our study horses. Our results are similar to these previous studies. Cravana and Fazio found that the plasma cortisol levels were not significant differences in therapeutic riding (Cravana et al., 2008a; Fazio et al., 2013). There has been reported that the salivary cortisol was decreased in therapeutic riding (Fazio et al., 2013). However, a previous study of 24-hour cortisol profile of horses show that the mean salivary cortisol was between 0.39 to 0.62 ng/ml (Bohák et al., 2013).Whereas the mean salivary cortisol level in this study was between 0.83 to 1.86 ng/ml, revealed twofold greater than usual. However, the normal range of salivary cortisol level for pony used in equine-assisted therapy still have not been reported in any studies. The circadian rhythm of cortisol level could be disrupted by changing the daily routine of the horses. A recent study show that the mean cortisol level was not significantly different between the different periods of time in therapeutic riding (Cravana et al., 2008a). However, one pony in our study showed a decrease in salivary cortisol level, due to the EAT had small effects on the HPA axis and the circadian rhythm probably not be disrupted completely. Then the salivary cortisol levels after EAT probably lower than before EAT by the circadian rhythm. In fact, numerous factors can also effect to the cortisol levels such as age, breed, sex, and unusual stimuli (PĂVĂLOIU and PAPUC, 2009).

In this study, the heart rate value was not significantly different between at rest and after EAT at 0 minute. The baseline of heart rate in the horses engaging in EAT has been recorded for 24 hour without using for riding or therapeutic lessons, the mean heart rate had a range 43 ± 8 bpm (Gehrke et al., 2011). Conversely in another study, the mean heart rate in the horses using for therapeutic sessions have been reported as a range 39 to 57 bpm (Cravana et al., 2008a). In our study, the mean heart rate was 39 to 45 bpm, which similarly to the range of baseline interval of Gehrke's study (high mean: 43-51 bpm). However, the heart rate showed higher values at 0 minutes, and 30 minutes during EAT in our study. This could be from mounting at the beginning of the therapeutic riding and dismounting after the therapeutic riding and also the instantly change of environment during a ride. For example, walked from a stable to an indoor arena or returned from a paddock to an indoor arena. However, This recent study implied no significant difference between the different periods of time (Cravana et al., 2008a). Furthermore, the level of salivary cortisol and heart rate were shown a little changes, but not significantly different between at rest and after EAT. This relationship indicates that EAT had little effect on the HPA axis. Consequently, our therapeutic plan did not induce the stress condition to our subjects.

The muscle segments were not significantly different between before and after EAT and were not significantly different between left and right side of the *longissimus dorsi*. However, the muscle tones were more stiffness at both T14 and T16 but less stiffness at T18 and L2. Similarly to this study, the muscle stiffness in thoroughbred horses has been compared between before and after training showed more stiffness at T16 and less stiffness at L2, respectively (Jewmongkonchai et al., 2010). Similar to another recent study, the muscle activity tends to decrease along spine in the caudal direction during trotting on the treadmill (Ritreuchai, 2009). This would imply that the muscle stiffness depends on the muscle activity of the horse during dynamic movement and the muscle tone seems to decrease at T18 and L2. One *in vivo* study at walk has showed that the amplitude of measured EMG was highest at T12 and decreased caudally (Licka et al., 2009). This is related to an *in vitro* study in which the range of possible movements gradually decreased cranio-caudally from T10 to L2 (Jeffcott, 1980). Another study *in vitro* study revealed that the muscle fascicle architecture predisposes the *longissimus dorsi* to different functions both along its length and between different regions (Ritruechai et al., 2008). The weight of rider and saddle caused the overall decrease in flexion and extension of the horseback that resulted in muscle tension (Cocq et al., 2004). Thus the muscle segment at T14 and T16 could have more muscle tension due to bearing the weight of a rider and a saddle. However, the weight of rider for EAT in this study was only 20 kilograms so the weight bearing of a rider had little effects on the horseback that result in no significantly different between before and after EAT. To our knowledge, the differences of muscle tone level in horse and pony have not been studied. However, the actual indenter readings were sensitive to many factors, such as the horse standing squarely on all four limbs, the head being held and external distraction (Wakeling et al., 2006).

Conclusion

Both salivary cortisol and heart rate were related together by show no significant differences between before and after EAT in this study. We conclude that EAT had small effects and workload on the HPA axis and heart rate response, thus indicated low stress levels in our study. And also the longissimus dorsi muscle tone after EAT has little effect on the horseback. For a further investigation, the comparative of stress response between ponies and horses is an interesting study because we widely use both ponies and horses in equine-assisted therapy nowadays.

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