

The immediate effects of cervical spine manipulation on pain and biochemical markers in females with acute non-specific mechanical neck pain: a randomized clinical trial

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ABSTRACT

Study Design: Randomized clinical trial with pre-test, post-test control group design.

Objectives: To examine the immediate effects of cervical spinal manipulation (CSM) on serum concentration of biochemical markers (oxytocin, neurotensin, orexin A, and cortisol).

Background: Several studies have found an association between spinal manipulation (SM) and pain perception. However, the mechanism by which SM modulates pain remains undefined.

Methods: Twenty-eight female subjects with non-specific mechanical neck pain were randomly assigned to one of two interventions (CSM versus sham CSM). Blood samples were drawn before and immediately after the respective interventions. Oxytocin, neurotensin, orexin A, and cortisol were measured from the blood and serum using the Milliplex Map Magnetic Bead Panel Immunoassay on the Luminex 200 Platform.

Results: In the CSM group, there were significant increases in pre- versus post-manipulation mean oxytocin (154.5 ± 60.1 vs. 185.1 ± 75.6 , $p = .012$); neurotensin (116.0 ± 26.5 vs. 136.4 ± 34.1 , $p < .001$); orexin A (52.2 ± 31.1 vs. 73.8 ± 38.8 , $p < .01$) serum concentration; but no significant differences in mean cortisol ($p = .052$) serum concentration. In the sham group, there were no significant differences in any of the biomarkers ($p > .05$).

Conclusion: The results of the current study suggest that the mechanical stimuli provided through a CSM may modify neuropeptide expression by immediately increasing the serum concentration of nociception-related biomarkers (oxytocin, neurotensin, orexin A, but not cortisol) in the blood of female subjects with non-specific mechanical neck pain.

KEYWORDS

Spinal manipulation; oxytocin; neurotensin; orexin A; cortisol; neck pain

Introduction

Neck pain is a common phenomenon and may produce varying degrees of disability. Spinal manipulation (SM) is a common technique for treatment of neck pain and has demonstrated various mechanical, neurophysiologic, and analgesic effects [1]. Females have a greater propensity to develop neck pain as opposed to males [2,3].

In the United States (US), opioid prescriptions to manage spinal pain have increased considerably since the 1990s and are now the most commonly prescribed drug class [4]. Complications from opioids range in severity from constipation, sexual dysfunction, and depression to addiction and overdose-related mortality [4–6]. In 2016, 116 individuals died every day from an opioid-related overdose [7]. Prescription opioid-related overdose deaths were five times higher in 2016 as compared to 1999 [8]. In 2017, the US Department of Health and Human Services declared an opioid-related public health emergency and announced a 5-Point Strategy to

Combat the Opioid Crisis [7]. Two of the strategies included expanding research focused on pain management and advancing better practices for pain management [7]. Due to the adverse side effects associated with prescription opioid usage, drug-free pain management strategies such as SM should be more readily considered for individuals with spinal pain [9].

SM is used by physical therapists and other health-care practitioners as an intervention to help relieve spinal pain and reduce disability [2,3]. SM has been shown to be an effective intervention for patients with non-specific mechanical neck pain (NS-MNP) [10], either alone or in combination with exercises as part of a multimodal management strategy [9,11,12].

The mechanism by which SM modulates pain remains poorly defined; however, there is evidence to suggest that analgesia may occur after SM [13,14]. There are a variety of observed and proposed phenomena that may explain the mechanisms for the psychological, mechanical, or neurophysiological responses from a SM associated with alterations in pain

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processing or sympathetic and motor systems' excitation [15,16]. Of these three mechanisms, the neurophysiological mechanism which triggers a cascade of changes in the peripheral and autonomic nervous systems and endocrine system, is the most widely accepted [9]. However, a single session of spinal mobilization or manipulation produces significant yet short-lived neurophysiological effects, typically lasting 5 min or less [17,18]. More recently, there is moderate emerging evidence supporting a modulation of biochemical mechanisms as well, suggesting that a mechanical stimuli provided by SM can trigger changes in analgesic and anti-inflammatory serum markers in asymptomatic individuals [9,19].

A recent study suggested that the mechanical stimulus produced during cervical manipulation seems to increase the serum concentration of neurotensin, oxytocin, and cortisol in asymptomatic subjects [19]. Oxytocin, neurotensin, and orexin A have antinociceptive effects while it has been well established that the endocrine-related steroid hormone cortisol serves a role in analgesia as it relates to stress response as well as immune parameters associated with the inflammatory process [9,19–23].

In a recent systematic review and meta-analysis, Kovanur-Sampath K, et al. investigated changes in biochemical markers following SM [9]. The authors defined biochemical markers as any chemical substance that modulates pain or inflammation and were classified into three broad biomarker categories: (1) neuropeptides, (2) inflammatory, and (3) endocrine. Out of the 1217 citations screened, only eight (8) met the inclusion criteria and were included in the final review. Of the eight studies, seven (7) were conducted on pain free, healthy volunteers. The single study, conducted 20 years ago, in subjects with pain was a four group randomized clinical trial with 40 male subjects total with 10 subjects in each group (i.e. pain-free SM, pain SM, pain-free sham, and pain sham) [24]. Their outcome measures were cortisol, adrenocorticotropic hormone (ACTH), and β -endorphin (an agonist of opioid receptors) and no changes in any outcomes were noted.

In light of the well-documented side effects of prescription opioids, drugless therapies such as SM may provide a viable treatment option for individuals in pain [9,25]. It is our understanding that to date, this is the first study to examine the immediate effects of cervical SM on biochemical markers in symptomatic females, the gender with the highest prevalence of cervical spine pain. In addition, this is the first study to investigate the effects on SM on the neuropeptides oxytocin, neurotensin, and orexin A in individuals with cervical pain.

Taking the above-mentioned findings into account, our purpose was to investigate whether cervical spinal manipulation (CSM) would result in

a consistent biochemical response or change neuropeptide and cortisol serum concentrations. Therefore, the purpose of this study was to evaluate the immediate effect of CSM on modulation of biochemical markers associated with pain perception and stress response (neurotensin, oxytocin, orexin A, and cortisol). We hypothesized that the mechanical stimuli initiated from a CSM may modulate key analgesic neuropeptide biomarkers in symptomatic female subjects.

Methods

Participants

A total of twenty-eight (28) females between the ages of 20 and 45 years with a primary complaint of NS-MNP were recruited for this study through flyers and word-of-mouth. Thirteen (13) subjects were randomly assigned to the experimental group (CSM) and fifteen (15) subjects to the sham group (sham manipulation). Inclusion criteria included: resting neck pain [as measured by the Numeric Pain Rating Scale (NPRS)] for ≤ 30 days that is aggravated by movement but does not extend distal to the shoulders and a score $\geq 10/50$ on the Neck Disability Index (NDI) [26–28]. Exclusion criteria were: serious medical conditions (e.g. cancer, spondylolisthesis, rheumatoid arthritis, ankylosing spondylitis, or other related autoimmune disease), cervical myelopathy signs (e.g. incoordination in hands, arms and legs, inability of walking at a brisk pace, bowel and bladder incontinence, etc.), nerve root compression (e.g. changes in sensation, muscle weakness, or decreased reflexes), working the night shift, steroid medication within three months, pregnancy or postpartum, pending legal action regarding their neck pain, history of whiplash associated disorder, neck pain from a traumatic event, and/or cervical spine surgery. All methods and procedures were approved by the Institutional Review Board (IRB) of Loma Linda University prior to commencing the study. This study was registered as a clinical trial at ClinicalTrials.gov Protocol Registration and Results System (#NCT 03176654).

Examination procedures

Since the majority of individuals with cervical spine pain lack an underlying pathology or abnormal anatomical condition, they are frequently classified as NS-MNP [29]. For this study, we have operationally defined NS-MNP as neck pain without traumatic onset with resting pain that is provoked with certain cervical movements and/or postures (aggravating factors) but relieved by moving out of the provocative position and/or postures (easing or ameliorating maneuver). In order to classify subjects with NS-MNP, each

subject received a thorough examination. Subjects provided demographic information, and completed self-reported measures to assess disability, pain, and stress levels at baseline. Self-reported measures included the NDI, NPRS [22,30,31], and Perceived Stress Scale (PSS) [28].

The Neck Disability Index: The NDI is the most widely used condition-specific disability scale for patients with neck pain. It consists of 10 items, 8 items addressing various aspects of function and 2 items addressing impairments (i.e. pain intensity, headaches), each scored from 0 to 5, with a maximum possible score of 50 points. A score of 10/50 is considered a mild disability. The NDI has been reported to be a reliable and valid outcome measure for patients with neck pain [28].

Numeric Pain Rating Scale: The subject's current resting pain level was established using the NPRS. The NPRS is an 11-point numeric pain intensity rating scale, ranging from 0 ('no pain') to 10 ('worst imaginable pain') [28]. The NPRS has been shown to be accurate as a screening test to identify primary care patients with clinically important pain. The NPRS exhibits fair to moderate test-retest reliability and has also showed adequate responsiveness in patients with mechanical neck pain. Also, the NPRS has adequate evidence of construct validity including sensitivity for both pain intensity and unpleasantness [22,30].

Perceived Stress Scale: The PSS is standardized widely used psychometric instrument for assessing the perception of stress. It is a measure of the degree to which situations in one's life are appraised as stressful. The PSS has established internal consistency (Cronbach's $\alpha = 0.87$) and good test-retest reliability (ICC = 0.86, $p < .001$) [28].

Next, the history and subjective examination was conducted. The examination was concluded with a thorough physical examination, when possible, using validated assessment tools [23] and diagnostic reasoning to classify the subject as having NS-MNP and to establish that the subject was appropriate to receive a CSM as well as to screen for potential red flags.

Outcomes measures

The primary outcome measures of this study were peripheral blood serum levels of oxytocin, neurotensin, orexin A, and cortisol pre- and post-CSM interventions.

Blood sample collection protocol

Blood sample collection

Subjects arrived to their blood sample collection nil per os for 10 h which took place between 8 AM and 11 AM. After the subject was assessed for heart rate, blood pressure and was determined appropriate to receive

SM, they were positioned supine on a high low treatment table with a single pillow under their head. Blood oxygenation was measured in the bilateral upper extremities with a pulse oximeter. Venipuncture was performed following standard protocol in the antecubital region of the upper extremity with the highest blood oxygenation using a BD Vacutainer® Safety-Lok™ Blood Collection 23-gauge butterfly needle and collected in serum separator tubes (BD Vacutainer®) by a phlebotomist with 9 years of experience. After needle placement, 6–8 mL of blood was collected in a Serum Separator tube. After the specimen was drawn, the blood collection device was stabilized while the SM or sham SM intervention took place. Care was taken to ensure that the subject was immobile with the exception of the cervical spine and head during the SM or sham SM. Standard blood specimen collection protocol was followed and specimens were promptly mixed after collection. The average time between collection of the pre-and post-blood specimens was 60 s and the average post-intervention collection started 20 s after the SM or sham SM since oxytocin plasma concentration has a half-life of approximately 1.5 min [32]. Prior to collecting post-intervention blood samples (one Serum Separator tube with 6–8 mL of blood) approximately 5 mL of blood were drawn and wasted to clear the blood collection device of pre-intervention blood product.

Blood sample pre-analytics

The Vacutainer tubes stood upright at ambient room temperature (22°C) for one hour before being centrifuged at 1000g for 10 min at 4°C (Allegra® X-15R, Beckman Coulter, USA). The serum was then aliquoted into multiple snap-cap micro Eppendorf freezer vials and stored at –80°C until analyzed.

Reagents and instrumentation

Study samples were analyzed for human oxytocin, neurotensin, orexin A, and cortisol using the Milliplex Map Magnetic Bead Panel Immunoassay on the Luminex 200 platform (MilliporeSigma, Burlington, Massachusetts 01803). The Human Neuropeptide Magnetic Bead set (cat. #HNP MAG-35K-03 with kit lot #2782901) and Human Circadian/Stress Magnetic Bead set (cat. # HNC SMAG-35K-01 with kit lot #2782855) were purchased from EMD Millipore (Burlington, Massachusetts). Samples were assayed by the clinically certified University of Minnesota Clinical Cytokine Reference Laboratory, Minneapolis, MN (CRL).

Randomization

Following subject assessment and acceptance into the study, subjects were randomly assigned; using

computer-generated randomized sequencing, into either the experimental (CSM) or sham CSM group. Subjects were blinded to the intervention group, however, based on the nature of the intervention; it was not possible to blind the treating therapist.

Treatment procedure

Subjects in the experimental group were treated for one session of cervical spine manipulation.

Cervical spine manipulation protocol and procedure

A CSM was applied to the site of pain and/or restriction with the patient in supine. This procedure was performed in both the right and left direction, first away from pain then toward pain. This CSM technique uses both primary levers (pre-manipulation rotation away, 30°–45°, from the side of pain or limitation) and secondary levers (side bending toward the side of pain coupled with lateral shift away, and then a posterior-anterior shift) (Figure 1). This is a bimanual technique. For the applicator hand, the anterolateral portion of the first or second phalanx of the second ray was positioned on the superior joint partner of the target vertebrae using a cradle hold. The other hand was placed on the posterolateral aspect of the occiput (above the ear). While maintaining these positions, the clinician performed the high velocity, low amplitude thrust with the arc of rotation dependent on the level of the target vertebrae. Dunning et al. have described the technique used in this study [33].

Sham protocol

Subjects in the sham group were instructed to lay on a plinth in the same position as the CSM group. The clinician conducted the same basic steps as the SM, localizing the appropriate vertebral landmarks but without moving the individual or carrying out the final thrust procedure. At the conclusion of the research session, all subjects in the sham group received CSM so as not to withhold an intervention that might be beneficial (without further blood sampling).

We did not experience a single adverse event from cervical spine manipulation in this study, suggesting that the subjects were screened adequately. Each subject experienced multiple cavitations during the thrust manipulation to both the left and the right. The procedures were performed by a physical therapy with 9 years of experience.

Sample size

The sample size was estimated using an effect size of 0.9 between the SM and sham group, a power of 0.8, and level of significance $\alpha = 0.05$. The sample size needed was estimated to be 30 (15 in each group).

Data analysis

Mean \pm SD was computed for quantitative variables and median (min, max) for ordinal variables. Normality of quantitative variables was assessed using Shapiro–Wilk test and box plots. Independent *t*-test was used for all continuous and independent variables in both groups at baseline. Mann–Whitney *U* test was used for



Figure 1. Cervical spinal manipulation.

ordinal variables in both groups at baseline. The dependent paired *t*-test was used to compare pre- and post-variables in both groups.

To examine the effect of intervention on biomarkers over time (pre- versus post), a 2 × 2 mixed factorial ANOVA (repeated measure) was conducted. Data were analyzed using SPSS Statistics Software version 24.0 (SPSS Inc, Chicago, IL, USA). All analyses were performed at an alpha level of .05.

Results

Out of the 30 participants screened, 28 subjects satisfied the eligibility criteria, agreed to participate, and were randomly assigned into the experimental group (*n* = 13) using a computer-generated random sequencing (Figure 2). Baseline characteristics of participants are shown in Table 1. None of the demographic variables were significant (as expected) for randomized design except for age (mean ± SD: 33.4 ± 7.2 years). This could be due to chance or small sample sizes. Normality assumption of quantitative variables was checked and it was satisfied.

The 2 × 2 mixed factorial ANOVA revealed no significant interaction of treatment by time. The between-group analysis revealed no statistical significance difference between the CSM Intervention & Sham. Therefore, the analysis was reduced to a paired *t*-test for each variable as it is shown in Table 2.

There was a significant increase in mean oxytocin (*p* = .012), neurotensin (*p* < 0.001), and orexin A (*p* = .005) over time for the intervention groups. However, there was no significant change in sham groups. Lastly, there was no significant difference in mean cortisol (*p* = .052) for the intervention group or for the sham group (*p* = .123) (Table 2, Figure 3).

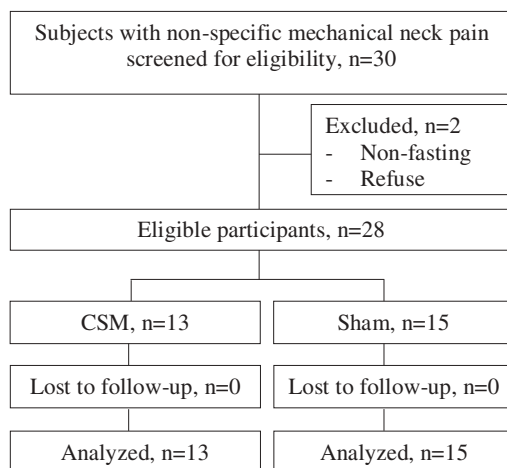


Figure 2. Flow chart of the methodological procedure.

Table 1. Selected characteristics of participants at baseline.

Characteristics	CSM Intervention (<i>n</i> ₁ = 13)	Sham (<i>n</i> ₂ = 15)	<i>p</i> -value ^a
Age	37.1 ± 7.3	30.1 ± 5.3	0.01*
SBP	115.3 ± 15.0	108.1 ± 9.8	0.14
DBP	82.8 ± 11.6	75.9 ± 6.6	0.06
HR	74.2 ± 7.8	76.7 ± 14.6	0.60
Oxytocin [§]	154.5 ± 60.1	158.8 ± 47.4	0.84
Neurotensin [§]	116.0 ± 26.5	125.6 ± 30.3	0.38
OrexinA [§]	52.2 ± 31.1	62.9 ± 28.5	0.35
Cortisol [‡]	9.58 ± 5.80	12.74 ± 5.86	0.16
NDI [^]	11(10, 12)	10 (10, 19)	0.79
NPRS [^]	4 (2, 5)	3 (1, 5)	0.17
PSS [^]	21 (17, 28)	22 (19, 30)	0.24

* *p* < 0.05.

SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; NDI: Neck Disability Index; NPRS: Numeric Pain Rating Scale; PSS: Perceived Stress Scale.

Values are presented as mean ± SD unless otherwise indicated.

^a Independent *t*-test.

[^]Median (min, max)

^bMann-Whitney *U* test

[§]Concentration of Oxytocin, Neurotensin, and Orexin A in serum samples (pg/mL).

[‡]Concentration of cortisol in serum samples (ug/dL).

Discussion

To our knowledge, this is the first study to evaluate the effectiveness of CSM on nociception-related biomarkers in females with NS-MNP. Our findings suggest that the mechanical stimuli from the CSM may modify neuropeptide expression in symptomatic subjects by statistically significant increase in the serum concentration of neurotensin, oxytocin, and orexin A in subjects with non-specific mechanical cervical spine pain.

Changes in neuropeptides

The results of the current study suggest that CSM may have an immediate effect in increasing three of the neuropeptides of interest in this study. Although the underlying mechanisms and functional role of SM remain undefined, our post-manipulation data allows us to postulate that the biomechanical force [34] created by SM may be capable of modulating these biomarkers [19].

Oxytocin

Oxytocin is produced within the hypothalamus and stored in Herring bodies of the posterior pituitary [35]. Oxytocin plays an endocrine role in modulating pain by blocking the activity of Aδ and C fibers without effecting non-nociceptive Aβ fibers [36,37]. The findings of this study show a significant increase in mean circulating serum oxytocin, a well-recognized biochemical analgesic, in the CSM group. Our findings were similar to the findings of Plaza-Manzano et al. [19] who reported a significant change in oxytocin following a SM study on asymptomatic individuals but not in the control group. Neuropeptides are released without delay or lag time since the

Table 2. Between/within group comparison of neuropeptide and cortisol levels.

Variable	CSM Intervention ($n_1 = 13$)			Sham ($n_2 = 15$)			
	Pre	Post	Mean difference (CI) ^a	Pre	Post	Mean difference (CI) ^a	Mean difference (CI) ^b
Oxytocin [§]	154.5 ± 60.1	185.1 ± 75.6	0.012*	158.8 ± 47.3	189.1 ± 46.4	0.053	0.940
Neurotensin [§]	116.0 ± 26.5	136.4 ± 34.1	30.6 (10.97, 72.63)	125.6 ± 30.3	131.6 ± 19.5	30.3 (-0.44, 61.05)	1.42 (-36.96, 39.80)
			$p < 0.001^*$			6.0 (-8.80, 20.81)	-2.43 (-22.41, 17.54)
Orexin A [§]	52.2 ± 31.1	73.8 ± 38.8	0.005*	62.9 ± 28.5	67.3 ± 30.1	0.526	0.851
Cortisol [‡]	9.57 ± 5.80	10.80 ± 7.18	21.60 (7.75, 35.46)	12.74 ± 5.86	13.97 ± 6.84	4.48 (-10.29, 19.25)	-2.12 (-25.19, 20.94)
			0.052			1.23 (-0.15, 2.48)	0.123
							-3.16 (-8.08, 1.75)

* $p < 0.05$.

Values are presented as mean ± SD.

[§]Concentration of Oxytocin, Neurotensin, and Orexin A in serum samples (pg/mL).

[‡]Concentration of cortisol in serum samples (ug/dL).

^a p -values for the null hypothesis that there is no difference between pre and post.

^b p -values for the null hypothesis that there is no difference between groups (CSM vs. Sham).

CI^a = 95% Confidence Interval for difference between pre and post.

CI^b = 95% Confidence Interval for difference between groups (CSM vs. Sham).

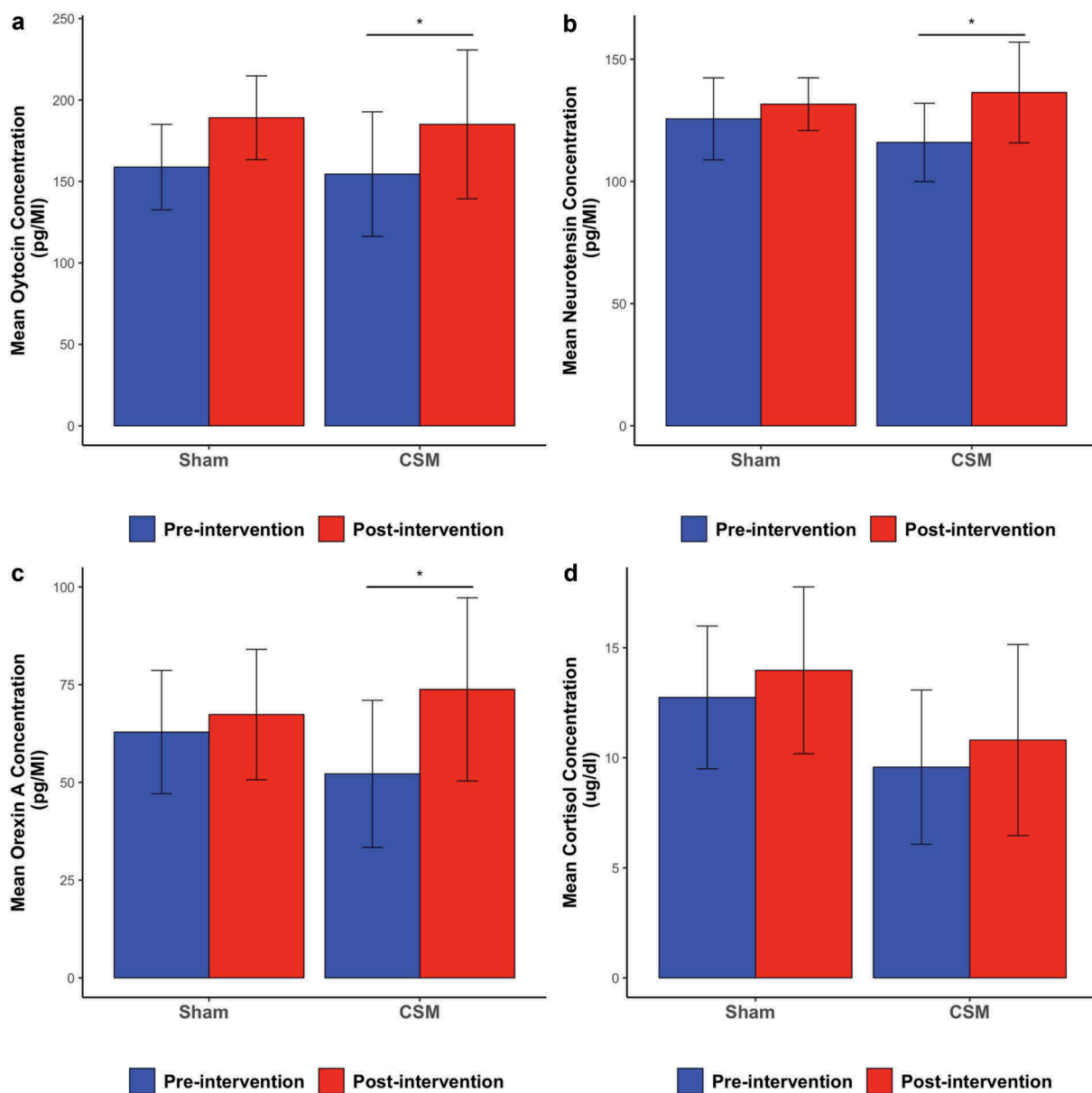


Figure 3. Mean and 95% confidence interval error bar for oxytocin, neurotensin, orexin A, cortisol concentration in blood samples.

neurotransmitter response needs to be immediate. Oxytocin plasma concentration has a half-life of approximately 1.5 min [32]. For this reason our second blood draw started within 20 s following the manipulation so that the third blood draw would be complete by 90 s post-manipulation. The second blood draw was drawn and wasted to clear any pre-intervention blood from the device.

Neurotensin

The findings of this study show a significant increase in mean circulating serum neurotensin, a well-recognized biochemical analgesic, in the SM group while no significant change was noted in the sham group. This tridecapeptide neuropeptide is extensively dispersed in the peripheral and central nervous systems [38]. Neurotensin has a wide range of actions, of which the one of greatest interest to this study being that of anti-nociception [39]. The analgesic actions of neurotensin is dissimilar to opioids making it a neurotransmitter of keen interest to pharma since it can be administered systematically or topically for pain management as an opioid alternative [39]. Neurotensin has analgesic impacts which are naloxone self-regulating and subsequently not reliant on opioids [16]. Neurotensin also affects the activity of the oxytocin-positive cells in the supraoptic nucleus of the hypothalamus [40]. Our findings are similar to those reported by Plaza-Manzano et al. in asymptomatic subjects [19]. To our knowledge, these are the only two studies that have investigated the activation of neurotensin with any form of manual therapy.

Orexin A

Of the neuropeptides assessed in this study, orexin A has been studied the least with respect to manual therapy. There is only one prior human study, Plaza-Manzano investigated the release of orexin A with SM in asymptomatic subjects [19]. There is, however, ample evidence that the central nervous system or intravenous administration of orexin A through injection suppresses rheumatoid arthritis-induced hyperalgesia, mechanical allodynia, and thermal hypersensitivity suggesting regulation of nociceptive processing in rats [41–43]. In another rat model, a significant change in orexin A was reported following electroacupuncture (EA) suggesting orexin A involvement in acupuncture analgesia [44]. These findings suggest that much like SM, EA can regulate descending inhibitory systems to achieve analgesic effects through changes in neuropeptide serum concentrations.

Our study has shown a statistically significant change in orexin A following mechanical stress provided by CSM in females with NS-MNP. No significant

difference was realized in the sham group. Even though orexin A has been shown to have hypoalgesic properties in rat studies, the first human study assessing the effect of SM on orexin A failed to show any significant difference while significant increases in the neuroxin and oxytocin neuropeptides in asymptomatic individuals were realized [19].

In addition to analgesic actions, in stress situations, orexin A activates glucocorticoid production [45]. In a rat model, injection of orexin elevated corticosterone levels at 15 min and these levels stayed elevated for a minimum of 60 min [46]. The increase in cortisol was dose dependent for orexin [46]. Note: Corticosterone is the primary glucocorticoid in rodents involved in stress responses and immune reactions, which is the equivalent to cortisol in humans. Our findings identified a significant increase in orexin A and no significant difference in cortisol immediately following CSM. Our findings are in line with animal study findings following stimuli [44] and with a recent systematic review reporting moderate evidence in favor of SM influencing neuropeptides and other biochemical markers [9]. Nevertheless, our findings differed from those reported by Plaza-Manzano [19] in regards to orexin A expression.

Cortisol

Our findings showed no significant differences in mean cortisol ($p = .052$) serum concentration. It has been well established that the endocrine-related steroid hormone cortisol serves a role in analgesia as it relates to the stress response [9,19–21]. In addition, cortisol is a potent anti-inflammatory and helps one cope with stress [47]. Cervical SM can influence inflammatory and immune biomarkers including cortisol [9,48,49]. Our findings differed from the findings of Plaza-Manzano et al. [19] who reported a significant change in cortisol following a SM study on asymptomatic individuals but not in the control group. One reason for this difference may be because our blood draw occurred immediately following manipulation rather than at delayed time intervals as described by Plaza-Manzano et al [19]. Oxytocin plasma concentration has a half-life of approximately 1.5 min while the half-life of cortisol is approximately 60–90 min [32]. The secretion of cortisol is not immediate and is controlled through one of the three inter-communicating regions of the body, the Hypothalamus-Pituitary-Adrenal (HPA) Axis. Our IRB limited the number of blood draws to three per subject for this study. Neuropeptides and cortisol behaviors are different. An immediate blood draw, necessary to capture neuropeptides, may have been too premature to fully capture changes in cortisol levels. The primary intent of this study was to assess the acute response of neuropeptides to SM.

In a human subject study examining the effect of SM in males on salivary cortisol, β -endorphin, and ACTH, they found no changes in any outcome measures 5 min and 30 min post-intervention [24]. This is in agreement with the Whelan et al. study that found no changes in basal cortisol levels in healthy males, as assessed through noninvasive salivary samples, at 5 and 60 min following 5-consecutive weeks of cervical SM. Whelan et al. concluded that neither SM nor the anticipation of the SM induces an adequate stress state to alter cortisol levels in asymptomatic subjects [50].

Our study design was similar to the Plaza-Manzano et al. [19] methodology in that both studies used venipuncture to collect serum cortisol levels. The use of venipuncture in itself can increase perceived stress levels and therefore cortisol [9]. Nevertheless, venipuncture was used in this study; however, other confounding variables such as circadian rhythm were controlled. The time of day can significantly affect cortisol levels. Therefore, for this reason, this study followed a strict time protocol for blood collection to reduce the effect of variance [51]. Individuals that work the night shift may have altered circadian rhythm and cortisol secretion. For these reasons, individuals working the night shift were excluded from the study [51]. All of our samples were collected in the early morning between 8 am and 10 am.

Stress, emotional or mechanical stimuli can alter cortisol levels. The baseline PSS scores were determined for both groups. At baselines, both groups' perceived stress levels were similar. The CSM group score was 21/40 while the sham group was just slightly higher at 22/40. Despite stress level homeostasis at baseline, the sham group had a higher, although not significant, baseline cortisol level. Chronic or intense pain can serve as a stressor that may intensify cortisol secretion [47]. The subjects in our study had relatively low pain intensity levels and the inclusion criteria included subjects with cervical pain ≤ 30 days. Pain magnification may be manifested as disability when pain is catastrophized due to a fear and avoidance behavior from a pain-provoking stimuli resulting in elevated cortisol levels [47]. Both the CSM and sham groups in our study had relatively low disability levels, 11/50 and 10/50, respectively. Lastly, the baseline serum cortisol were 9.6 and 12.7 $\mu\text{g/dL}$ for the CSM and sham groups, respectively, which are in the low end of the normal range for morning serum cortisol levels (10–20 $\mu\text{g/dL}$).

Limitations

There were numerous limitations in this study. The small sample size was the main limitation. In addition, although there was a sham group, there was a lack of

a control group with no touch contact. The vertebral pressure applied during the set-up for the thrust (pre-load) in the sham group could affect paraspinal proprioceptive mechanisms [52] and, as a result, may alter biomarkers' concentrations. Another limitation was that there was no minimal pain level established for subjects to be included in the study. To be included in the study, subjects needed to have cervical pain at rest that was aggravated by certain movements or postures, which may be a study limitation. Subjects in the sham group had resting pain as low as 1/10 and in the CSM group as low as 2/10. The mean resting pain level was relatively low for both groups. Even though the subjects matched the operational definition for NS-MNP, they might not match the typical patient seeking physical therapy services. Despite the relatively low NPRS, the median pain rating from Section 1 of the NDI (Pain Intensity) was 'The pain is moderate at this moment' for both groups.

This study focused only on the immediate effects of CSM without a short- or long-term follow-up and this is a potential limitation. In addition, since this study was conducted on females with NS-MNP, findings may not be generalizable to their male counterparts. Another limitation was the use of serum cortisol testing rather than salivary cortisol since venipuncture can increase perceived stress levels and potentially cortisol. However, this study was faced with the same dilemma as Plaza-Manzano group [19]: The three neuropeptides of interest in our study required venipuncture in order to obtain serum samples. Once the skin was broken to obtain baseline neuropeptide measurements, the post-CSM salivary cortisol testing was no longer viable. Also, the fact that the half-life of oxytocin is very short, as compared to cortisol, the final blood draw concluded within 90 s following the SM may have been too premature for cortisol to cross the HPA axis and enter into the blood stream. Additional blood draws would have been advantageous. Lastly, the diagnostic label of NS-MNP suggests that this entity is a heterogenic condition [29]; however, the findings of the current study may not be generalizable to individuals with known underlying anatomical structures or mechanisms (e.g., cervical spondylosis, herniated disc) responsible for patients' pain impairments.

Suggestions for future research

Further studies should consider recruiting a diverse age and gender population. Future studies to compare the concentrations of circulating serum biochemical markers following SM to other forms of manual therapy such as spinal mobilization may be warranted. The dosage needed to cause pain modulation has not been determined. Additional blood draws at longer time intervals are recommended for future studies.

Future research is needed to see if individuals in pain respond to mechanical stimuli differently than healthy controls within the same study. A recent article suggested that research studies using small samples of healthy subjects should give way to randomized controlled trials on subjects with pain [17]. The authors of this study concur and recommend a follow-up study with a larger sample size of symptomatic individuals.

Conclusion

The results of the current study suggest that the mechanical stimuli provided through a CSM may modify neuropeptide expression by immediately increasing the serum concentration of neurotensin, oxytocin, and orexin A in the blood of female subjects with NS-MNP. These findings could contribute to the body of knowledge with more information on the effects of CSM and its relationship to neurochemical changes related to stress and pain perception. Furthermore, this study may aid to validate past studies by providing the potential mechanism responsible for the positive physical therapy outcomes related to neck pain and CSM. The findings of this study, combined with the findings of others may support a biochemical-neurophysiological mechanism for pain modulation following CSM.

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