

Pilot study on the influence of osteopathic manual therapy on cortisol and testosterone concentrations in horses

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Abstract

We hypothesized that osteopathic manipulation therapy (OMT) can regulate the activity of the hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-gonadal (HPG) axes, resulting in positive physiological and hormonal changes. This study investigated the effects of OMT on cortisol and testosterone concentrations in horses. Experimental longitudinal, randomized, controlled study. Healthy stallions from the same stable selected on the basis of their age, breed, weight, health, training level, and daily routines were divided into an OMT group ($n = 19$) and a control group receiving no therapy ($n = 19$). Horses in the OMT group underwent OMT, while those in the control group underwent regular handling, and horses in both groups underwent measurement of blood serum cortisol and testosterone concentrations and the testosterone/cortisol (T/C) ratio at three time points (before, after, and 1 h after the intervention) at 2-week intervals (0, 14, 28 days). The OMT group showed significant changes on day 14, with a 31.24% decrease in cortisol concentration, a 27.34% increase in testosterone concentration, and a 1.97% increase in the T/C ratio from the baseline levels ($P < 0.001$). The results of this study provide insights into the potential of OMT as a non-pharmacological method for modulating endocrine function and improving overall health, stress, and pain management.

Equine osteopathy, stress index, anabolic index

Manipulation therapy is commonly used in the horse industry to relieve pain and improve mobility. Manipulation is a manual procedure wherein a guided impulse pushes a joint or vertebral segment beyond its physiological range of motion without exceeding the anatomic limit of articulation. It is also known as osteopathy, high-velocity chiropractic manipulation, or low-amplitude controlled thrust technique (Haussler 2016). Over the past few decades, research on equine osteopathic manipulation therapy (OMT) has been primarily based on a biomechanical paradigm measuring joint movement and gait differences (Haussler et al. 2010; Acutt et al. 2019) and objective pain measures (Haussler 2020; Tabor and Williams 2020).

The biomechanical paradigm mentioned above has gradually given way to a neurophysiological model as more information has become available. The framework of this model is based on studies showing the effects of manipulation therapy on the autonomic nervous system (Birt et al. 2015; Laeng and Alnaes 2019; Vokietytė-Vilėniškė et al. 2023), neuroimmune function (Haavik et al. 2021; Lutke Schipholt et al. 2021) and neuroendocrine mechanisms (Sampath et al. 2015; Sampath et al. 2017a; Sampath et al. 2021). In this regard, the hypothalamic-pituitary-adrenal (HPA) axis is generally recognised to maintain homeostasis by changing the cortisol concentration in the body via the circadian rhythm (Bohák et al. 2013) and as a defence against stress and other threats in the environment (Sampath et al. 2019). For example, an increase in the blood cortisol concentration during exercise suppresses cytokine release and

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prevents pathological processes (Arfuso et al. 2022). In contrast, a long-term increase in blood cortisol concentration is linked to chronic pain and can cause muscle tissue breakdown through catabolic processes (Sampath et al. 2019; Grzędzicka et al. 2023). Another study on horse environments found that horses kept in solitary conditions had lower cortisol concentrations after stimulation than horses kept in groups and on pastures (Mehta and Josephs 2010). Moreover, Sauer et al. (2019) found that horses over 15 years of age had significantly higher cortisol concentrations than younger horses, suggesting body adaptivity.

Testosterone, another hormone widely used to monitor the hypothalamic-pituitary-gonadal (HPG) axis in human athletes, serves as a stress factor and adaptation model by affecting muscular tissue in the anabolic (stimulating protein synthesis) and anti-catabolic (protecting muscle proteins and facilitating recovery) pathways. The testosterone concentration in horses is mostly linked to behavioural issues (Sauer et al. 2019), male development, reproductive success (Olvera-Maneu et al. 2021; Medill et al. 2023) and performance (Witkowska-Piłaszewicz et al. 2021; Dąbrowska et al. 2022; Grzędzicka et al. 2023). In a study by Grzędzicka et al. (2023), experienced racehorses showed increased blood serum testosterone concentration after exercise, indicating that anabolism occurs during recovery and is more effective in regularly trained horses.

A thorough understanding of the stress response function requires the measurement of multiple stress-related biological processes owing to the involvement of multiple systems (Kivlighan and Granger 2006). In this regard, the connection between the HPG and HPA axes is particularly significant. Inhibition of the stress hormone cortisol releases the reproductive sex hormone testosterone and vice versa (Hayes et al. 2010). Therefore, studies in sports and exercise science have measured the testosterone/cortisol (T/C) ratio, called the anabolic index (Hug et al. 2003; Hayes et al. 2010; Sampath et al. 2019; Grzędzicka et al. 2023). The balance between the anabolic and catabolic processes in the body depends on the interaction between cortisol and testosterone. These hormones are highly responsive to both acute and chronic exercise. It can also provide insights into behavioural changes, overreaching, overtraining, strength gain, recovery, and adaptation to subsequent exercise (Grzędzicka et al. 2023). In fact, in athletes monitored over an extended period, a decrease of > 30% in the T/C ratio from the baseline may signal overtraining (Adlercreutz et al. 1986). A recent study on the anabolic index found that it varies according to the type of exercise, making it a better marker for monitoring racehorses than endurance athlete performance (Grzędzicka et al. 2023). A study by Dąbrowska et al. (2022) on leisure horses concluded that the T/C ratio increased when the horses underwent high exploitation, and their results showed a link with increased testosterone concentration, suggesting horse behavioural issues.

In the thoroughbred racing industry, horses often undergo intense training in stressful environments that can lead to injuries, overtraining, and conditions such as EGUS syndrome. The response of an athlete's body to pain and stress during races, including the role of races in performance, has been the topic of an increasing number of studies (Hayes et al. 2010; Witkowska-Piłaszewicz et al. 2021; Grzędzicka et al. 2023). Pain is associated with poor performance (Haussler et al. 2020) therefore, pain management is key to productivity and animal welfare. Non-invasive techniques, such as manipulation therapy, and their connection to decreasing pain (nociception) show considerable potential for inclusion in athlete healthcare.

This is the first study to investigate the effects of structural OMT on cortisol and testosterone blood serum concentrations, T/C ratio, and potential stress and pain management in horses.

Materials and Methods

The study was conducted according to the guidelines by the Ethics Committee under the study approval number B6-(19)-1476. This research was carried out by the provisions of the Law of the Republic of Lithuania—order number 8–500 on the protection, maintenance, and use of animals, of November 6, 1997 (the Official Gazette ‘Valstybės žinios’ number 108–6595, dated November 28, 1997); order number 4–361 of December 31, 1998 of the State Veterinary Service of the Republic of Lithuania on breeding, care, and transportation of laboratory animals; and order number 4 of 18 January 1999 of the State Veterinary Service of the Republic of Lithuania on the use of laboratory animals for scientific tests. The study approval number was B6-(19)-1476.

Experimental protocol

This trial employed an experimental longitudinal, randomized, controlled design of 6 weeks’ duration, with randomization at the starting point on day 0. A washout period of 14 days was deemed sufficient between the intervention sessions since carryover effects were not expected or considered negligible. This study aimed to assess the immediate and long-term effects of OMT; therefore, osteopathic treatment was performed three times on days 0, 14, and 28.

Animal selection

Three days before the experiment, all 38 horses underwent clinical examination by an accredited equine osteopath (EDO certification on December 20, 2015) to assess their joint range of motion. During the initial clinical assessment, various vital signs were assessed, including heart rate, respiratory rate, capillary refill time, rectal temperature, mucous membranes, and lymph nodes (Costa 2017). A comprehensive orthopaedic examination was performed using the horse lameness evaluation protocol (Davidson 2018) and the working horse back pain evaluation protocol (Martin and Klide 1999). All horses ($n = 38$) had a limited joint range of motion and a sudden, firm end-feel, indicating an osteopathic movement disorder (Haussler 2016).

On the experiment day, the horses were randomly divided into the OMT group ($n = 19$) and the control group ($n = 19$). Blinding of the therapist to the intervention was impossible due to the nature of the intervention. All procedures were performed in horse boxes to reduce stress. In the OMT group, OMT was performed once between blood sampling measurements. In the control group, no OMT or rechecking of the joint range of motion was performed during the experimental period. Light touch has been demonstrated to activate low-threshold mechanoreceptors through C-tactile fibres, which can modify autonomic nervous system functions (Cerritelli et al. 2016).

Blood samples were collected from each horse by the same veterinarian before (1), after (2), and 1 h after OMT (3). For horses in the control group, the first blood sample was collected (1), and then the horses were calmly held in a stable box for 20 min for the next sampling (2), which was performed after the average time required to administer OMT to each horse in the OMT group, and the last sample was obtained 1 h later (3). Experiments were conducted at 8:00–12:00 h to avoid circadian changes in the cortisol and testosterone ratio (Hayes et al. 2010) and performed three times at two-week intervals (i.e., days 0, 14, and 28) to assess the long-term effects of OMT on blood serum testosterone and cortisol concentrations (Sampath et al. 2019). The treatment was performed in winter (January) to avoid the influence of severe seasonal testosterone changes (Olvera-Maneu et al. 2021).

A group of 38 thoroughbred stallions from a Lithuanian stable were enrolled in this study. The horses were fed three times a day, and a total food amount of approximately 2.5% of the horse’s body weight and water were always available. The mean age in both groups was 6 ± 1.033 years, and the mean weight was 450.20 ± 14.274 kg. None of the horses were currently participating in flat racing competitions. Horses were ridden four times a week by professional riders; walked with a walker for 30 min five days a week; and spent 2–3 h a day in the paddock with access to hay and water. Three days prior to the experiments, the horses were not ridden, and their daily routine included 30 min of walking with a walker and spending 2–3 h in the paddock. During the breaks between experimental sessions, the horses underwent the same training program as before, and 3 days prior to the experiment, they were not ridden again.

None of the horses were excluded from the study. The exclusion criteria were abnormal clinical examination values, lameness, back pain, use of drugs (steroids, non-steroidal anti-inflammatory drugs) or regenerative medicine within the last six months, and any surgery within the last six months. None of the horses had previously undergone osteopathic treatment. No other therapy was administered to the horses during the study. None of the horses were in the breeding season.

Horses in the OMT group were assessed on the day of the experiment. The EDO performed a joint motion check using the hands-on technique, passively moving the joint from a neutral position to flexion, extension, lateral bending, or axial rotation, depending on the joint; a joint movement disorder (MD) was indicated if there was a sudden, firm-end feel in either direction (Haussler 2016). The EDO used controlled high-velocity, low-amplitude thrust techniques to target restrictions in the cervical, thoracic, lumbar, and scapular joints, as well as the sacrum, shoulder, and hip joints on both sides of the horse’s body during the OMT group session (Pascal 2002; Pusey et al. 2010; Haussler 2016).

Biochemical analyses

Blood collection was performed by jugular vein puncture and two BD Vacutainer® Plus 6-ml plastic serum tubes containing additive silica, a clot activator, with a red lid (Eysins, Switzerland). All samples were immediately

placed in a transportable cooler bag and transported to the laboratory for analysis. All samples were centrifuged at $2000 \times g$ for 10 min to obtain sera. An automatic competitive fluorescent enzyme immunoassay AIA-360 analyser (Tosoh Bioscience, San Francisco, CA, USA) was used to assess serum cortisol concentration (nmol/l). Siemens ADVIA Centaur XP immunoassay analyser (Siemens Healthcare Diagnostics, New York, NY, USA) with direct chemiluminescence testing methodology using advanced acridinium ester technology was used to estimate the serum testosterone concentration (nmol/l). In both analyses, daily checks, calibration curves, and maintenance procedures were performed according to instructions outlined in the system operator's manuals.

Statistical analyses

Statistical analysis was performed using IBM SPSS Statistics for Windows (version 25.0; IBM Corp., Armonk, NY, USA). The data showed a normal distribution (Shapiro-Wilk test). Comparisons of cortisol and testosterone concentrations and their ratios were performed three times and analysed with repeated-measures analysis of variance for comparison of means across the investigated variables based on blood sampling times (time 1, before therapy; time 2, immediately after therapy; and time 3, 1 h after therapy). The T/C ratio was calculated using the following formula: $T/C \text{ ratio} = \text{cortisol (nmol/l)} \div \text{testosterone (nmol/l)} \times 100$, and the results were expressed as a percentage. Differences in mean values between groups (investigated and control groups) were analysed using Student's *t*-test. A probability of less than 0.05 was considered reliable ($P < 0.05$). Cortisol, testosterone, and their ratios were compared between days 0, 14, and 28 of the experiment using repeated-measures ANOVA. Each comparison was tested separately for blood sampling time (1, before; 2, immediately after; 3, 1 h after) and the studied groups (OMT, control). Capital letters (X, Y, Z) indicated differences with $P < 0.05$ between 0, 14, and 28 days of the experiment.

Results

The cortisol concentration was higher in the OMT group than in the control group on day 14 at times 1 and 2. In contrast, the cortisol concentration was lower in the OMT group than in the control group on day 28 only at times 2 and 3. When comparing the findings for different time points of blood collection, the cortisol concentration was higher at time 2 than at time 3 on day 0. Similarly, it was higher at time 1 than at time 3 on days 14 and 28, as well as in the control group on day 0. No other differences were observed between the groups for different times of blood collection. When comparing the findings for different days of blood collection (0, 14, 28), the cortisol concentration in the OMT group was higher on day 0 than on day 28 at time 2 and on day 14 at time 3. The cortisol concentration in the control group was higher at time 3 on day 28 than on days 0 and 14 (Table 1).

Table 1. Descriptive statistics of cortisol concentration measured at different time points. Cortisol concentration is expressed in nmol/l and the values are presented as mean $\pm$ SD.

Time of blood collection	Osteopathic manual therapy group	Control group
Day 0		
1	138.58 $\pm$ 30.42	131.57 $\pm$ 23.64 ^A
2	145.63 $\pm$ 18.62 ^{A,X}	131.19 $\pm$ 31.51 ^A
3	123.54 $\pm$ 20.87 ^{B,X}	112.97 $\pm$ 27.78 ^{B,X}
Day 14		
1	155.06 $\pm$ 18.51 ^{A,a}	128.12 $\pm$ 25.13 ^b
2	134.20 $\pm$ 18.52 ^B	130.97 $\pm$ 33.44
3	106.62 $\pm$ 12.53 ^{C,Y}	113.67 $\pm$ 29.47 ^Y
Day 28		
1	147.57 $\pm$ 15.03 ^A	137.79 $\pm$ 20.97
2	129.03 $\pm$ 16.01 ^{B,Y}	137.45 $\pm$ 28.35
3	113.85 $\pm$ 18.16 ^{C,a}	138.81 $\pm$ 23.39 ^{b,Z}

Lower-case superscripts (^a, ^b) indicate differences between groups. Uppercase superscripts (^A, ^B, ^C) indicate differences between the time points of investigation. ^X, ^Y, and ^Z indicate differences between days of the experiment ($P < 0.05$). 1 - before OMT; 2 - after OMT; 3 - after 1 hour.

The testosterone concentration was lower in the OMT group than in the control group on day 0 at time points 2 and 3. However, testosterone concentration in the OMT group was higher than the corresponding values in the control group at time 2 and time 3 on days 14 and 28.

In comparisons based on the time of blood collection, testosterone concentration was lower at time point 2 than at time points 1 and 3 on day 0, and testosterone concentration was lower at time point 1 than at time points 2 and 3 on days 14 and 28, as well as in the control group on day 0. In comparisons of the findings for different days (0, 14, 28), testosterone concentration in the OMT group was higher on days 28 and 14 for time 2 and time 3 than it was on day 0. No other differences were found between the days in the control group (Table 2).

Table 2. Descriptive statistics of testosterone concentration measured at different time points. Testosterone concentration was expressed in nmol/l and the values are presented as mean $\pm$ SD.

Time of blood collection	Osteopathic manual therapy group	Control group
Day 0		
1	2.81 $\pm$ 0.66 ^A	3.03 $\pm$ 0.58 ^A
2	2.33 $\pm$ 0.41 ^{B,a,X}	3.34 $\pm$ 0.57 ^{B,b}
3	3.05 $\pm$ 0.44 ^{A,a,X}	3.39 $\pm$ 0.52 ^{B,b}
Day 14		
1	2.95 $\pm$ 0.68 ^A	3.15 $\pm$ 0.55
2	3.85 $\pm$ 0.39 ^{B,a,Y}	3.27 $\pm$ 0.45 ^b
3	4.06 $\pm$ 0.45 ^{B,a,Y}	3.25 $\pm$ 0.51 ^b
Day 28		
1	3.07 $\pm$ 0.66 ^A	3.04 $\pm$ 0.51
2	4.00 $\pm$ 0.54 ^{B,a,Z}	3.39 $\pm$ 0.6 ^b
3	4.22 $\pm$ 0.39 ^{B,a,Z}	3.11 $\pm$ 0.63 ^b

Lower-case superscripts (^a, ^b) indicate differences between groups. Uppercase superscripts (^A, ^B, ^C) indicate differences between the time points of investigation. ^X, ^Y, and ^Z indicate differences between days of the experiment ($P < 0.05$). 1 - before OMT; 2 - after OMT; 3 - after 1 hour.

Table 3. Testosterone/cortisol ratio at the three measurement points on different days.

Time of blood collection	Osteopathic manual therapy group Testosterone/cortisol ratio, percentage	Control group Testosterone/cortisol ratio, percentage
Day 0		
1	2.32 ^A	2.43 ^A
2	1.61 ^{B,a,X}	2.72 ^b
3	2.53 ^{A,a,X}	3.18 ^{B,b,X}
Day 14		
1	1.96 ^{A,a}	2.60 ^{A,b}
2	2.95 ^{B,Y}	2.74
3	3.93 ^{C,a,Y}	3.03 ^{B,b,X}
Day 28		
1	2.11 ^A	2.28 ^A
2	3.17 ^{B,a,Y}	2.57 ^b
3	3.79 ^{C,a,Y}	2.32 ^{B,b,Y}

Lower-case superscripts (^a, ^b) indicate differences between groups. Uppercase superscripts (^A, ^B, ^C) indicate differences between the time points of investigation. ^X, ^Y, and ^Z indicate differences between days of the experiment ($P < 0.05$). 1 - before OMT; 2 - after OMT; 3 - after 1 hour.

The mean T/C ratio in the OMT group was higher than that in the control group at time 2 on day 28 and at time 3 on days 14 and 28. However, the T/C ratio in the OMT group was lower than that in the control group at time 2 and time 3 on day 14 and at time 1 on day 0. On day 0, the T/C ratio in the OMT group at time 2 was lower than that at time 1 and time 3. In addition, the T/C ratio at time 1 was lower than that at time 2 and time 3 on days 14 and 28. In the control group, the T/C ratio at time 1 was lower than that at time 3 on days 0 and 14, and the T/C ratio at time 1 was lower than that at time 2 on day 28. In comparisons of the findings obtained on days 0, 14, and 28, the T/C ratios at time 2 and time 3 in the OMT group were higher on days 14 and 28 than on day 0. In the control group, the T/C ratio at time 3 was higher on days 0 and 14 than on day 28 (Table 3).

Discussion

Cortisol is a stress-induced biochemical factor that reduces local oedema and discomfort. In addition, elevated cortisol concentrations are believed to promote wound healing by stimulating gluconeogenesis (Hannibal and Bishop 2014) and regulating the inflammatory response during exercise (Arfuso et al. 2022). Therefore, slightly increased plasma cortisol levels during prolonged physical activity are associated with a normal, short-term response to intense exercise known as ‘good stress’ (Dąbrowska et al. 2022). The stress response is activated when the HPA axis is stimulated. Bassett et al. (1987) demonstrated that the stress level of a subject is correlated with the cortisol concentration. The HPA axis regulates cortisol levels through feedforward and feedback loops (Sampath et al. 2017a). The glucocorticoid-mediated feedback loop occurs over at least three temporal domains: quick (seconds to minutes), intermediate (1–10 h), and slow (hours to days) (Keller-Wood and Dallman 1984). Studies in humans have reported inconsistent changes in plasma or salivary cortisol concentrations following spinal manipulation (Whelan et al. 2002; Sampath et al. 2015; Sampath et al. 2017a; Sampath et al. 2017b, Lohman et al. 2019; Sampath et al. 2019; Valera-Calero et al. 2019; Farrell et al. 2023). However, animal experiments have shown that innocuous stimulation (such as spinal manipulation) can inhibit adrenal gland activity (Budgell et al. 1997; Henneman 2022; Vokietytė-Vilėniškė et al. 2023). Our data were consistent with these previous findings. We observed a decrease in cortisol concentration from baseline on days 14 and 28.

Additionally, our findings showed a notable increase in cortisol concentration on day 0 immediately after therapy, followed by a decrease 1 h later. In our study, the cortisol levels increased slightly before OMT therapy on days 14 and 28. This increase in cortisol levels could be linked to the venipuncture procedure since it involves puncturing a vein, causing stress, and increasing cortisol levels (Weckesser et al. 2014). Although both groups underwent the procedure, only the OMT group showed an increase before and a decrease after the intervention. Thus, the presence of a control group ensured that these results could be linked to the technique used.

In sports medicine, testosterone concentration has been reported to be associated with protein synthesis (anabolic effect) and inhibition of protein degradation (anti-catabolic effect) (Vingren et al. 2010; Witkowska-Piłaszewicz et al. 2021; Grzędzicka et al. 2023). Testosterone has been also reported to play a significant role in the stress response: the testosterone concentration has been shown to increase in response to competitive stressors, performance tasks, or physical challenges. In horses, testosterone concentration varies based on horse usage, athletic ability, and type of sport (Dąbrowska et al. 2022; Grzędzicka et al. 2023). Manipulation therapy has been suggested to lead to an increase in testosterone concentration and cause faster amino acid uptake, promoting muscle growth, recovery, and adaptation (Sampath et al. 2017a; Sampath et al. 2021).

In our study, on day 0, the testosterone concentration decreased after OMT. This could be explained by the interaction with cortisol concentration, since the two axes interact with each other (Sampath et al. 2019). Moreover, we noticed an increase in testosterone concentration on days 14 and 28 in the OMT group, which could lead to the hypothesis that manipulation therapy affects the autonomic nervous system by activating the HPG axis and increasing testosterone concentrations (Sampath et al. 2019).

The T/C ratio is a biomarker commonly used in sports and exercise science to indicate stress, overtraining, recovery (Adlercreutz et al. 1986; Hug et al. 2003; Mehta and Josephs 2010; Sampath et al. 2019; Sampath et al. 2021; Grzędzicka et al. 2023), as well as behavioural abnormalities (Dąbrowska et al. 2022). On comparing the concentrations of these hormones after spinal manipulation, some authors observed a decrease in the T/C ratio in healthy men (Sampath et al. 2017a), while others found an increase in the ratio in symptomatic patients of both sexes after therapy (Bohák et al. 2013, Sampath et al. 2021). However, our longitudinal study yielded different findings. In the OMT group, the T/C ratio decreased immediately after therapy on day 0, while it increased immediately after therapy on days 14 and 28. On all days, the T/C ratio increased 1 h after therapy. These changes may indicate a more favourable environment for recovery, tissue healing, and collagen synthesis (Demling 2005), which could lead to the hypothesis that extended osteopathic manual therapy could facilitate the healing process immediately after the procedure.

This study had several limitations that warrant consideration. While the control group was chosen to provide a consistent baseline for observing the effects of OMT, sham treatment was not included in the study. The information from a sham group could have been valuable for understanding how the HPA and HPG axes react to OMT.

Female horses (mares) were not included in the current study. In humans, the responses of the HPA and HPG axes differ between men and women. Therefore, animals of both sexes should be included when evaluating OMT efficacy.

Finally, this study focused solely on neuroendocrine responses to OMT. However, it did not evaluate the effectiveness of OMT in improving patient outcomes, such as pain and physical function, or measure the clinical effects related to hormone changes.

Despite these limitations, this study provides initial insights into the effects of structural OMT on horses' cortisol and testosterone concentration changes and its potential usefulness for pain and stress management. More robust studies are required to understand the implications of OMT fully. When healthy horses received OMT, their bodies responded with cortisol, testosterone, and T/C ratio changes, indicating a neuroendocrine response. This response was visible with prolonged treatments (3 times two weeks apart). Thus, OMT could be beneficial for athletic horse health care. Since this is the first study in horses, additional research is needed to assess the effects of OMT in both healthy and unbalanced (pathological) horses.

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