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Neuroendocrine Response Following a Thoracic Spinal Manipulation in Healthy Men

Spinal manipulation (SM) is a specific hands-on approach commonly used by several different therapeutic professions to treat spinal pain.[4,35] Though the exact mechanisms through which SM operates are still unclear,[4] various mechanisms have been proposed, including changes in the autonomic nervous system (ANS), sympathetic nervous system (SNS), and the endocrine system (hypothalamic-pituitary-adrenal [HPA] axis).[24]

Several studies have been undertaken to investigate the effects of SM on the SNS.[8,9,77,87,89] However, there is no gold standard of noninvasive measurement of changes in activity.[93] One reliable, noninvasive technique to measure SNS activity is the use of near-infrared spectroscopy (NIRS).[12,26,37] This method uses light in the near-infrared spectrum (600-1000 nm) from an optode attached to the skin. Different wavelengths of this light are absorbed by either oxygenated hemoglobin (O_2Hb) or deoxygenated hemoglobin, and changes in the absorption of light can be used to calculate changes in levels of O_2Hb and deoxygenated hemoglobin.[27,36] This, in turn, can be used to measure blood flow (vasoconstriction/dilation) of arterioles in skeletal muscles. As the autonomic regulation of skeletal muscle blood flow is dominated by the SNS,[29,57] O_2Hb changes in skeletal muscles as measured by NIRS may provide a reliable estimate of SNS activity.

Most tissues innervated by the SNS also receive innervation by the parasympathetic nervous system (PNS)[20]; therefore, the interplay between these 2 systems affects how the tissues respond to stress/ damage. Heart rate variability (HRV), the beat-to-beat variation in heart rate, is a well-established method of assessing

● **STUDY DESIGN:** Controlled laboratory study.

● **BACKGROUND:** Spinal manipulation (SM) can trigger a cascade of responses involving multiple systems, including the sympathetic nervous system and the endocrine system, specifically, the hypothalamic-pituitary axis. However, no manual therapy study has investigated the neuroendocrine response to SM (ie, sympathetic nervous system–hypothalamic-pituitary axis) in the same trial.

● **OBJECTIVE:** To determine short-term changes in sympathetic nervous system activity, heart rate variability, and endocrine activity (cortisol, testosterone, and testosterone-cortisol [T/C] ratio) following a thoracic SM.

● **METHODS:** Twenty-four healthy men aged between 18 and 45 years were randomized into 2 groups: thoracic SM (n = 12) and sham (n = 12). Outcome measures were salivary cortisol (micrograms per deciliter), salivary testosterone (picograms per milliliter), T/C ratio, heart rate variability, and changes in oxyhemoglobin concentration of the right calf muscle (micromoles per liter). Measurements were done before and at 5 minutes,

30 minutes, and approximately 6 hours after intervention.

● **RESULTS:** A statistically significant group-by-time interaction was noted for T/C ratio (*P*<.05) and salivary cortisol (*P*<.01) concentrations. Significant between-group differences were noted for salivary cortisol concentration at 5 minutes (mean difference, 0.35; 95% confidence interval: 0.12, 0.6; interaction: *P*<.01) and for T/C ratio at 6 hours postintervention (mean difference, −0.09; 95% confidence interval: −0.16, −0.04; *P* = .02). However, SM did not differentially alter oxyhemoglobin, testosterone, or heart rate variability relative to responses in the sham group.

● **CONCLUSION:** Thoracic SM resulted in an immediate decrease in salivary cortisol concentration and reduced T/C ratio 6 hours after intervention. A pattern of immediate sympathetic excitation was also observed in the SM group. *J Orthop Sports Phys Ther 2017;47(9):617-627. Epub 13 Jul 2017. doi:10.2519/jospt.2017.7348*

● **KEY WORDS:** *autonomic nervous system, cortisol, spinal manipulation, sympathetic nervous system, testosterone*

autonomic function.[55] Analysis of HRV in the frequency domain reveals 3 oscillatory components in the spectral profile: (1) a very low–frequency band (less than 0.04 Hz), (2) a low-frequency (LF) band (0.04-0.15 Hz), and (3) a high-frequency (HF) band (0.15-0.4 Hz).[55,56] While the HF component is related to the PNS efferent activity, the LF component corresponds to both SNS and PNS efferent activity.[56,61] Therefore, the LF/HF ratio is interpreted as an indicator of cardiac autonomic neural activity.[56] The use of the LF/HF ratio has been criticized,[32,45] as it oversimplifies the complex nonlinear interactions between the SNS and the PNS divisions of the ANS.[5] Despite this, the LF/HF ratio still remains a widely used tool to assess autonomic cardiovascular regulation.[49,61,66,73] Previous investigations into the effects of thoracic SM on HRV have produced conflicting results.[9,87] While Budgell and Polus[9] reported that a thoracic SM resulted in changes in HRV, Ward et al[87] did not report any changes in ANS function following a thoracic SM.

Cortisol is released by the activation of the HPA axis.[14] The use of salivary sampling as a noninvasive tool for the measurement of free cortisol, and thereby HPA axis activity, has been well established.[46] Being a hormone, cortisol has a known circadian rhythm that could be influenced by a number of confounding factors, including timing of sampling, number and nature of study days, day of data collection (weekend versus weekdays), drinking habits (eg, coffee and alcohol), eating habits (food with high sugar content), dental hygiene/tooth brushing, smoking, and intense physical activity before data collection.[15,81] Therefore, adequate control of these factors is crucial for reliably measuring changes in cortisol before and after an intervention. It is unclear how these confounding factors were addressed in previous studies that have investigated the effects of SM on the HPA axis,[65,67,90] making it difficult to interpret the findings.

It has been proposed that models include multiple measurements of stress-related biological processes to assess the role of the individual's response to stress.[48] For example, the hypothalamic-pituitary-gonadal (HPG) axis and the HPA axis are known to interact with testosterone and cortisol, mutually inhibiting each other.[3,52] The balance between testosterone and cortisol (T/C ratio) may therefore provide a better estimation of hypothalamic-pituitary (HP) axis activity[28] and has been used as a hormonal biomarker to determine overtraining in athletes[41,50] and susceptibility to certain diseases.[31,78]

When an individual is presented with a stressor, such as a painful injury, the hypothalamus coordinates the stress response by activation of the HP axis and/or a fast-acting neural component involving the SNS.[15] Therefore, the hypothalamus maintains homeostasis by the activation of the neuroendocrine system (SNS-HP axis).[15] It has been shown that SM results in rapid hypoalgesia with concurrent SNS activation.[82,86] Given their integrated function, it could be hypothesized that SNS changes following SM would be accompanied by changes in the HP axis.[71] To our knowledge, no manual therapy study has investigated the neuroendocrine response to SM (ie, SNS-HP axis) in the same trial. Moreover, studies have only explored immediate changes (30-120 minutes) in HPA axis activity. Therefore, changes in the HPA axis longer than 2 hours following SM are still unknown.

We hypothesized that a thoracic SM would result in neuroendocrine response (SNS-HP axis). Activation of the SNS is demonstrated by an immediate vasoconstriction (reduced O_2Hb) of skeletal muscle in the calf, measured by NIRS. By measuring salivary cortisol and testosterone, it may be possible to understand whether changes in SNS activity are accompanied by changes in HP axis activity (T/C ratio). This may provide important mechanistic information that could be of interest to physical therapists for 2 important reasons: (1) it has been well established that the neuroendocrine mechanisms (HPA axis-SNS) play a crucial role in the modulation of pain and inflammation,[15] hence, neuroendocrine response can be a potential mechanism of pain inhibition and tissue healing following SM; (2) as argued previously,[4] identification of SM mechanisms may increase the acceptance of these techniques by health care providers. Therefore, the objectives of this study were to determine the short-term changes in SNS activity (using NIRS); SNS/PNS balance (using HRV); and changes in cortisol, testosterone, and the T/C ratio following a thoracic SM.

METHODS

Study Design

THIS WAS A LABORATORY EXPERIMENT using sham control (by random allocation) within a repeated-measures study design. The ethical approval for this study was obtained from the University of Otago Human Ethics Committee (H14/150). All participants signed an informed-consent form prior to participation in the study.

Participants

Participants were recruited from the wider community through flyers, posters, and e-mail. Healthy men between 18 and 45 years of age with no history of significant pain or illness were eligible to participate in the study. Exclusion criteria were history of serious pathologic or psychiatric disorder; previous spinal (or other relevant) surgery; any bone weakness or thoracic spine conditions, such as osteoporosis, osteopenia, Scheuermann's disease, and ankylosing spondylitis; currently on any medications; and any contraindication to SM, such as rib/spine fractures.

Randomization and Blinding

The randomization schedule was prepared by a research administrator using a computer-generated random-number table.[70] Participants were then block randomized to either the intervention

(SM) group or a sham intervention group. This was done by the use of sealed opaque envelopes. A research assistant not otherwise involved in the study did initial screening and randomization. This ensured that group allocation was concealed, and that the outcome assessor and the participant were blinded. However, due to the nature of the intervention, it was not possible to blind the manual therapist to the intervention.

Procedure

Participants were invited to attend a screening session with a research assistant not otherwise involved in the study. Participants who met the inclusion criteria were given salivary collection devices, an instruction sheet about saliva collection, and the Perceived Stress Scale (PSS-10).[17] The PSS-10, a self-reported instrument designed to measure one's level of perceived stress, has been widely used in research and shown high reliability and validity.[79] The PSS-10 was used in the present study, as perceived stress is known to influence both the HPA axis and ANS activity.[15,17] Scores of 20 or higher are considered high stress. The participants were also given a sheet to self-report (1) number of hours slept during the 2 days of data collection, (2) time of saliva sample collection, and (3) any alcohol or medication usage that was not anticipated.

The experiment took place in a laboratory where lighting, temperature, and humidity (24°C, 40%) were kept constant, as environmental factors have been shown to influence HRV recordings.[42] Upon arrival, the participant's height and weight were recorded, and any contraindications to SM were screened. Participants returned the completed PSS-10 scale and were given a 5-minute rest period, following which preintervention salivary samples (beta 2) were collected. The participant lay supine upon a treatment plinth with the legs extended. During the data recording, participants were asked to lie as still as possible to avoid interference with data recording.[80]

Salivary Protocol

Saliva samples were collected via unstimulated passive drool, as cotton-based sampling devices may introduce bias into the measurement.[76] The time of saliva sample collection was standardized such that participants collected saliva at approximately the same time on days 1 and 2. For complete collection procedures and protocol, please refer to the **APPENDIX** (available at www.jospt.org).

Intervention

The participant was asked to cross the arms in front of the body, placing each hand on the opposite shoulder so that elbows were on top of each other in front of the lower chest (**FIGURE 1**). The physical therapist then rolled the participant over on his or her side and placed the fixating hand, curled into a fist with the thumb and index finger extended (commonly called the pistol grip),[18] at the level of the fifth thoracic vertebra, an area known to contain preganglionic neurons of the SNS.[8,34,87] The therapist then rolled the participant back to the supine position. High-velocity, low-amplitude thrust was delivered through the participant's upper extremity and thorax upon expiration.[18] A single thrust was used to standardize the intervention.

For the sham intervention, the same setup was used, but the therapist did not place a fixating hand against the thoracic spine and no thrust was given. Both the intervention and sham intervention took approximately 10 seconds.

Outcome Measures

Salivary Cortisol and Testosterone Salivary cortisol and testosterone have been shown to be valid and reliable surrogate measures of the activities of the HPA and HPG axes, respectively.[23,40] From these measurements, the T/C ratio can be calculated.

O_2Hb Changes Using NIRS Near-infrared spectroscopy has been shown to demonstrate high degrees of validity and intrasubject reproducibility.[51] The standard error of measurement for O_2Hb in skeletal muscle by NIRS was calculated at 8.53%.[51] For this study, the point of largest circumference of the right calf was identified and measured. The NIRS optode was placed one third of this distance medially from the anterior border of the tibia along the line of largest circumference. This placed the optode over the medial belly of the gastrocnemius, which has previously been shown to be a reliable placement of a NIRS optode[80] (**FIGURE 1**). The leg was supported on pillows under the knee and ankle to just suspend the NIRS optode from the table, to eliminate any movement of the optode in relation
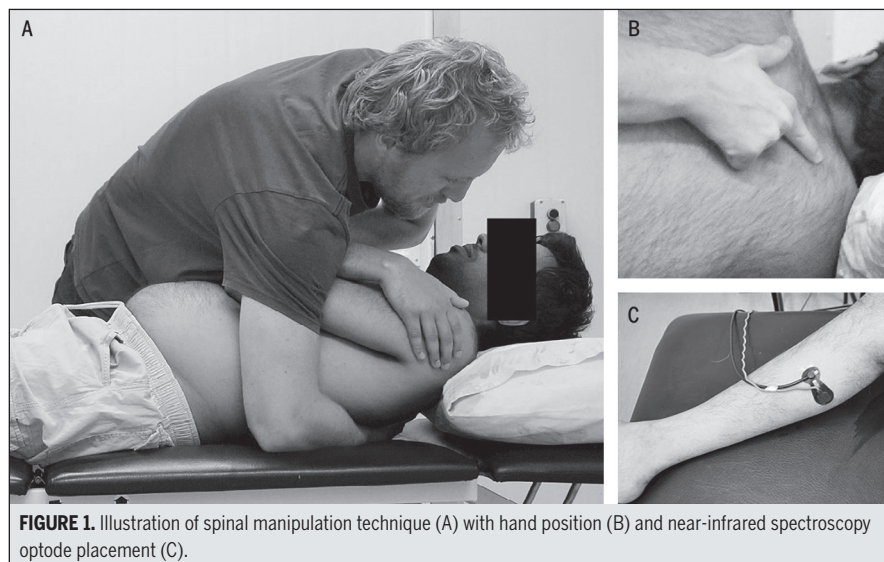
FIGURE 1. Illustration of spinal manipulation technique (A) with hand position (B) and near-infrared spectroscopy optode placement (C).

to the skin. The NIRS optode was held in place by double-sided adhesive tape, and an elastic bandage was loosely wrapped around the calf to eliminate disturbances from any outside light.
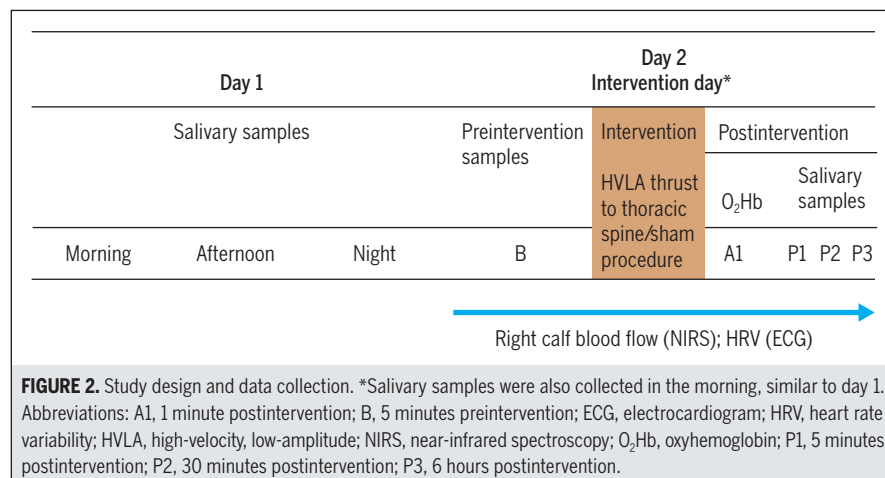
HRV Measurement For changes in HRV, the LF/HF ratio was the outcome measure, as this has been suggested to best represent changes in autonomic activity.[55] Heart rate variability was recorded using single-use disposable electrodes (Blue Sensor SP; Ambu Ltd, St Ives, UK), and a lead-2 electrocardiogram (ECG) setup was used. The ECG signal was fed through a Bio Amp and PowerLab 16/30 (ADInstruments Pty Ltd, Bella Vista, Australia).

Data Recording

Data were recorded using LabChart 8 (ADInstruments Pty Ltd). Near-infrared spectroscopy and ECG data were continuously recorded from 5 minutes preintervention to 30 minutes postintervention. Salivary samples were collected at 8 different time points on 2 consecutive weekdays to reduce the within-subject variation of situational factors such as daily schedules.[15] On day 1, participants collected baseline salivary samples at home at 3 different time points (morning, afternoon, and night). On day 2, salivary samples were collected at preintervention (B: 5 minutes before intervention) and postintervention at 3 time points (P1: 5 minutes; P2: 30 minutes; and P3: night, approximately 6 hours postintervention). The experimental timeline is given in **FIGURE 2**.

Data Processing

For NIRS data, mean 30-second blocks of O_2Hb before intervention were calculated, as this has been recommended to achieve accurate readings.[36] This was set to zero, as the equipment used in this study calculates change from baseline. Change scores from baseline were then calculated immediately (1 minute) and at 5 minutes and 30 minutes after the intervention. For HRV, data collected at 5 minutes before, 5 minutes after, and 30 minutes after the intervention were

FIGURE 2. Study design and data collection. *Salivary samples were also collected in the morning, similar to day 1. Abbreviations: A1, 1 minute postintervention; B, 5 minutes preintervention; ECG, electrocardiogram; HRV, heart rate variability; HVLA, high-velocity, low-amplitude; NIRS, near-infrared spectroscopy; O_2Hb, oxyhemoglobin; P1, 5 minutes postintervention; P2, 30 minutes postintervention; P3, 6 hours postintervention.

analyzed, as analysis of 5-minute blocks is recommended for short-term ECG recordings.[55]

Salivary Biomarker Analysis

The levels of testosterone and cortisol in all the test samples were measured using a commercially available enzyme-linked immunosorbent assay kit (Salimetrics, LLC, College Township, PA), according to the manufacturer's instruction. The **APPENDIX** provides a brief description of lab procedures used.

Power Calculations

Of the 3 outcome measures (the minimum clinically important differences of which are currently unknown), cortisol was considered primary. From previous literature,[13,65,67,90] an effect size of 0.25 was considered practical in studies involving measuring cortisol in normal individuals. Based on the repeated-measures mixed design of the current study, a power calculation was performed using G*Power (Version 3.0.1.0; Heinrich-Heine Universität, Düsseldorf, Germany). A sample size of n = 24 was required to provide 80% power at the 5% level of significance for an effect size of 0.25.

Statistical Analysis

Data for continuous variables were expressed as mean ± SD. Raw cortisol values were log transformed to address nonnormality and skewness.[43] Any out-

liers were handled by Winsorizing the data point.[54] The Shapiro-Wilk and Levene tests were performed to assess normality and homoscedasticity.[68] A 2-way mixed model with absolute agreement was used to calculate the intraclass correlation coefficient ($ICC_{3,1}$) for the interday reliability (days 1 and 2) of morning and afternoon salivary cortisol and testosterone values. An ICC of 0.20 or less was defined as poor, an ICC between 0.21 and 0.40 was defined as fair, an ICC between 0.41 and 0.60 was defined as satisfactory, an ICC between 0.61 and 0.80 was defined as good, and an ICC of 0.81 or greater was defined as an excellent agreement between both evaluations.[88] A repeated-measures analysis of variance was performed to test the effect of the between-group factor (sham or SM) and within-group factor (time) on the dependent variables (salivary cortisol, salivary testosterone, T/C ratio, HRV, and O_2Hb).[68] The hypothesis of interest was the group-by-time interaction. A post hoc pairwise comparison between groups at different time points was also carried out. A Bonferroni correction was used to adjust for multiple post hoc comparisons.[1] Eta-square was used to measure effect sizes. Management and analysis of data were performed using the statistical package SPSS for Windows Version 22.0 (IBM Corporation, Armonk, NY). The level of statistical significance was set at $P<.05$.

RESULTS

THE FLOW OF PARTICIPANTS IS SHOWN in **FIGURE 3**. Data collection was performed from March to June 2015. Data collection was stopped after the 24th participant, as no loss to follow-up was anticipated given the one-off nature of the intervention.

There were no missing data. Participants did not vary in baseline characteristics, except for height (**TABLE 1**). No differences were found in the number of hours slept during the 2 days of data collection or in alcohol or medication usage between groups.

The ICC calculated between days 1 and 2 for salivary cortisol samples indicated strong agreement (0.71) between the morning measures and a satisfactory agreement (0.47) between the afternoon measures. The ICC calculated for salivary testosterone indicated a fair agreement (0.4) between the morning measures and a strong agreement in the afternoon samples (0.78).

T/C Ratio

There was a statistically significant interaction of group by time for the T/C ratio ($P<.05$), with an effect size of 0.11. Between-group analysis (**TABLE 2**) showed a statistically significant difference between the sham and SM groups at only 1 postintervention time point: night (mean difference, −0.09; 95% confidence interval: −0.16, −0.04; $P = .02$). **FIGURE 4** shows group changes in T/C ratio.

Salivary Cortisol

There was a statistically significant interaction of group by time for salivary cortisol concentration ($P<.001$), with an effect size of 0.28. Pairwise comparison between groups (**TABLE 2**) at each postintervention time point revealed a statistically significant difference between the sham group and SM group at 5 minutes postintervention (mean difference, 0.35; 95% confidence interval: 0.12, 0.6). However, no statistically significant interaction of group by time was found
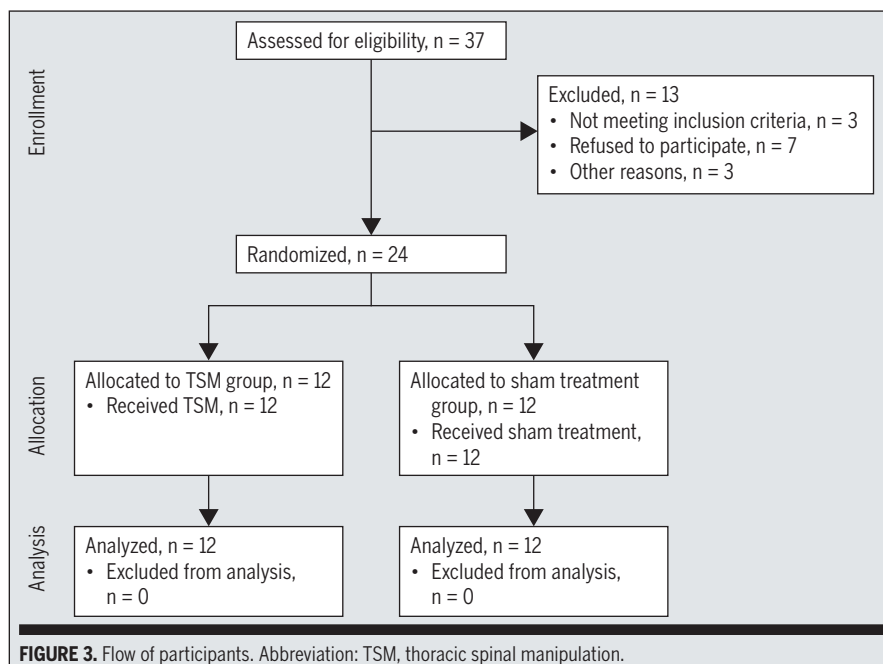
for salivary cortisol at 30 minutes or 6 hours (night) postintervention. Within-group comparison showed that salivary cortisol levels were lower (significant) postintervention (5 and 30 minutes) compared to preintervention in the SM group ($P<.05$).

Salivary Testosterone

No statistically significant interaction of group by time was found for salivary testosterone levels ($P = .33$), with an effect size of 0.05. No statistically significant difference was found between or within

groups at any of the postintervention time points (**TABLE 2**).

Heart Rate Variability

No statistically significant interaction of group by time was found for HRV ($P = .75$), with an effect size of 0.13. No statistically significant difference (between or within groups) was found for HRV at any of the postintervention time points (**TABLE 3**).

Oxyhemoglobin

No statistically significant interaction of group by time was found for O_2Hb ($P = .32$),

FIGURE 3. Flow of participants. Abbreviation: TSM, thoracic spinal manipulation.

TABLE 1	BASELINE CHARACTERISTICS OF PARTICIPANTS*

Characteristic	Sham (n = 12)	Spinal Manipulation (n = 12)
Age, y	27.33 ± 6.8	28.33 ± 5.8
Weight, kg	78.44 ± 7.73	74.79 ± 15.2
Height, m	1.79 ± 0.06	1.72 ± 0.07
PSS-10	15.0 ± 6.20	13.92 ± 4.2
T/C ratio	0.36 ± 0.03	0.29 ± 0.03
Cortisol	−0.987 ± 0.25	−0.783 ± 0.24
Testosterone	149.0 ± 68.6	115.0 ± 45.4
HRV	1.32 ± 0.33	1.25 ± 0.29

Abbreviations: HRV, heart rate variability; PSS, Perceived Stress Scale; T/C, testosterone-cortisol.
Values are mean ± SD.

TABLE 2	Hormonal Changes After Intervention*					
Time Point/Group	**Cortisol†**	**P Value‡**	**Testosterone§**	**P Value‡**	**T/C Ratio‖**	**P Value‡**
B						
SM (n = 12)	−0.78 ± 0.24		115 ± 45.4		0.29 ± 0.036	
Sham (n = 12)	−0.99 ± 0.25		149 ± 68.6		0.36 ± 0.036	
P1						
SM (n = 12)	−0.99 ± 0.27		151 ± 58.7		0.34 ± 0.032	
Sham (n = 12)	−0.64 ± 0.29		141 ± 67.6		0.30 ± 0.032	
Difference¶	−0.35 (−0.59, −12)	.005	10.08 (−43.5, 63.8)	.70	0.04 (−0.05, 0.13)	.38
P2						
SM (n = 12)	−0.93 ± 0.29		157 ± 67.4		0.35 ± 0.033	
Sham (n = 12)	−0.73 ± 0.32		164 ± 59.3		0.35 ± 0.033	
Difference¶	−0.20 (−0.46, 0.05)	.11	−6.29 (−60, 47.5)	.81	0.00 (−0.09, 0.09)	1.0
P3						
SM (n = 12)	−1.07 ± 0.26		81 ± 25.6		0.30 ± 0.026	
Sham (n = 12)	−1.31 ± 0.42		94 ± 51.8		0.39 ± 0.026	
Difference¶	0.23 (−0.06, 0.53)	.11	−12.87 (−47.5, 21.8)	.45	−0.09 (−0.16, −0.04)	.02

Abbreviations: B, preintervention samples; P1, postintervention samples (5 minutes); P2, postintervention samples (30 minutes); P3, postintervention samples (6 hours); SM, spinal manipulation; T/C, testosterone-cortisol.
Values are mean ± SD unless otherwise indicated.
†*Effect size, 0.28 (P<.01). Log-transformed values.*
‡*Group-by-time interaction.*
§*Effect size, 0.05 (P = .33).*
‖*Effect size, 0.11 (P<.05).*
¶*Between-group difference (SM − sham). Values are mean difference (95% confidence interval).*

TABLE 3	Changes in O₂Hb and HRV After Intervention*			
Time Point/Group	**O₂Hb†**	**P Value‡**	**HRV§**	**P Value‡**
A1/B‖				
SM (n = 12)	−12.7 ± 25.6		1.25 ± 0.33	
Sham (n = 12)	−2.54 ± 19.5		1.32 ± 0.29	
Difference¶	−10.2 (29.5, 9.0)	.28		
P1				
SM (n = 12)	4.9 ± 20.8		1.23 ± 0.52	
Sham (n = 12)	4.1 ± 13.2		1.34 ± 0.31	
Difference¶	0.89 (13.9, 15.6)	.9	−0.11 (−0.47, 0.25)	.54
P2				
SM (n = 12)	46.8 ± 59.0		1.35 ± 0.36	
Sham (n = 12)	31.6 ± 48.4		1.34 ± 0.30	
Difference¶	15.2 (−30.5, 60.9)	.49	0.01 (−0.26, 0.28)	.95

Abbreviations: A1, postintervention samples (1 minute); B, preintervention samples; HRV, heart rate variability; O₂Hb, oxyhemoglobin; P1, postintervention samples (5 minutes); P2, postintervention samples (30 minutes); P3, postintervention samples (6 hours); SM, spinal manipulation.
Values are mean ± SD unless otherwise indicated.
†*Effect size, 0.05 (P= .32).*
‡*Group-by-time interaction.*
§*Effect size, 0.13 (P=.75).*
‖*Time point for O₂Hb was A1 and for HRV was B.*
¶*Between-group difference (SM − sham). Values are mean difference (95% confidence interval).*

with an effect size of 0.05. No statistically significant difference (between groups) was found in O₂Hb concentration at any postintervention time point (**TABLE 3**). However, in the SM group, a statistically significant increase in O₂Hb concentration was found at 30 minutes postintervention ($P<.05$) compared to O₂Hb values at baseline, 1 minute, and 5 minutes postintervention.

DISCUSSION

WE HYPOTHESIZED THAT A THO-racic SM would result in a neuroendocrine response (change in HP axis activity), as observed by the T/C ratio, salivary cortisol, and O₂Hb concentration. We found a statistically significant interaction of group by time in the T/C ratio approximately 6 hours postintervention and in salivary cortisol levels at 5 minutes postintervention. Though statistical significance between groups was not achieved for O₂Hb or HRV, de-

creases in O_2Hb levels suggest an immediate vasoconstriction in the SM group.

T/C Ratio

Our study found that the T/C ratio was significantly lower in the SM group compared to the sham group at 6 hours postintervention. This may be due to changes in cortisol levels, as testosterone levels were similar in both groups at 6 hours following intervention. When compared with the sham group (mean difference, −0.58), a lesser drop in salivary cortisol levels was evidenced from 30 minutes postintervention to 6 hours postintervention in the SM group (mean difference, −0.14). This may indicate activation of the HPA axis following SM.

Cortisol has widespread effects on glucose, fat, and protein metabolism and can stimulate gluconeogenesis, which can be used as a building block of tissue repair,[25] thereby playing an important role in tissue healing. It is important to note that tissue healing is a complex process and may be influenced by other hormones produced by the pituitary, such as growth hormone and thyroid hormone.[64] However, to measure these was beyond the scope of this study. On the other hand, an elevated cortisol level has been associated with dysregulated insulin, impaired glucose metabolism, abdominal obesity, osteoporosis, hyperlipidemia, and hypertension.[7,53,91] Therefore, our findings should be interpreted with caution and need to be verified by future studies using a series of interventions replicating clinical practice.

The T/C ratio has been widely used in sports and exercise science research as an indicator of overtraining and recovery. To our knowledge, this is the first study to investigate the effects of thoracic SM on the T/C ratio. Previously, studies have used only cortisol as an indicator of HPA axis activity.[13,65,67,90] However, it is now clear that the HPA axis can be influenced by other endocrine mechanisms (the HPG axis, specifically). Hence, it has been advocated that the balance between these 2 hormones may provide a better estimation of HPA axis activity.[3,19] The ex-
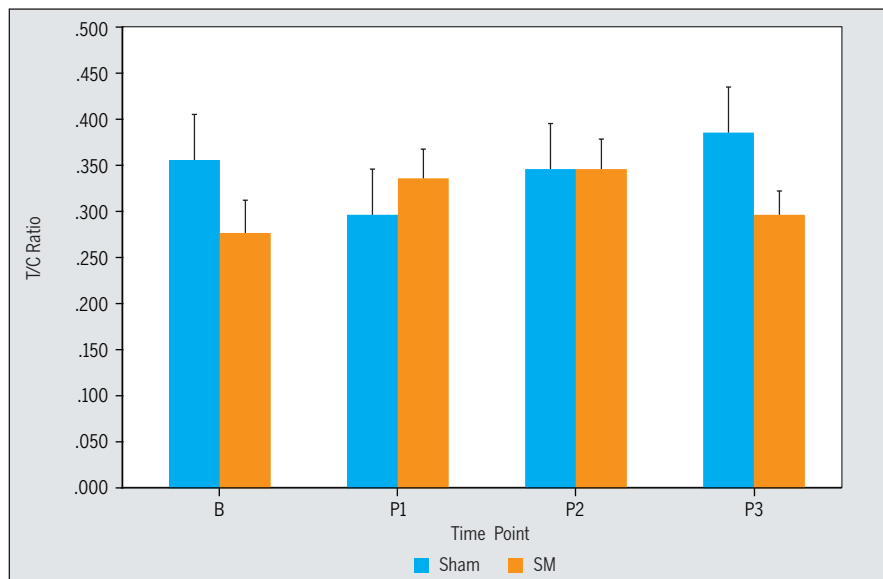
FIGURE 4. Changes in T/C ratio between groups following intervention. Abbreviations: A1, postintervention samples (1 minute); B, preintervention samples; P1, postintervention samples (5 minutes); P2, postintervention samples (30 minutes); P3, postintervention samples (6 hours); SM, spinal manipulation; T/C, testosterone-cortisol.

act clinical utility of changes in the T/C ratio noted in our study is unclear. Nevertheless, our findings may provide a platform for future studies to explore further in this area of manual therapy research.

Salivary Cortisol and Testosterone

There was a significant decrease in salivary cortisol levels at 5 minutes postintervention in the SM group compared to the sham group. Cortisol is regulated by the HPA axis through feedforward and feedback loops.[11,92] The glucocorticoid-mediated feedback loop operates in at least 3 time domains: fast (within seconds to minutes), intermediate (2 to 10 hours), and slow (over hours to days).[44] Animal models further demonstrate that an innocuous stimulus (such as SM) applied to the spine can have inhibitory effects on adrenal gland activity.[10] Therefore, it could be argued that an immediate (5 minutes) drop in cortisol level occurred due to reflexive inhibition in adrenal activity via SM. This is in contrast with findings from previous studies[13,90] that have reported no change in cortisol levels immediately following an SM. The intervention used in the present study was a thoracic SM, whereas Whelan et al[90]

used an upper cervical SM. It is unclear whether Christian et al[13] used a cervical or a thoracic SM, or both. Our data collection occurred in the afternoon, when cortisol levels are shown to be more stable, whereas the salivary/plasma cortisol samples were collected during late morning in the other studies,[13,90] which may partly explain the difference in findings.

The drop in cortisol at 5 minutes postintervention may then trigger feedback loop mechanisms, resulting in an increase in cortisol concentration observed 6 hours following SM. However, it is to be noted that many factors, including the circadian rhythm, ultradian rhythms, and stress levels (to name a few), may influence blood corticosterone concentrations.[59] The PSS-10 scores were similar in both groups. Hence, the difference in cortisol levels may not be due to differences in baseline perceived stress levels. Most participants in our study received SM for the first time. It could be argued that the participants perceived the act of SM as stressful. If this was true, then an increase in cortisol levels should have been noticed immediately rather than many hours following SM. However, we found no significant changes in salivary cortisol levels between the

2 groups at 30 minutes postintervention. This is in agreement with previous findings.[67] Therefore, our finding strengthens the fact that the act of SM itself may not induce a state of stress, as noted previously.[85] Alternatively, it could be argued that SM had an influence on the opioid system, thereby influencing the HPA axis.[6] However, this can only be speculated, as we did not measure the opioid system as part of this study.

The baseline salivary cortisol and testosterone levels reported in our study are consistent with those in the literature.[38] Both cortisol and testosterone exhibit circadian rhythmicity, with peak concentrations in the morning and reduced concentrations in the evening and overnight.[84] As mentioned previously, the circadian rhythmicity of hormones can be affected by a number of confounding factors.[15,81] It is unclear how these confounding factors were addressed in previous studies,[13,65,90] which may also partly explain their conflicting results. Participants in the present study were required to follow a strict protocol before saliva collection (**APPENDIX**), and data collection was conducted on Wednesday and Thursday, as opposed to the weekend, which has been shown to influence stress and hence cortisol levels.[46] Therefore, calculating the interday reliability (ICC) for both of the hormones assessed was a crucial aspect of the present study's design. The ICCs calculated for morning and afternoon cortisol and testosterone concentrations in this study are consistent with those reported in the literature.[39,90] Establishing the ICC enabled reliable analysis of preintervention and postintervention cortisol and testosterone levels on day 2.

Oxyhemoglobin

Findings from this study indicate that, within the SM group, O_2Hb reduced immediately (1 minute) after thoracic SM and steadily rose, with changes at 30 minutes being statistically different from baseline, 1-minute, and 5-minute values. It is to be noted that between-group changes did not reach statistical signifi-

cance. Previously, studies investigating the effects of SM have routinely used skin blood flow as a measure of SNS activity. However, as skin blood flow is mediated not only by the SNS but by other factors,[20] interpretation of changes has recently been questioned.[93] A systematic review by Zegarra-Parodi et al[93] found that the included studies were heterogeneous in nature, but the extent to which changes in skin blood flow reflected SNS changes in deeper tissues remains unclear.[93] We used NIRS to measure O_2Hb changes in calf muscle as an indicator of SNS activity, and NIRS has been shown to reliably measure changes in skeletal muscle blood flow when compared to established methods, such as Doppler ultrasound[26] and functional magnetic resonance imaging.[21]

Our study did not find significant between-group changes in O_2Hb of calf muscle. Research has shown that SM elicits extrasegmental effects, and changes in SNS activity have been suggested as one possible explanation for these effects. By measuring blood flow in calf muscle, which has no segmental connection to the thoracic spine, extrasegmental effects of thoracic SM via the SNS could be assessed. Absence of significant between-group changes in O_2Hb found in our study may reflect ongoing challenges with regard to measuring autonomic activity.[93] Further, the precise location and extent of vasoconstriction within skeletal muscle following SNS activation can be difficult to ascertain.[83] Alternatively, other reliable markers of sympathetic activity, such as salivary alpha-amylase,[69] may be considered for future studies exploring these changes following SM.

Heart Rate Variability

No significant difference in HRV (LF/HF ratio) between the sham and the SM groups was found. A thoracic SM could have a stimulatory action on the ANS (especially the SNS), considering the anatomical and physiological relationship of the thoracic spine and the SNS. Similar to previous findings,[77,87] we found no changes in HRV values following a thoracic SM.

However, the findings contrast those of Budgell and Polus,[9] who reported that a prone high-velocity, low-amplitude thrust of the thoracic spine may influence the autonomic output to the heart that was not duplicated by a sham procedure. It is important to note that the SM technique used in our study (supine high-velocity, low-amplitude thrust) is different from that used in the other study (prone high-velocity, low-amplitude thrust). Therefore, the results may not be comparable. There have been disagreements over the accuracy of the LF/HF ratio as a marker of cardiac autonomic activity.[5,58,74] For instance, some authors dispute the direct contribution of sympathetic activity to LF components of the HRV and attribute it to the modulating baroreflex activity.[33,62] The inaccuracy of the test may also be a reason why the findings are different between studies. However, ratios of spectral power (LF/HF ratio) continue to be used in research to provide better information about sympathovagal balance, especially in short-term recordings.[61]

The findings of the present study indicate that, following SM, there is a quick response from the SNS, followed by a longer-lasting slow response from the neuroendocrine system. This may provide justification for the use of SM in the early phases of injury, to enhance physiological processes either before the patient can start exercising or as an adjunct to exercise, regardless of the site of injury. Previous manual therapy studies have explored individual biomechanical[30] and neurophysiological effects,[86] with the potential interaction of these effects often being overlooked.[4] In chronic pain populations, however, dysregulation of both the ANS and the HPA axis has been reported.[75] Therefore, our findings may provide preliminary evidence for the combined effects (ie, neuroendocrine) of thoracic SM, the therapeutic benefits of which need further investigation in symptomatic populations.

Strengths and Limitations

One of the major strengths of the study is its design. We used a randomized, sham-controlled, repeated-measures

experimental design, collecting salivary samples at different time points, as suggested by a review.[2] We were able to successfully address various methodological factors that could confound hormonal measurements. The atmospheric chamber used during the experiment allowed us to control various parameters, such as the temperature and humidity, which are known to have an effect on endocrine as well as ANS functions. Further, measuring testosterone to enable calculation of the T/C ratio, an indicator of HPA activity, is also unique to our study. This may improve our understanding of the complex interactive hormonal response to SM. The use of NIRS to measure SNS activity is also novel to our study. Because of the one-off nature of the intervention used in this study, there were no missing data or attrition of participants.

The study is not without its limitations. The effect sizes for the T/C ratio (0.11) and cortisol (0.28) changes noted in our study could be considered "medium."[16,22] This may be because the response of the HP axis is known to be minimal in healthy individuals and has been shown to be amplified in painful populations.[60,63] Therefore, the magnitude of effects should be bigger in symptomatic populations and needs to be verified by future research. Women were not included in the current study because it is well known that the HPA axis and HPG axis response differs between men and women, especially during menstrual cycles and contraceptive usage.[19,47] Future research should either stratify participants by sex or adjust the hormonal data (women) for known confounders. We found changes in HP axis activity following SM and measured only 2 hormones.

An a priori power calculation to determine the sample size was undertaken to achieve an effect size of 0.25. This effect size could be considered "medium."[22] Further, a study[72] indicated that a minimum of 24 participants are required for studies involving HRV. Hence, a sample size of 24 was considered adequate for this mechanistic study. However, it is to be noted that the power calculations were not based on the minimum clinically important difference of the primary outcome measures, which are currently unknown. Therefore, the possibility of a type II error among the nonsignificant findings cannot be ruled out.

CONCLUSION

THORACIC SM HAS AN EFFECT ON HPA axis activity, as indicated by changes in salivary cortisol 5 minutes after SM and in the T/C ratio many hours following SM. A thoracic SM may also have immediate effects on the SNS, as indicated by O_2Hb levels. The clinical implications of these changes are, however, unclear. More research is therefore warranted in this area. ◉

■ KEY POINTS

FINDINGS: Thoracic spinal manipulation can reduce salivary cortisol levels soon after intervention and the testosterone-cortisol ratio many hours following intervention.

IMPLICATIONS: This study may provide preliminary evidence that thoracic spinal manipulation may influence tissue healing via modulation of the neuroendocrine system.

CAUTION: This study's findings are based on healthy male participants. Therefore, the nature of neuroendocrine response in females is still unknown. Future research in symptomatic populations is required to ascertain the clinical utility of the neuroendocrine response following spinal manipulation noted in this study.

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APPENDIX

SALIVARY PROTOCOL

The participants were asked to lean slightly forward, tilt their heads down, and slowly dribble saliva into a polypropylene cryovial (2-mL capacity). The participants were requested to use minimal orofacial movement and to let saliva dribble into the collection tube.[23] In line with previous recommendations,[24] the participants were asked to (*a*) refrain from brushing the teeth prior to each of the saliva collections; (*b*) rinse the mouth with water and swallow to remove food residue before sample collection; (*c*) systematically inspect the salivary samples at the point of collection and, if visibly contaminated with blood, exclude the samples; (*d*) avoid food with high sugar or acidity for at least 2 hours before sampling; (*e*) avoid vigorous exercise for at least 2 hours, preferably longer, before sampling; (*f*) avoid alcohol consumption and caffeine (if possible) for 24 hours prior to all experimental and baseline measures; (*g*) carefully register the time of sampling and obtain samples at the same time of day; (*h*) immediately store the salivary samples in the refrigerator; and (*i*) record and report any medication usage during data collection.

Free-standing polypropylene vials (capacity of 2 mL) were used for collecting salivary samples. Small polypropylene labels with information regarding participant ID and sample time and day ensured sample identification during saliva collection and analysis. A cryostorage box designed to promote better sample organization and storage was used to store the salivary vials.

Salivary Biomarker Analysis

In brief, 25 μL of standards, controls, and saliva samples were added in duplicates into the appropriate wells. Testosterone (1:1000 dilution) or cortisol (1:1600 dilution) enzyme conjugate was diluted with the assay diluent, and 150 μL of the diluted conjugate were added into each well and mixed on a plate rotator (250 rpm for 5 minutes), followed by incubation at room temperature for 1 hour. Following this incubation, the plate was washed 4 times with 100 μL of 1X washing buffer at room temperature. Next, 200 μL of chromogenic substrate tetramethylbenzidine were added for horseradish peroxidase enzyme detection of testosterone and cortisol antibodies. After incubating the plate for 25 minutes at room temperature in the dark, 50 μL of stop solution were added to each well using a multichannel pipette, and mixed on a plate rotator for 3 minutes at 250 rpm until all wells turned to a yellow color. The plate was read within 10 minutes after the addition of the stop solution in a plate reader at 450 nm. Average optical density (OD) values of all the duplicate wells were determined and subtracted from the OD values from nonspecific binding wells. Percent bound for each of the standard and test samples was calculated by dividing the OD of each well by the average OD of a blank. Finally, the concentration of testosterone and cortisol was calculated using the 4-parameter nonlinear regression curve (Prism software; GraphPad Software, Inc, La Jolla, CA).