Kovanur Sampath, K., Treffel, L., P. Thomson, O., Draper-Rodi, J., Fleischmann, M. and Tumilty, S., 2023. Changes in biochemical markers following a spinal manipulation–a systematic review update. Journal of Manual & Manipulative Therapy, pp.1-23.

Abstract

Objective: The aim of this systematic review was to update the current level of evidence for spinal manipulation in influencing various biochemical markers in healthy and/or symptomatic population.

Methods: This is a systematic review update. Various databases were searched (inception till May 2023) and fifteen trials (737 participants) that met the inclusion criteria were included in the review. Two authors independently screened, extracted and assessed the risk of bias in included studies. Outcome measure data were synthesized using standard mean differences and meta-analysis for the primary outcome (biochemical markers). The Grading of Recommendations, Assessment, Development and Evaluation (GRADE) was used for assessing the quality of the body of evidence for each outcome of interest.

Results: There was low quality evidence that spinal manipulation influenced various biochemical markers (not pooled). There was low quality evidence of significant difference that spinal manipulation is better (SMD -0.42, 95% CI - 0.74 to -0.1) than control in eliciting changes in cortisol levels immediately after intervention. Low quality evidence further indicated (not pooled) that spinal manipulation can influence inflammatory markers such as interleukins levels post-intervention. There was also very low-quality evidence that spinal manipulation does not influence substance-P, neurotensin, oxytocin, orexin-A, testosterone and epinephrine/nor-epinephrine.

Conclusion: Spinal manipulation may influence inflammatory and cortisol post-intervention. However, the wider prediction intervals in most outcome measures point to the need for future research to clarify and establish the clinical relevance of these changes.

Introduction

Spinal manipulation (SM) is a specific hands-on approach used by several different healthcare disciplines commonly for the intended purposes of reducing spinal pain and reducing disability [1-5]. Early theories on the mechanisms of therapeutic effects following SM centred within a biomechanical paradigm. According to the biomechanical model, a SM can cause changes in the biomechanics of the spine which allows it to function in a more optimal state[6,7]. However, accumulating evidence clearly demonstrates a shift towards a neurophysiological paradigm [8-25]. According to the neurophysiological paradigm, a mechanical input such as a SM may trigger a cascade of neurophysiological response at both spinal and supraspinal levels [7,10,14,24].

Pain modulation following SM is a net result of complex neural interactions between various physiological systems involving different biochemical mediators [26]. Several neuropeptides such as substance-P (SP), neurotensin, oxytocin and orexin-A influence pain modulation through widespread effects in the nervous system [27,28]. As these chemicals are primarily released at the injury site, they also influence the initiation of inflammatory process. This in-turn results in the production of numerous pro-inflammatory and immuno-regulatory cytokines and neurotransmitters (e.g., tumor necrosis factor α (TNF-α); interleukins (IL)) [29,30]. Furthermore, endogenous opioids (ex: β-endorphins); hormones (e.g., cortisol) and catecholamine's (epinephrine and nor-epinephrine) modulate several immune parameters associated with the inflammatory process [31-33].

It has been hypothesised that SM activates the liberation of various biochemical markers such as SP, TNF-α from neural tissues resulting in its hypoalgesia and/or anti-inflammatory effects [34]. This is based on evidence that have demonstrated that SM can influence biochemical markers such as SP [35]; neurotensin and oxytocin; β-endorphins [10] and hormones such as cortisol [15,36]. A systematic review undertaken by our team previously established a

'moderate' level evidence that SM may influence various biochemical markers following SM [37]. Specifically, SM can increase substance-p, neurotensin, oxytocin and interleukin levels and may influence cortisol levels post-intervention. [37].

Our previous systematic review [37] employed valid methods and has been widely cited suggesting that our review is current and topical. Further, since the publication of our review, there has been significant interest in this topic area with several new studies published. Taking into consideration these factors and a possibility that the level of evidence may change with the findings from new studies, we considered that it was timely to provide an update of our systematic review as recommended previously [38,39].

The aim of this systematic review update was to provide an update on:

- The effects of SM on biochemical markers in humans.
- Establish the level of evidence for changes in biochemical biomarkers following a SM.

Operational definitions:

Systematic review update: The update of a systematic review is defined as "a new edition of a published systematic review with changes that can include new data, new methods, or new analysis to the previous version" [38]. This may include the following: updating the search; updating risk of bias tools; synthesis of new papers; adjusting the conclusions of a review [39].

Biochemical Markers: For the purpose of this systematic review update, biochemical markers were classified into the following three categories: (1) neuropeptides (2) inflammatory and (3) endocrine biomarkers.

Methods

This review has been reported based on Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines [40]. The review protocol was prospectively registered on the International Prospective Register of Systematic Reviews (PROSPERO: CRD42016049473).

Types of studies:

Randomised controlled trials (RCT) or controlled clinical trials that involved humans (healthy or painful), measured biochemical markers were eligible for this review. Only articles published in English language were included. Further, published conference abstracts, pilot studies and dissertations were excluded.

Types of participants:

Studies involving humans were eligible. There were no restrictions based on age, gender and severity of pain.

Types of intervention:

The intervention of interest was SM provided either by a physiotherapist, osteopath, or chiropractor. SM is defined as a high-velocity, low-amplitude thrust technique that is often associated with a cavitation [41]. The comparator (control) group could be any of the following: no intervention, usual care group, GP care, sham therapy or any other therapy.

Types of Outcome(s):

The outcome measures of interest included the following biochemical markers: (1) neuropeptides (e.g., neurotensin, oxytocin, SP) (2) inflammatory (e.g., TNF, IL) and (3) endocrine (e.g., cortisol, epinephrine, nor-epinephrine) biomarkers from any body fluids.

Search strategy:

In consultation with a librarian, it was decided that the previous search strategy was relevant and no changes were required. A replacement approach as recommended by Cochrane was utilized where the previous review was used as one source of studies. A bibliographic search (Table 1) was performed through the following databases: Medline, AMED, EMBASE, CINAHL, SPORTSDiscus, PubMed, Cochrane Library, Web of Science, Physiotherapy Evidence Database, and SCOPUS (from inception till May 2023).

Table 1: Search strategy

Phase 1	Phase 2	Phase 3
1. Exp. (manual N5 thrap*)	12. Exp. Biological marker	28. Exp. Randomized clinical trial/
2. Exp. "physical therap*"	13. Biochemical markers	29. Controlled clinical trial/
3. Exp. physical therapy modalities	14. Exp. Pain	30. Clinical study/
4. Exp. chiropractic	15. Exp. stress	31. Clinical article/
5. Exp. osteopathy	16. Stress biomarker	32. Multicenter study/
6. Manipulation N5 treatment	17. Endocrine*	33. random allocation/
7. therap* N5 manipulat*	18. Sympathetic nervous system	34. single-blind procedure/
8. traction manip*	19. Hormone	35. placebos/
9. thoracic manip*	20. cortisol	36. or/ 28-35
10. mobilization	21. oxytocin	37. assign*
11. Or/ 1-10	22. β-endorphins	38. allocate*
	23. catecholamine	39. blind*
	24. neuropeptide	40. control
	25. ACTH	41. random*
	26. OR/12-25	42. or/ 37-41
	27. 11AND 26	43. 36 OR 42
		44. Not animal
		45. 43 AND 44
		46. 27 AND 45

Data Management:

Articles obtained by the systematic search in the above-mentioned databases were exported to Covidence (Covidence systematic review software, Veritas Health Innovation, Melbourne, Australia; www.covidence.org) and managed in Covidence throughout the review process.

Study selection:

Duplicates were automatically detected and removed by Covidence. However, one reviewer (KSK) went through the titles to ensure all duplicates were removed. Full texts of the remaining articles were then screened by two independent reviewers (KSK and LT). Any disagreements between reviewers at any stage of the selection process were resolved through consensus and discussion. A third reviewer was available if required.

Data extraction and management:

Three reviewers (KSK, JDR and LT) collected data independently from included studies using a standardized data collection form in Covidence. The following were extracted: (1) study characteristics: funding, settings, design and country (2) patient characteristics: age, gender, severity of condition (if applicable) (3) intervention characteristics: number of intervention groups, content of each intervention (4) Outcome/data results: outcome measures (biomarkers) used, time points used and duration of follow-up (Table 2). Any disagreements were resolved by reaching a consensus.

Risk of bias:

The Cochrane Collaboration's tool for assessing risk of bias [42] available as part of Covidence was used by two reviewers (KSK, LT, JDR and OT) independently to assess the risk of bias in the included studies. Any disagreements were resolved through consensus. If consensus could not be obtained a third reviewer was available to enable a final decision. A study was considered to have low risk of bias if the random sequence generation, allocation concealment and incomplete outcome data domains were adequately met. While the use of the recent

Cochrane's risk of bias (RoB 2) tool [43] has been encouraged, it was not mandatory to use RoB-2 for a review update.

Summary measures:

Meta-analyses were performed where it was appropriate to pool data from multiple studies at two time points (1) immediate and (2) short-term. For the purpose of this review, immediate was defined as the measurement point immediately (up to 30 minutes) after intervention and short-term was defined as the measurement point up to 24 hours after intervention. Mean and standard deviations for outcome measures were extracted into Cochrane's online Review Manager (RevMan Web, version 1.22.0) [44] software to analyse the comparative data between each intervention effect.

Measures of treatment effects: All outcomes of interest were examined as a standardized mean difference (SMD) and a random effects model was used whereby the overall effects are adjusted to include an estimate of the degree of variation or heterogeneity across studies. An effect size (Cohen's d; small – 0.2; medium – 0.5 and large – 0.8) [45] and a 95% confidence interval were calculated for each treatment comparison.

Dealing with missing data: the authors were contacted in cases of missing data. For data that were graphically displayed, a software tool (https://automeris.io/WebPlotDigitizer/) was used, which is consistent with the original review.

Assessment of heterogeneity: clinical heterogeneity was evaluated by determining if different clinical factors (characteristics of participants, interventions, outcome measure) varied between trials and could potentially influence the treatment effect. Statistical heterogeneity was determined using Chi-square and I^2 statistics (25%, 50% and 75% representing low, moderate and high heterogeneity respectively). If the heterogeneity was more than 50% (representing moderate heterogeneity), a sensitivity analysis was conducted to identify the cause of statistical heterogeneity.

Prediction Interval: We calculated prediction interval (PI) as I2 statistics may not point to the clinical implications of the observed heterogeneity. The PI represents interval within which the effect size of a new study would fall if the new study was randomly selected from the same population of studies that are included in the meta-analysis[46]. Reporting a prediction interval in addition to the summary estimate, CI and I2 statistics have been recommended to capture the range of true effects that can be expected in future settings[47,48]. The formula to calculate PI is available [49]; however, a pre-set template that is available from www.meta-analysis.com was used for calculating PIs in this review.

Assessment of reporting biases: Funnel plot has been recommended to assess publication bias in included studies. However, the funnel plot was not performed as the required statistical conditions were not met (10 or more studies).

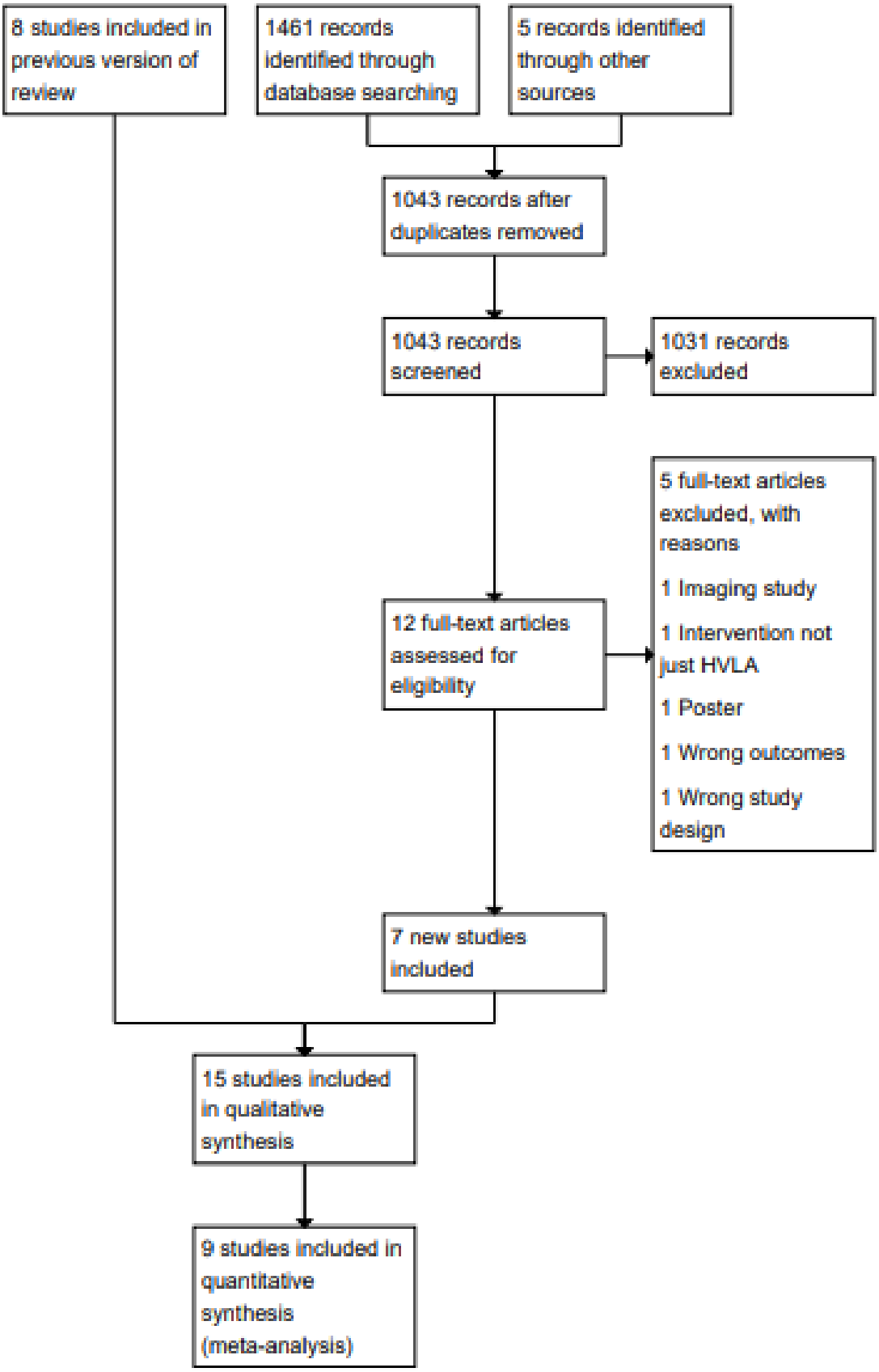
Data synthesis:

The Grading of Recommendations, Assessment, Development and Evaluation (GRADE) system [50] was used to determine the overall quality of the evidence (high, moderate, low and very low).

Results

An updated search retrieved a total of 1466 records. After removal of duplicates, 1043 records were screened. Of the 12 full-text records that were assessed for eligibility, a total of seven studies that met the inclusion criteria were included in review. Together with the eight studies from the original review, a total of 15 studies were part of this systematic review update (refer figure 1)

Figure 1: PRISMA Diagram of Included Studies


8 studies included in previous version of review
1461 records identified through database searching
5 records identified through other sources
1043 records after duplicates removed
1043 records screened
1031 records excluded
12 full-text articles assessed for eligibility
5 full-text articles excluded, with reasons
1 Imaging study
1 Intervention not just HVLA
1 Poster
1 Wrong outcomes
1 Wrong study design
7 new studies included
15 studies included in qualitative synthesis
9 studies included in quantitative synthesis (meta-analysis)

Summary of included studies:

A full description of included studies has been provided in the 'characteristics of included studies' (refer Table 2).

Table 2: Characteristics of included studies

Author, year	Methods/participant characteristics	Intervention	Outcome Measure(s)/ time points	Findings	Notes
Achalan dabaso 2014	3 groups RCT Randomized: 30 healthy subjects volunteers Gender: 16 male – 14 female subjects Age: 27.6 - 29.8 - 28.6 (y, mean 3 groups Ctrl, Cerv, Th) Settings: healthy students from the University of Jaen	Placebo SM *vs* SM (cervical-Thoracic) **Placebo SM** Control group: n=10 received following the cervical manipulation protocol with regard to hand contact, without intention of mobilization, nor application of tissue tension by the operator **Cervical group**: n=10 received HVLA thrust at C4 and C5 cervical spine in supine, with left rotation and right-side bending **Thoracic Manipulation**: n=10 received HVLA thrust at levels T3-T4 and T4-T5	Blood samples (plasma and serum) CPK, LDH, CRP, Troponin-I, Myoglobin, NSE, aldolase Before and right after intervention and 2 hours after	No changes in any of the studied damage markers	

Brennan 1991	3 groups RCT. Randomised: 99 healthy volunteers. Gender: 67 males, 32 females Age: 26.2 (mean) Setting: Research department, Chiropractic college.	SM (vs) sham (vs) soft tissue **SMT group**: 42 participants received a thoracic SMT (T1 to aT6). **Sham group:** 38 participants received sham manipulation (low velocity, low amplitude thrust). **Soft tissue group:** 19 participants received soft tissue manipulation to either the left or right gluteal area.	plasma concentration CBC SP 15 minutes pre and 15 minutes post-intervention	↑SP in SM group	Funded by a grant from the Foundation for Chiropractic Education and Research.
Christian 1987	4 groups RCT. Randomised: 40. 20 with pain and 20 pain-free. Gender: only male participants Age: 18 to 30 (range) Setting: chiropractic teaching clinic	Pain-free SM group (vs.) pain SM group (vs.) pain-free sham group (vs.) pain sham group. **Pain-free SM group:** 10 asymptomatic participants received chiropractic SMT. **Pain SM group:** 10 participants with pain received chiropractic SMT. **Pain-free sham group:** 10 asymptomatic participants received sham intervention	Plasma samples Cortisol ACTH β-endorphin Pre-intervention, 5 and 30 minutes post-intervention.	No changes in any outcome measures	Supported by a grant-in aid from NHMRC, Australia

		where a very slight pressure was exerted on the neck. **Pain sham group:** 10 participants with pain received sham intervention.			
Duarte 2022	3 groups RCT 99 healthy young adults mostly chiropractic students Gender: 10 female – 89 male Age: 25.6 years (mean) Setting: Canadian Memorial Chiropractic College Simulation Laboratory and Life Science Laboratory	Spinal manipulation therapy vs Control Single intervention **Control** (preload only): n= 33 **Single thoracic SMT** with a total peak force of 400N: n=33 **Single thoracic SMT** with a total peak force of 800N: n=33	14 different inflammatory biomarkers (pro, anti, dual role, chemokine, and growth factor) was assessed by multiplex array GM-CSF IFN-γ IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-17A, IL-23 TNF-α	Select plasma pro-inflammatory and dual-role cytokines were elevated by higher compared to lower SMT force btw-group (800N *vs* 400N) difference was observed on interferon-gamma, IL-5, IL-6, while a within-group difference (800N: immediately *vs* 20 minutes post-intervention) was observed on IL-6	This research project was funded by the Internal Research Support Fund at Canadian Memorial Chiropractic College

Kovanur Sampath 2017	RCT:2 Groups 24 healthy men Age: 18-45 y Setting: Controlled laboratory study	Thoracic SM *vs* Sham **SM**: n=12 received HVLA thrust at T5 vertebra upon expiration (single thrust) **Sham**: n=12 same setup without HVLA thrust	Salivary Cortisol Salivary Testosterone T/C Ratio HRV Oxyhemoglobin concentration (right calf muscle) Before, at 5 minutes, 30 minutes and approximately 6 hours after intervention	Thoracic SM resulted in an immediate decrease in salivary cortisol concentration and reduced T/C ratio 6h after intervention. SM did not differentially alter oxyhemoglobin, testosterone, or HRV *vs* responses in the sham group	Funded by a grant from the New Zealand Manipulative Physiotherapists Association
Kovanur Sampath 2021	Randomized 2-sequence, 2-period crossover trial 24 participants with Achilles tendinopathy >3mo Age: 48 ±7 y Gender: Male: 10 ; Female: 14 Setting: University Laboratory with washout period of 1week	SM *vs* sham 2 session in cross-over Sequence 1 (sham intervention and then thoracic spinal manipulation) or sequence 2 (thoracic spinal manipulation and then sham intervention) Session duration : 10 seconds **SM**: n=24 received thoracic spinal manipulation HVLA on T5 vertebra upon expiration **Sham intervention**: n=24 received same setup, not place a fixating hand against	Salivary samples T/C Ratio (Salivary samples) HRV (/ECG) Total oxygenation index calf muscle and Achille tendon (/near-infrared spectroscopy) TC Ratio: Pre-intervention, at 5 minutes, 30 minutes, and 6 hours post-intervention	Statistically significant condition by time interaction was found for the T/C ratio (mean difference: −0.16;CI:−0.33 to 0.006: P < .05) and TOI (mean difference: 1.35; CI:−1.3 to 4.1: P < .05) of calf muscle but not for Achilles tendon (P = .6); No difference was found	Funded by a grant from the New Zealand Manipulative Physiotherapists Association

		thoracic spine and without HVLA thrust		for heart rate variability (P = .5)	
Lohman 2019	RCT 2 groups Randomized 28 female subjects with non-specific mechanical neck pain Age: 37.1 – 30.1 (CSM – Sham) Setting: Loma Linda University	Cervical SM *vs* sham CSM One session **CSM**: n=13 received a cervical spine manipulation HVLA thrust in rotation **Sham CSM**: n=15 received sham CSM without moving the individual or carrying out the final thrust procedure	Serum concentration using the Milliplex Map Magnetic Bead Panel Immunoassay on the Luminex 200 Platform Oxytocin Neurotensin Orexin A Cortisol	CSM group, significant increases in pre *vs* 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) but no significant differences in mean cortisol (p= .052) (Serum concentration)	Supported by Loma Linda University.
Molina-Ortega 2014	3 groups RCT Randomised: 30 healthy volunteers Gender: 16 male, 14 female Age: 27.8 (mean)	Control (vs) Cervical SM (vs) Thoracic SM **Control group:** 10 participants received no intervention. **Cervical manipulation group:** 10 participants	Serum samples NO2 SP PPT (Algometer) Pre-intervention, immediately after and	↑SP, ↑PPT in CSM group No effects on NO2	

	Setting: University Research Department	received cervical manipulation. **Thoracic manipulation group:** 10 participants received thoracic manipulation.	2 hours post-intervention.	
Pascual-Vaca 2017	Randomized controlled blinded clinical study 46 patients suffering from renal lithiasis ; 27 men (59%) and 19 women (41%) with an average age of 38.5 (SD=6.80) and a Body Mass Index (BMI) of 25.07 (SD=3.12) **Settings/location:** Nephrology Departments of 2 hospitals and one private consultancy of physiotherapy in Valencia (Spain)	The experimental group (EG, n=23) received a spinal manipulation of the thoracolumbar junction, and the control group (CG, n=23) received a sham procedure **EG**: High speed movement with low amplitude, bilaterally on T12-L1 at the end of ROM rotating patient **CG:** The therapist placed one hand on the sacrum and the other hand on the middle thoracic region, without performing any action for 90 seconds. A rest time of 10 minutes was also taken before taking the post intervention measurements.	PPT algometer (spinous process T10 to L1 and quadratus Lumbarum) Urinary pH Pre-Post (immediately after intervention)	significant changes in PPT in both quadratus lumborum ($P<0.001$) as well as in the spinous processes of all of the evaluated levels ($P<0.05$). No changes in urinary pH were observed ($P=0.419$)
Plaza-Manzano 2014	3 groups RCT. Randomised: 30 healthy participants.	Control (vs.) cervical manipulation (vs.) thoracic manipulation	Serum samples neurotensin oxytocin	↑neurotensin, ↑oxytocin in CSM and TSM groups immediately. ↑cortisol in CSM group immediately.

	Gender: 16 males, 14 females Age: 27.8 (mean) Setting: University Research setting.	**Control group:** 10 participants received no intervention. **Cervical manipulation group:** 10 participants received cervical manipulation. **Thoracic manipulation group:** 10 participants received thoracic manipulation.	orexin A cortisol. Samples were collected before, immediately after and 2 hours after manipulation.	No changes in orexin-A.	
Puhl 2012	2 group RCT. Randomised: 56 healthy participants. Gender: 19 males, 17 females. Age: 26.1(mean). Setting: Chiropractic teaching clinic.	SMT (vs.) Sham**.** **SMT group**: 18 participants received a thoracic SMT. **Sham group:** 18 participants received sham manipulation (identical setup like SMT but without the thrust).	Plasma samples NE E Pre-intervention, immediately after and 15 minutes post-intervention.	No changes in E or NE levels.	Only 36 included in final analysis. 2 subjects developed adverse reaction (vertigo) post-randomisation during catheter insertion. No adverse events after intervention Funded by Research division, Canadian Memorial Chiropractic College

Teodorczyk-Injeyan 2006	3 groups RCT. Randomised: 64, healthy participants Gender: 28 males, 36 females Age: 24.7 (mean) Setting: Chiropractic College	SMT (vs.) Sham (vs.) control**.** **SMT group**: 24 participants received a thoracic SMT. **Sham group:** 20 participants received sham manipulation (identical setup like SMT but without the thrust). **Control group:** participants (n=20) did not receive any treatment.	Serum samples TNF-α SP IL-1 Pre-intervention, 20 minutes and 2 hours post-intervention.	↓ IL-1$^{\beta}$ No effects on TNF-α or SP	Funded by Public Health Services Grant, Canada
Teodorczyk-Injeyan 2010	3 groups RCT. Randomised: 74 healthy participants Gender: 31 males, 43 females Age: 24.7 (mean) Setting: Chiropractic College	SMT-C (vs.) SMT-NC (vs.) control**.** **SM with cavitation group**: 27 participants received a thoracic SMT with an audible cavitation. **SM without cavitation:** 25 participants received sham manipulation (identical setup like SMT but without cavitation). **Control group:** participants (n=22) in this group did not receive any treatment.	Serum samples PBMC IgG IgM Pre-intervention, 20 minutes and 2 hours post-intervention.	↑IgG, ↑IgM in SM-C group at 20-minutes and 2 hours post-intervention.	Funded by Public Health Services Grant, Canada

Valera-Calero 2019	3 groups RCT 83 patients with chronic mechanical neck pain **Age:** Mean±SD cMAN 35.64±8.11 cMOB 37.25±10.54 Sham 36.96±8.89 **Gender** : 51 women, 32 men **Setting:** University of Alcala de Henares: outpatient (referrals from office workers)	Cervical manipulation *vs* cervical mobilization *vs* sham manipulation in patients with chronic mechanical neck pain. **Cervical spine manipulation** (n=28) velocity, mid-range, left rotational force to C5-C6, with right side bending and left rotation **Cervical mobilization** (n=28) grade III postero-anterior joint oscillatory mobilization technique applied to the articular pillar of C5/6 on the subject's symptomatic side **Sham manipulation** (n=27) eliminated the joint preload and thrust component	Salivary cortisol levels Pre-Post intervention	A significant and comparable increase in cortisol levels was observed immediately after cervical manipulation and mobilization (both P<0.001) Reduced neck pain and decreased disability immediately after manipulation.	
Whelan 2002	3 groups RCT. Randomised: 30 healthy student volunteers Gender: only male participants Age: unavailable	**Control Group:** 10 participants were just supine lying. No manipulation or vertebral positioning done. **Sham group:** 10 participants were lying supine with their cervical spine positioned but without any manipulation.	Salivary samples Cortisol 5 consecutive weeks. Week-1: 5 consecutive days. Week 2-5: pre-intervention, 5 and 60	No effects on basal cortisol levels.	Supported by New York Research Committee

	Setting: Research Department, Chiropractic college.	**CM Group:** An upper cervical manipulation was performed on 10 participants.	minutes after intervention

ACTH – Adreno-Corticotropic Hormone, C – Control, CSM – Cervical Spinal Manipulation, I – Intervention, Ig – Immunoglobulin, IL – Interleukin, NO2 – Nitric Oxide, PBMC – Peripheral Blood Mononuclear Cells, PPT – Pressure Pain Threshold, SM – Spinal Manipulation, SM-C – Spinal Manipulation with Cavitation, SM-NC – Spinal Manipulation with No Cavitation, SP – Substance-P, ST – Soft Tissue, TSM – Thoracic Spinal Manipulation, TNF – Tumour Necrosis Factor, VC – Venipuncture Control.

Methods

Out of 15 studies[15,18-21,35,36,51-58], nine studies were RCTs with three groups[21,35,36,51,52,54,56-58]; five studies were RCTs with two groups[15,18-20,55]; and one study had four groups[53].

Sample Size

A total of 737 participants were examined in the studies. The sample size in the included studies ranged from 30 to 99 with only five studies recruiting more than 50 participants. All studies recruited participants in a single center.

Participants

The mean age of participants across all studies was 29.7 years. While 11 studies[15,20,21,35,51,53-58] included both male and female participants; three studies[19,36,52] included only male participants; and one study[18] included only female participants. Of the 15 studies, ten included healthy volunteers[15,19,21,35,36,51,52,54,56,57], four[18,20,53,58] included participants with pain (3 with neck pain and 1 with Achilles tendinopathy) and one study incuded participants with renal lithiasis.

Interventions

Two interventions were used by the researchers (1) cervical spine manipulation (either directed to atlanto-axial joint or cervical spine) (2) thoracic spine manipulation (either directed to T1 to T6, T12 or at the therapist's discretion). In eight out of 15 studies (53%), thoracic spinal manipulation was the intervention used[15,19-21,52,54,55,57]. Four out of 15 studies (27%) used cervical manipulation[18,36,53,58] as the intervention and three out of 15 studies (20%) made use of both cervical and thoracic spinal manipulation interventions. While low velocity low amplitude thrust (mobilization) or setup for a thrust without manipulation was the commonly used sham procedure (n=8), touch with no pressure was used as control (n=7).

Outcome measures

A diverse range of outcome measure were reported in the studies including SP, neurotensin, cortisol, epinephrine/nor-epinephrine, interleukins, TNF, oxytocin and orexin-A. Most studies provided follow-up assessments at two time points: immediately (up to 30 minutes) and short-term (hours) after intervention.

Safety

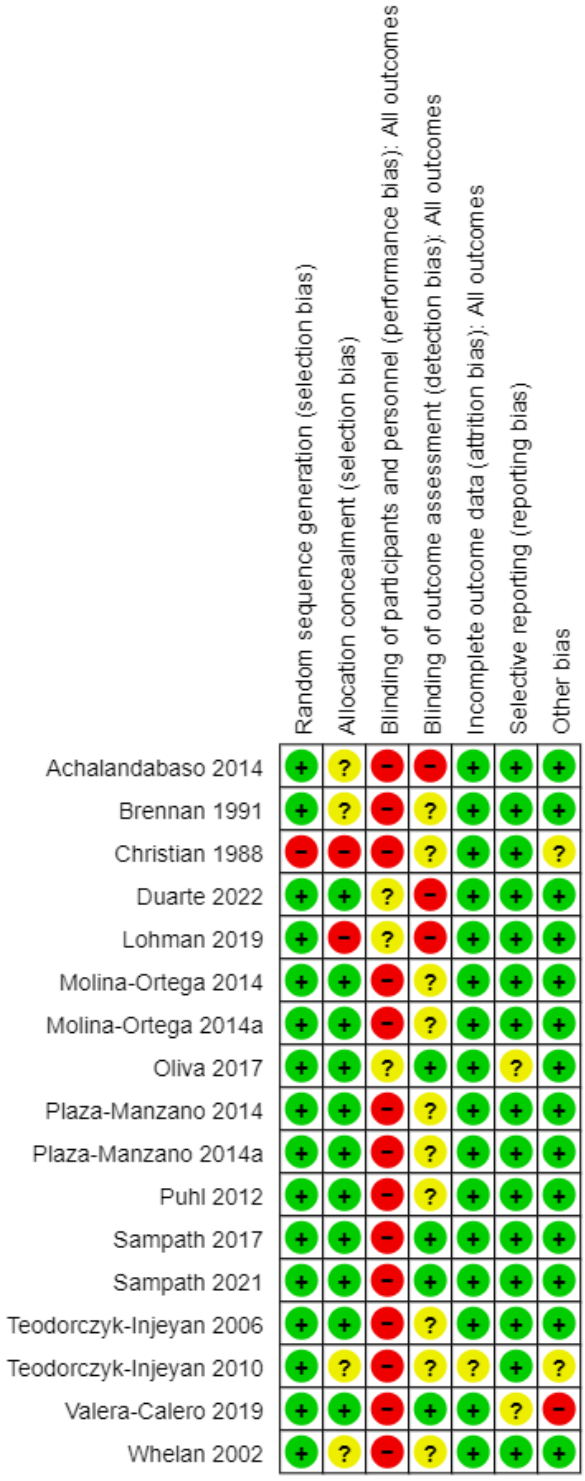
Only one study [15] reported about withdrawal/adverse events. Another study [51]investigated changes in tissue damage markers after a spinal manipulation, which can be considered as an investigation about safety of spinal manipulation. Other studies did not report the presence/absence of adverse events and/or safety of spinal manipulation.

Risk of bias in included studies:

The risk of bias was analysed for all individual studies. Figure 2 provides a summary of the judgements of each methodological quality item for each study except for one study [53], random sequence generation was adequate in all other studies. Allocation concealment was considered 'unclear' in four studies [21,36,51,52]; 'inadequate' in two studies [18,53] and 'adequate' in nine studies [15,19,20,35,54-58]. In manual therapy studies, blinding of participants and practitioners may not be possible. Hence all studies were rated as either 'high' risk or 'unclear' risk for this domain. Blinding of outcome assessors was explicit and considered 'low' risk in four studies [19,20,55,58], 'unclear' risk in eight studies[15,21,35,36,52,53,56,57] and 'high' risk in three studies [18,51,54]. Except for one study [15] in which participants withdrew post randomisation, attrition bias was not detected in other studies. One study [58] was rated 'high risk' for other bias as there was considerable deviation from the study protocol. Of the 15 studies, 10 studies [15,18-21,36,52-54,57] received either full or partial funding. Five studies [35,51,55,56,58] did not report source of funding.

One study [53] was rated ‘high risk’ overall as it did not meet random sequence generation and allocation concealment criteria.

Figure 2: Risk of bias in included studies



Note: Molina-Ortega 2014 and 2014a are one study; Plaza-Manzano 2014 and 2014a are one study.

Effects of interventions:

A summary of findings table was created to summarise the overall quality of evidence using GRADE (Tables 3, 4 and 5).

Spinal manipulation (vs) control/sham in influencing biochemical markers:

Data from 15 studies (total of 737 participants) [15,18-21,35,36,51-58] (not pooled) demonstrated a 'low' quality evidence that SM was better than control in eliciting changes in biochemical markers (Table 3).

Table 3: Summary of findings (GRADE)

Spinal manipulation compared to Control/Sham in influencing biochemical markers

Patient or population: healthy or symptomatic participants
Settings: Primary care, outpatient, community
Intervention: Spinal Manipulation
Comparison: Control/sham
Biochemical markers (follow up: mean 2 hours; assessed with: plasma or serum or saliva)

ainty assessment							№ of patients		Effect		Certainty
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	[SM]	[Other Intervention]	Relative (95% CI)	Absolute (95% CI)	
15	randomised trials	not serious	serious[a]	serious[b]	not serious	None	357	380	-	see comment	⊕⊕◯◯ Low

CI: confidence interval

Explanations

a. Known Heterogeneity across studies, not pooled

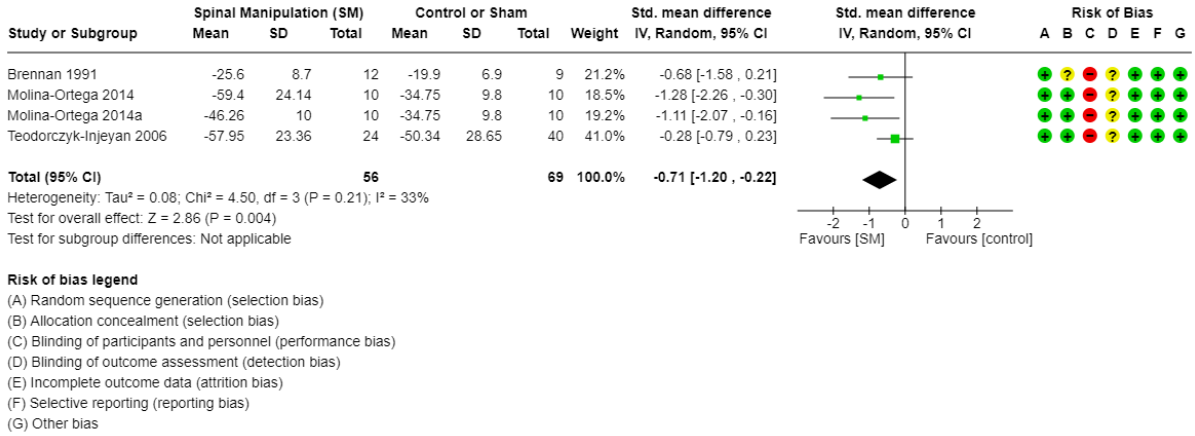
b. Different settings/context/outcome measures

Spinal manipulation (vs.) control/sham in influencing neuropeptides:

Data from three studies (125 participants) [15,35,52] showed (Figure 3) that there was a 'very low' quality evidence of no difference that SM is better than control/sham (SMD -0.71, 95% CI – 1.22 to -0.22; PI: -2.33 to 0.91) in increasing SP levels immediately after intervention. Although, the effect size and associated CIs indicate statistical significance, the prediction intervals are wide and point to lack of clear benefit from SM. Further, there was 'very low' quality evidence from two studies (104 participants) of no significant difference that SM is better than control (SMD -01.16, 95% CI – 2.53 to 0.21) (Table 4) in eliciting changes in SP levels at short-term after intervention. Between-study heterogeneity was high (86%).

There was 'very low' quality evidence from two studies (68 participants) [18,56] of no significant difference that SM is better than control/sham (SMD -0.52, 95% CI – 1.01 to -0.03; PI -3.69 to 2.65) in increasing neurotensin after intervention. Although, the effect size and associated CIs indicate statistical significance, the prediction intervals are wide and point to lack of clear benefit from SM. However, 'very low' quality evidence from two studies (68 participants) [18,56] demonstrated no significant difference between SM and control/sham (SMD -0.47, 95%CI -1 to 0.06) in influencing oxytocin and orexin-A (SMD -0.59, 95% CI -1.48 to 0.29).

Figure 3: Forest plot of comparison:
SM vs control/sham , outcome: Substance-P (Immediate)



Spinal manipulation (vs.) control in influencing inflammatory biomarkers:

Data were extracted from four studies (192 participants; not pooled) that compared the effectiveness of SM with control on inflammatory biomarkers such as interleukins. There was 'low' quality evidence that SM is better than control in influencing inflammatory markers such as interleukins (Table 4).

Table 4: Summary of findings (GRADE)

Spinal manipulation compared to Control/Sham in influencing neuropeptides and inflammatory biomarkers

Patient or population: Healthy or symptomatic participants
Settings: Primary care, outpatient, community
Intervention: Spinal Manipulation
Comparison: Control/sham

Quality assessment							№ of patients		Effect		Quality
№ of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**Spinal Manipulation**	**Control**	**Relative (95% CI)**	**Absolute (95% CI)**	
Substance-P, immediate changes (assessed with blood)											
3	randomised trials	not serious	not serious	not serious	very serious[1]	none	56	69	-	SMD **0.71 lower** (1.22 lower to 0.22 lower)	⊕⊕◯◯ LOW

Quality assessment							№ of patients		Effect		Quality
№ of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**Spinal Manipulation**	**Control**	**Relative (95% CI)**	**Absolute (95% CI)**	
Substance-P, short-term changes (assessed with blood)											
2	randomised trials	not serious	serious[2]	not serious	serious[3]	none	44	60	-	SMD **1.16 fewer** (2.53 lower to 0.21 higher)	⊕◯◯◯ VERYLOW
Neurotensin (assessed with: Blood)											
2	randomised trials	not serious	not serious	not serious	very serious[4]	none	33	35	-	SMD 0.52 **lower** (1.01 lower to 0.03 lower)	⊕◯◯◯ VERYLOW
Oxytocin (assessed with: Blood)											
2	randomised trials	not serious	not serious	not serious	very serious[4]	none	33	35	-	SMD 0.47 **lower** (1 lower to 0.06 higher)	⊕◯◯◯ VERYLOW
Orexin-A (assessed with: Blood)											
2	randomised trials	not serious	not serious	not serious	very serious[4]	none				33	
Inflammatory Biomarkers (TNF, IL-2; assessed with: Blood)											
4	randomised trials	not serious	serious [5]	not serious	not serious	none	107	85	-	See comment	⊕⊕◯◯ LOW

CI: Confidence interval; **SMD:** Standardised mean difference

1. Graphical data retrieved using software and SD imputed.
2. Heterogeneity = 86%
3. Sample size < 100. Findings based on single study.
4. Sample size < 100
5. Known Heterogeneity, studies not pooled

Spinal manipulation (vs.) control in influencing endocrine biomarkers:

Cortisol: Data was pooled from seven studies (239 participants) to determine the effects of SM on cortisol levels (Figure 4). Between-study heterogeneity was moderate (I^2 = 63%). Hence a sensitivity analysis was done, and two studies were removed from the meta-analysis, which reduced the heterogeneity (I^2 = 0%) (Figure 4a) There was a 'low' quality evidence (Table 5) of statistically significant difference that SM is better than control/sham in eliciting changes in cortisol levels (SMD -0.42, 95% CI -0.74 to -0.10; PI -0.83 to 0.0) immediately after intervention.

Figure 4: Forest plot of comparison: SM vs control/sham , outcome: Cortisol (Immediate)

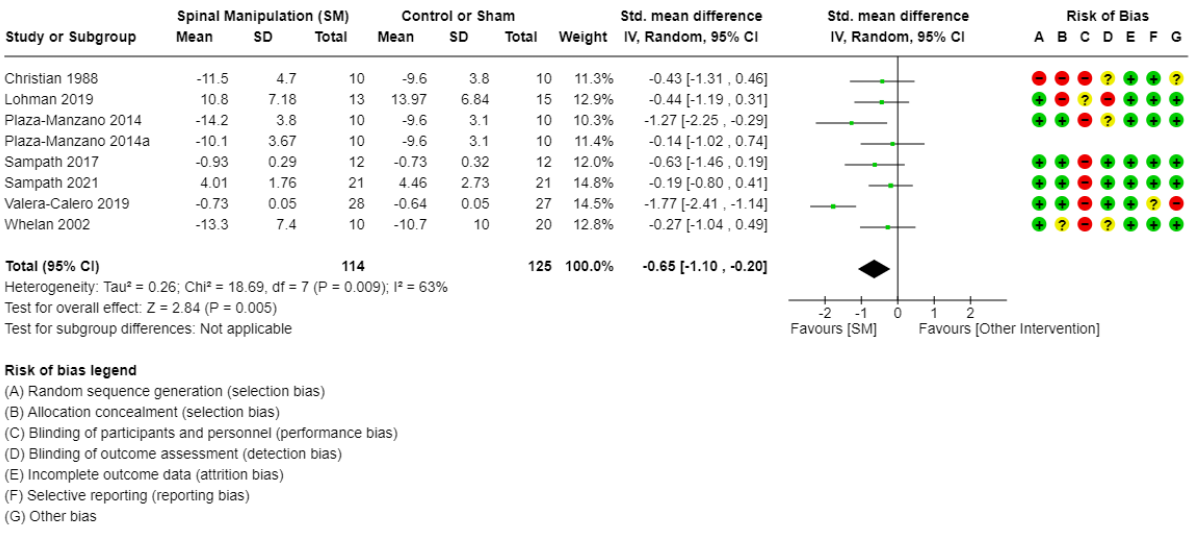
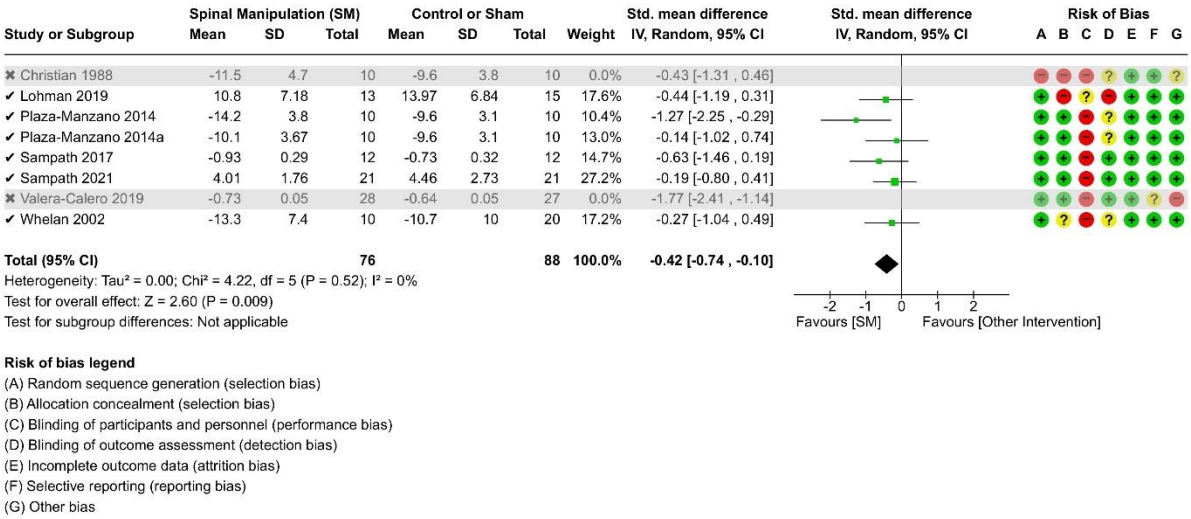
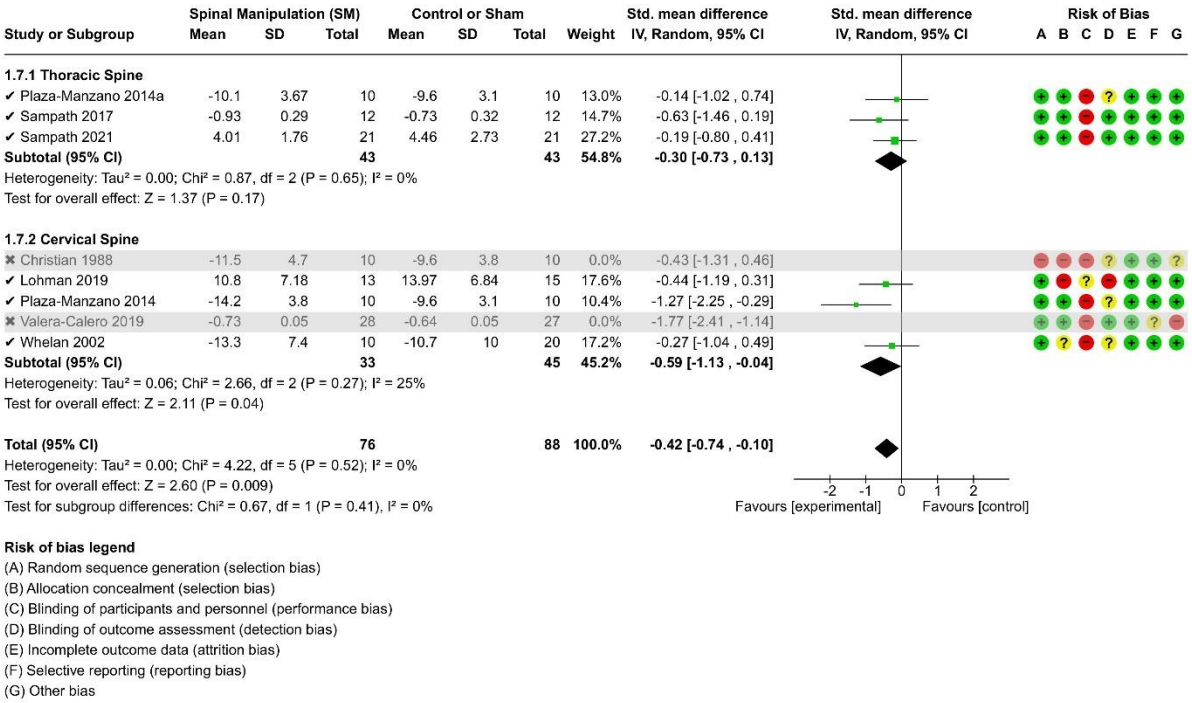


Figure 4a: Forest plot of comparison (sensitivity analysis): SM vs control/sham, outcome: Cortisol (Immediate)



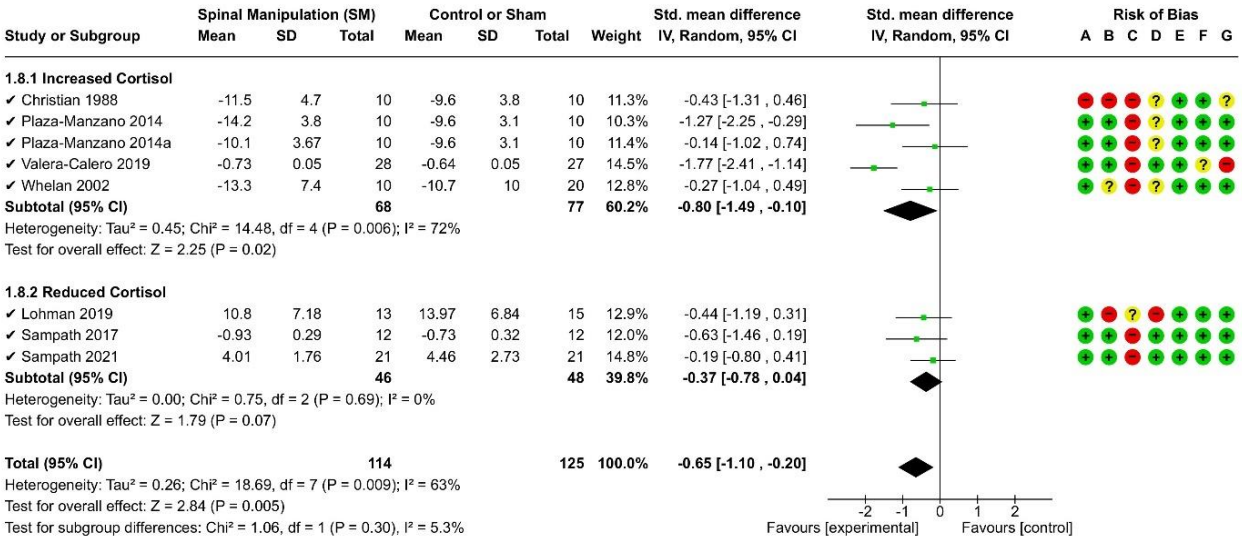
Segmental response: A subgroup analysis was undertaken to determine if the response in cortisol was different based on the region of spine manipulated (thoracic vs cervical in this instance). The results demonstrated that cervical spine manipulation cortisol levels compared to a thoracic spine manipulation (SMD- -0.65, 95% CI -1.10 to -0.2; PI -2.01 to 0.7) (refer figure 5).

Figure 5: sub-group analysis (thoracic vs cervical manipulation). Outcome: cortisol (immediate)



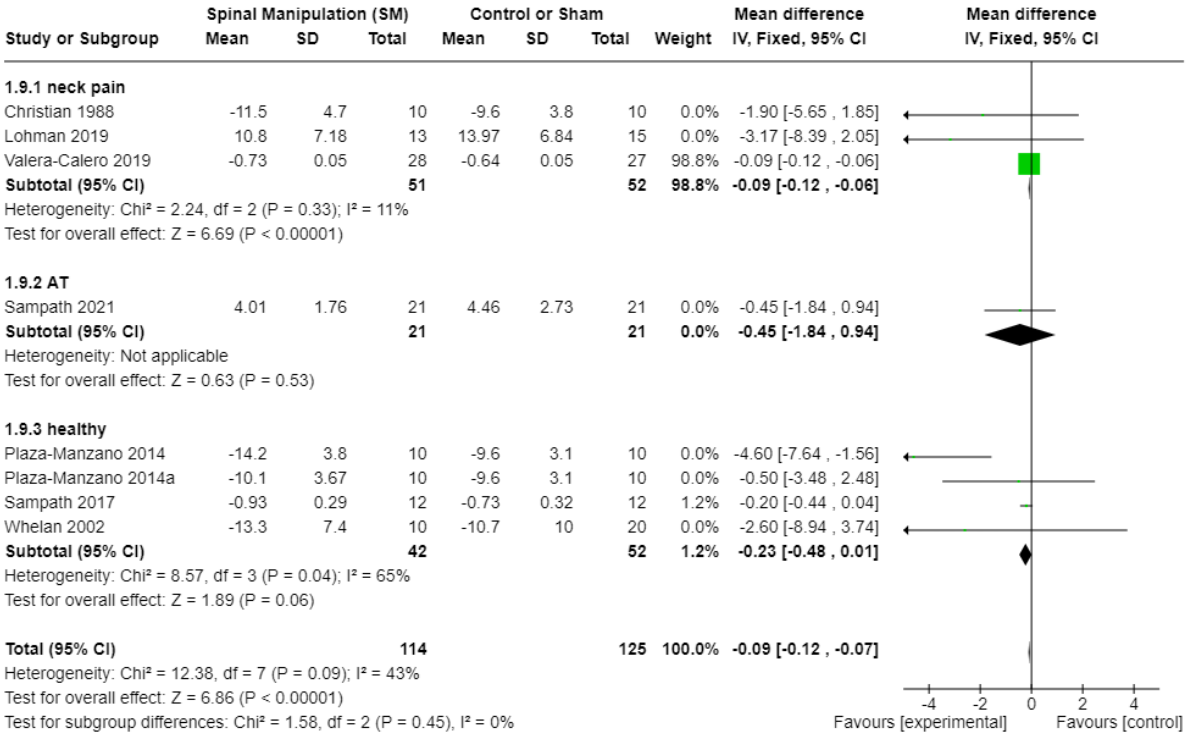
Direction of effect: another subgroup analysis was undertaken to determine the direction of effect (increase or decrease) of cortisol following a spinal manipulation. The subgroup analysis indicates that cortisol levels increase immediately following a spinal manipulation despite the segment being manipulated (SMD -0.65, 95% CI -1.10 to -0.2; PI -2.08 to 0.79) (figure 6).

Figure 6: sub-group analysis (direction of effect – increase or decrease). Outcome: cortisol (immediate)



Healthy vs painful population: subgroup analysis demonstrated that changes in cortisol following a SM is statistically significant in people with pain (especially neck pain) compared to healthy volunteers (SMD -0.09, 95% CI -0.12 to -0.07; PI -1.4 to 1.2) (figure 7).

Figure 7: **sub-group analysis (healthy vs pain). Outcome: cortisol (immediate)**



Cortisol (short-term): Low quality evidence from four studies (136 participants) demonstrated no significant difference that SM is better than control (SMD -0.45, 95% CI –0.79 to -0.1; PI: -1.21 to 0.31) in eliciting changes in cortisol levels at short-term after intervention (Table 5). Although, the effect size and associated CIs indicate statistical significance, the prediction intervals are wide and point to lack of clear benefit from SM in short-term changes in cortisol.

Table 5: Summary of findings (GRADE)

Spinal manipulation compared to Control in influencing endocrine markers
Patient or population: Healthy or symptomatic participants
Settings: Primary care, outpatient, community
Intervention: Spinal Manipulation
Comparison: Control

Quality assessment							№ of patients		Effect		
№ of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**Spinal Manipulation**	**Control**	**Relative (95% CI)**	**Absolute (95% CI)**	**Quality**
Cortisol, immediate changes (assessed with blood or saliva)											
7	randomised trials	not serious	serious[1]	not serious	Serious[2]	none	114	115	-	SMD **0.42 lower** (-0.74 lower to -0.1 lower)	⨁⨁◯◯ LOW
Cortisol, short-term changes (assessed with blood or saliva)											
4	randomised trials	not serious	Serious[3]	not serious	Serious[4]	none	63	63	-	SMD **0.45 lower** (-0.79 lower to 0.1 lower)	⨁⨁◯◯ LOW
Testosterone (assessed with: saliva)											
2	randomised trials	not serious	serious	not serious	very serious[3]	none	33	33	-	SMD - **0.01 lower** (-0.14 lower to 0.12 higher)	⨁◯◯◯ VERYLOW
Testosterone (assessed with: saliva)											

Quality assessment							№ of patients		Effect		
№ of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**Spinal Manipulation**	**Control**	**Relative (95% CI)**	**Absolute (95% CI)**	**Quality**
2	randomised trials	not serious	serious	not serious	very serious [3]	none	33	33	-	SMD - **0.04 lower** (-0.06 lower to 0.14 higher)	⨁◯◯◯ VERYLOW

CI: Confidence interval; **SMD:** Standardised mean difference

1. Sample size < 100
2. Heterogeneity

Sample size < 50. Findings based on single study.**Testosterone:** 'Very Low' quality evidence from two studies (66 participants) demonstrated no significant difference that SM is better than control in eliciting changes in testosterone levels immediately (SMD -0.01, 95% CI -0.14 to 0.12] and at short-term after intervention (SMD -0.04, 95% CI -0.06 to 0.14] (Table 5). Findings from single studies indicates no change in epinephrine or nor-epinephrine and urinary pH level following spinal manipulation.

Discussion

Summary of main results

This review updates the previous review published in 2017[59], comparing spinal manipulation against control in influencing biochemical markers. The updated review now includes 15 studies (737 participants) compared to eight studies (325 participants in the 2017 review). It also includes different types of participants (healthy volunteers, people in pain or disease); various types of spinal manipulation (cervical, thoracic and lumbar); a wide range of outcome measures (inflammatory markers, pain markers, urinary pH and T/C ratio), thus providing a comprehensive analysis of spinal manipulation in influencing biochemical markers. The findings from this review update established 'low' level evidence in support of SM in influencing biochemical markers such as cortisol (immediate changes) and inflammatory markers but not for substance-p, neurotensin, testosterone, oxytocin and orexin-A. Further, subgroup analyses established that: (1) cervical SM influences cortisol compared to thoracic SM; (2) cortisol levels increase immediately after intervention despite the segment being manipulated; and (3) response differ in people with pain (especially neck pain) compared to healthy volunteers. The key differences between the original review and this review update have been provided in appendix 1.

Overall completeness and applicability of evidence

The data from this review can be considered relevant to current clinical practice as we found evidence that SM may influence various biochemical markers such as cortisol and inflammatory markers. It is important that these findings are interpreted with caution and in consideration of prediction intervals (discussed later). Further, 10 out of 15 studies[15,19,21,35,36,51,52,54,56,57] have been done on healthy volunteers, which makes it difficult to

ascertain the applicability of the evidence in clinical practice. Although four studies[18,20,53,58] included participants with pain, the effect of SM on the magnitude and duration of biochemical responses in symptomatic patients (e.g. pain population or inflammatory disorders) needs further scrutiny and is an ongoing area of investigation[20,35,58]. Cervical or thoracic spinal manipulation are the common techniques utilized in the studies, with a subgroup analysis demonstrating that cervical SM may have more influence on cortisol levels. However, this is based on five studies[18,36,53,56,58] and should be verified by future studies that may have direct comparison between the two techniques. There was no adverse events/harm associated with SM. One study [51]measured tissue damage markers and demonstrated that there was no tissue damage associated with SM.

Quality of the evidence

As reflected by the GRADE ratings, the overall quality of the evidence in this review update was 'low' to 'very low' for all outcomes. This is because included trials studied a wide range of interventions, outcome measure, data collection techniques and post-intervention time points. Therefore, we were unable to pool data due to heterogeneity, especially for inflammatory markers. In addition, the sample size (being low in most studies), wide confidence intervals and prediction intervals led to issues of imprecision and inconsistency. It is important to note that we have downgraded the level of evidence compared to the original review. Although, eight more studies were part of this review update and points to growth in the evidence base, it also has resulted in further heterogeneity. Except for immediate changes in cortisol, the broad prediction intervals for other outcomes may indicate the existence of setting where SM may have suboptimal effects. Ten out of fifteen studies were small scale RCTs (less than 50 participants) done on healthy volunteers where there is a chance for overly positive trends for interventions due to inflated effect sizes. A review [60] has shown that trials

with fewer than 50 participants had effect estimates larger than trials with more participants (48% more on average). Hence, it has been recommended that trials with fewer than 19 participants in each trial arm be excluded from systematic reviews due to risk of bias associated with small RCTs [61]. We did not downgrade the risk of bias for blinding therapists as this is very difficult to achieve in manual therapy setting. While blinding of participants was done in some studies, it was unclear in other studies. Keeping in line with recent recommendations [62], future studies should concentrate on better blinding of participants and also therapists in maintaining blinding including adding a measure of blinding effectiveness. Only one study [58] had reported using the Template for Intervention Description and Replication (TIDieR) guidelines [63]. Therefore, it has to be re-emphasised that the overall quality of reporting of manual therapy studies still requires considerable improvement.

Potential biases in the review process

We consider the review process to be robust and expect minimal biases in extracting and reporting of data. A minimum of two reviewers acted independently through the various phases of the review and a third reviewer was available to resolve any disagreements if required. We undertook extensive search to identify new studies that may be included in this review update. We did not downgrade the risk of bias based on 'publication' bias as we had only 15 studies included in the review. It is well noted that existing ways to publication bias are unsatisfactory and funnel plot was not considered appropriate in this instance. Further, only publications done in English language were included in the review, thereby, raising the possibility of language bias [64]. In turn, this may limit the usefulness of the review's findings as we may miss out important cultural contexts [65]. Hence, recommendations have been made to include studies published in languages other than English (LOTE) [66]. However, due to lack of resources both in terms of funding and/or access to members who can fluently speak/read

LOTE, we had to limit our review to studies published only in English, as identified previously [64].

Agreements and disagreements with other studies or reviews

The findings from this review update remains partly consistent with our original systematic review findings. However, we decided to downgrade the quality of evidence from 'low' to 'very low' compared to the original review, largely due to inconsistency, indirectness and imprecision introduced by the inclusion of these studies.

Our review update established very low evidence that SM does not influence neuropeptides such as SP, neurotensin, oxytocin and orexin-A immediately after intervention. This is in contrast with the previous findings [35,37,52]. These neuropeptides are found in many regions of the CNS and are known to induce analgesia directly or indirectly. Molina-Ortega et al. (2014) further reported a positive correlation between SP levels and pressure pain threshold suggesting that high levels of serum SP before SM are associated with increased pressure pain threshold after SM. Hence, the review findings may be of importance. It has to be noted however that only on a few studies [18,56] have investigated these neuropeptides. Hence, the lack of beneficial effects of SM may be due to low number of studies in this area highlighting the need for further research investigating these biomarkers.

Our review findings indicate the SM may influence cortisol levels immediately (< 30 minutes) but not at short-term (many hours) after intervention. This is in agreement with our original review that demonstrated changes in cortisol levels immediately but not at short-term after intervention. The number of studies investigating the effects of SM on cortisol have increased in the last 5 years that may explain the difference. Emerging pattern from the current review update indicates that cortisol level may increase immediately after intervention despite the

segment manipulated. However, this is based on only two studies [56,58] that had used a cervical spine manipulation involving rotational thrust. Further, a cervical spine manipulation may influence cortisol levels immediately in people with neck pain. The changes in cortisol were shown to be positively correlated with reduced neck pain and reduced disability in one study [58]. It was noted that recent studies have considered various methodological factors that may influence cortisol levels and have outlined strategies to mitigate these variables, which is consistent with previous recommendations [37,67].

Our review update demonstrated no significant difference that SM is better than control in eliciting changes in testosterone levels immediately and at short-term after intervention. Testosterone was measured in the studies as interactions between the end products of the gonadal (e.g. testosterone) and the adrenal axis (cortisol) have been well documented [68]. Hence, the balance between testosterone and cortisol represented as T/C ratio may therefore provide a better estimation of the HPA axis activity[69]. Although not often used in manual therapy research, T/C ratio has been widely used in sports and exercise science research as valid outcome measure for stress response[69]. Hence, T/C ratio is an area of future research interest.

Findings from our review of four studies indicate that SM is better than control in influencing various inflammatory/immune markers such as interleukins (especially, IL-1, IL-2, IL-6), TNF-α, IgG and IgM. The regulation of inflammation and immunity involve complex interactions between the nervous system and the immune system mediated by the action of numerous neurotransmitters and cytokines [29,30,70]. This is consistent with previous findings and suggest that a central anti-inflammatory mechanism might be activated following a SM. However, it must be noted that some of the studies were done more than 10 years previously

indicating a dearth of recent investigation in this area. Hence, our findings must be interpreted with caution.

Implications for clinical practice and research:

Two common themes are consistent with our previous systematic review (1) clinical utility: while the changes in endocrine markers (especially cortisol) and inflammatory markers shed light into mechanisms through which SM may work, the clinical utility of such changes (especially short-term) is still largely unknown. Hence, it will be helpful to investigate long-term changes in these biochemical markers and their association with symptom improvement. (2) The mean age of participants explored across studies was 29.2 year (up from 26 years in the original review). Therefore, the generalisability and clinical application of our findings could be questioned. Hence, future studies may target participants across different age groups. The methodology used for collecting hormone samples and the reporting of protocol have improved since our previous review.

The wider prediction interval found in our meta-analysis may have important implication for clinical practice and research. Despite statistically significant findings as demonstrated by effect size and confidence intervals, the wide prediction intervals reduce the confidence in findings. That is, the effects of intervention may vary substantially depending on the setting or population used. This clearly emphasises the need for more well controlled studies to clarify our findings. The rationale for calculating prediction intervals could be criticised as there are less than ten studies as part of our meta-analysis [47]. However, we decided to calculate prediction intervals for a few reasons (1) there is still no consensus on what a sufficient number of studies would be to generate reliable prediction intervals. Some evidence[46] indicate that a minimum of three studies is enough to calculate prediction intervals (which we meet); (2) it is important to demonstrate the variability/heterogeneity to enable meaningful

interpretation of our findings by clinicians and researchers; and (3) it is better to highlight the heterogeneity and therefore the need for further research than to erroneously conclude that the intervention is beneficial (as demonstrated by effect size and CIs alone). Finally, we did not propose GRADE based recommendations due to the heterogeneity, which can be considered another important limitation.

Author's Conclusion

This review established low level evidence that SM influences various inflammatory markers and cortisol. Specifically, we found that SM can increase cortisol levels immediately post-intervention. Hence the beneficial effects of SM such as pain relief and reduced inflammation could potentially be modulated through these mechanistic pathways. However, well powered trials targeting symptomatic populations are required to validate our review findings.

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