International Journal of Osteopathic Medicine 33–34 (2019) 24–30

Contents lists available at ScienceDirect

International Journal of Osteopathic Medicine

journal homepage: www.elsevier.com/locate/ijosm

Review

Stress axis and osteopathy: A dual hormone approach

Kesava Kovanur Sampath[a,*], Rajesh Katare[c], Steve Tumilty[b]

[a] Department of Nursing, Midwifery and Allied Health, Ara Institute of Canterbury, Christchurch, New Zealand
[b] Centre for Health, Activity, and Rehabilitation Research, School of Physiotherapy, University of Otago, Dunedin, New Zealand
[c] Department of Physiology-Heart Otago, University of Otago, New Zealand

ARTICLE INFO

Keywords:
HPA axis
Cortisol
HPG axis
Testosterone
T/C ratio
Osteopathy

ABSTRACT

Manual therapy initiates a cascade of neurophysiological changes in various systems including the peripheral nervous system, autonomic nervous system and the endocrine system. Of particular focus of this review was the hypothalamic-pituitary-adrenal (HPA) axis. When faced with a stressor, the HPA axis provides the frontline of defence resulting in the production of cortisol, measurement of which has been shown to be a reliable indicator of HPA axis activity. Manual therapy has been shown to influence the HPA axis. However, a functional cross-talk has been clearly demonstrated between the HPA axis and the hypothalamic-pituitary-gonadal (HPG) axis, with cortisol and testosterone inhibiting each other at all levels. This mutual inhibition may mean that multiple measures across these two systems (HPA-HPG axis) should be considered to provide an accurate representation of stress physiology. The balance between testosterone and cortisol represented as a ratio termed T/C ratio has been used for this purpose. Given the implicit role of the HPA-HPG axis in pain and inflammation and an apparent gender difference in pain perception and treatment response, T/C ratio may be of interest to osteopaths as it may enable us to capture the holistic effects of osteopathy.

This review revisits the function of the HPA axis in pain and inflammation, presents a dual hormone approach in measuring the HPA axis and evaluates the implications for osteopathic practice and research.

Introduction

A common reason for using manual therapy is to diminish pain and improve mobility. Various systematic reviews [1–3] have concluded that manual therapy leads to clinically significant improvements in pain and function in various musculoskeletal conditions. However, the exact physiological mechanisms responsible for the clinical benefits of manual therapy are varied [4]. Initial theories centred on the biomechanical paradigm, where in addressing altered biomechanics due to tissue damage through manual therapy results in normal functioning [5]. However, the biomechanical model has been questioned as the relationship between structural dysfunction and pain has not been established, especially in people with chronic pain [6].

With evidence accumulating, there has been a gradual shift from the biomechanical model to the neuro-physiological model. The framework for this model is based on the findings from several studies that have shown the effects of manual therapy at the spinal level [7–9]; the supraspinal level [10]; the sympathetic nervous system [11]; enhanced production of biochemical markers such as tumour necrosis factor (TNF-α) and substance P [12,13]; and the hypothalamic-pituitary-adrenal (HPA) axis. Of particular focus of this review was the HPA axis.

Life exists by maintaining a complex dynamic equilibrium commonly referred to as homeostasis [14]. Walter Canon offered the first model of homeostasis as the "*coordinated physiological processes which maintain most of the steady states in the organism*" [15]. Homeostasis is constantly challenged by various intrinsic or extrinsic adverse forces called the stressors. The body's response to restore homeostasis termed as 'allostasis' (first proposed by McEwen in 1998) incorporates individual response to perceived stress and behavioural changes necessary to establish stability through change. The hypothalamus plays a key role in allostasis as it co-ordinates the defence response of the body by activating the ANS, especially the sympathetic nervous system (flight, flight or freeze) and the HPA axis [16], which in turn activates necessary physiological and behavioural responses for survival [17].

The activation of HPA axis results in the production of adrenocorticotropic hormone (ACTH) into the systemic circulation [18]. Upon release, the ACTH stimulates the zona fasciculata cells of the adrenal cortex to release glucocorticoid namely cortisol [19]. The effects of cortisol on cell metabolism are diverse and act at numerous levels to redirect bodily energy resources. Cortisol also plays an integral role in glucose, fat, and protein metabolism. Cortisol not only acts during early stages of inflammation by decreasing local oedema and pain but is also

* Corresponding author. Department of Nursing, Midwifery and Allied Health, Ara Institute of Canterbury, Christchurch, New Zealand.
E-mail address: Kesava.KovanurSampath@ara.ac.nz (K.K. Sampath).

https://doi.org/10.1016/j.ijosm.2019.05.001
Received 29 November 2018; Received in revised form 14 May 2019; Accepted 15 May 2019

believed to increase the rate of healing by stimulating gluconeogenesis [14]. Given its implicit role in pain and inflammation (expanded below), the HPA axis could be of interest to osteopaths. In this context, objective measurements of biomarkers (e.g. cortisol) that mediate and/or modulate the HPA axis response before and after osteopathic intervention may not only help us to validate the role of osteopathic treatment not only on stress physiology but also on pain and/or inflammation. The aim of this review therefore are to (1) review the role of HPA axis in pain and inflammation (2) review available evidence on the role of manual therapy in influencing the HPA axis and (3) present a dual hormone approach in measuring the HPA axis.

HPA axis – role in inflammation and pain

Injury to a tissue leads to changes in chemical environment of the peripheral terminals of nociceptors. In addition to activating the nociceptive terminals, some of the biochemicals also sensitize the terminals, so they become hypersensitive to subsequent stimuli [20]. This hypersensitivity to stimuli is termed as peripheral sensitisation and it often is a result of tissue damage and inflammation. Hence, chemical mediators of inflammation such as bradykinin, histamine, nerve growth factor and serotonin play an important role in peripheral sensitisation. On the other hand, central sensitisation is defined as an augmented central nervous system response to peripheral noxious stimuli [21]. The instigating factor of central sensitisation could originate in the periphery eventually leading to long-term potentiation in the spinal cord as well as structural changes in the brain.

Two processes may play a role in central sensitisation namely 'hyperalgesic priming' and 'wind up' [22]. Hyperalgesic priming occurs when a peripheral nociceptor is exposed to an injury or other priming event that may result in establishment of long-term potentiation in the central nervous system. 'Wind-up' involves repetitive low frequency input to peripheral c-fibers leading to temporal summation and activation, ultimately generating a pain response [22]. This leads to hypersensitivity characterized by lowered thresholds necessary to elicit and maintain wind-up. Nerve growth factor and substance P have been implicated in both "hyperalgesic priming" and "wind up" processes as a mediator of sensitisation of dorsal horn neurons and long-term potentiation [23].

Mast cells are a critical part of the innate immune system and are highly responsive to activation of the HPA axis [24]. Mast cells are observed adjacent to unmyelinated nerves throughout the body as well as in direct contact with nerve fibers in the dura mater [25]. These afferents express receptors involved in nociception that are expressed on neurons with cell bodies in the dorsal root ganglia, trigeminal ganglia, and nodose ganglia [26]. Increased activation of these receptors enhances pain-related afferent input to the central nervous system. Mast cells are a significant source of both nerve growth factor and substance P [27], which can influence central sensitisation as explained above. Further, mast cells also generate and maintain peripheral neurogenic inflammation by releasing neuropeptides, including substance P and calcitonin gene related protein, which perpetuates inflammatory mediator release in the proximate milieu. Therefore, increased peripheral corticotropin releasing hormone release due to dysregulated HPA axis activity may influence both peripheral and central sensitisation (Fig. 1).

Pain as an emotion has important physiological implications as pain represents a threat to homeostasis. Allostasis may be facilitated by the fact that multitudes of ascending tracts in the spinal cord ensure transmission of pain signals to reach multiple sites in the brain that not only process nociception but also discriminative aspects of pain such as memory; and the integration of the cognitive and behavioural aspects of pain including emotional arousal [28]. In the short run, increased vigilance in a hostile environment may be adaptive [29]; however, in the long run may lead to maladaptation, as is the case for chronic anxiety or depressive disorders [30].Further, experimental work in animal models has established that both acute and chronic stress contribute to the underlying cause of cardiovascular disease, specifically, development of atherosclerosis, which is generally mediated by an increased inflammatory response to a stressor [31]. In summary, the HPA axis plays a key role in both pain and inflammation through its modulation of mast cells and other mediators of pain and inflammation such as TNF-α, substance P, nerve growth factor, interleukins (IL1, IL 2 and IL 6).

Evidence of manual therapy in influencing HPA axis activity

Various studies have explored the effectiveness of manual therapy in influencing biomarkers of pain and inflammation including cortisol. For the purpose of this review, spinal manipulation was defined as a high velocity low amplitude (HVLA) thrust. It is neither the aim nor within the scope of this article to systematically review the literature. Studies that were considered interesting are presented here (Table 1). Two studies [32,33] were not randomised controlled trials.

On the other hand, a well-designed trial [34] demonstrated an increase in substance P levels following a cervical spinal manipulation. Another well-designed RCT [35] established an increase in neuropeptides involved in pain modulation such as neurotensin and oxytocin following a thoracic spinal manipulation; and an increase in cortisol levels following a cervical spinal manipulation. A recent study [36] showed that salivary cortisol levels dropped immediately and a reduced Testosterone to cortisol (T/C) ratio 6 h after a thoracic spinal manipulation. However, the study by Whelan et al. (2002) did not demonstrate any changes in basal cortisol levels following spinal manipulation. Further, manual therapy has been shown to influence endogenous opioids such as $_{\beta}$-endorphins [37], TNF- α, interleukins (IL-6, IL-10), and adrenaline [38–40]. Taken together, available evidence clearly supports the role for central mechanisms of inflammation and pain modulation following manual therapy.

HPA-HPG interaction – a dual hormone approach

It is well known that under normal conditions, cortisol, the end product of the HPA axis, provides the frontline of defence against stress or any threats to homeostasis [14]. However, as multiple systems interact in producing a response, it has been proposed that models need to include multiple measurements of stress-related biological processes in order to understand the role of an individual's response to stress [41]. Of particular importance is the interaction between the HPG axis and the HPA axis [42]. This can be easily observed by the inhibitory effects of stress on reproductive behaviour, including sex steroid release [43]. Rodent models clearly demonstrate that central administration of corticotropin releasing hormone inhibits the synthesis of sex hormones [44]. Further, apparent interactions between the end products of the gonadal (e.g. testosterone) and the adrenal axis (cortisol) have also been demonstrated [42]. In other words, activation of HPA axis (as in stress) generally inhibits the HPG axis.

The HPA-HPG axis interaction is certainly not unidirectional [45]. That is the HPA axis is subjected to gonadal influences and is reciprocally inhibited by the HPG axis [45]. Sex differences in both basal and stress induced HPA axis activity has been observed in a number of species with males showing lower levels of cortisol under both conditions [46]. The sex differences in HPA axis response may be attributed to the inhibitory effects of testosterone on the HPA axis [47]. For example, the ACTH and cortisol response to stress were found to be higher in male rats that had undergone gonadectomy and these effects were shown to be reversed with testosterone replacement therapy [47,48]. Evidence further indicates that sex steroids can also influence the magnitude of ACTH response to stress through their actions on both feedforward and feedback elements of the HPA axis [45].

The HPA axis has been shown to be dysfunctional in a number of chronic conditions including depression, central obesity,

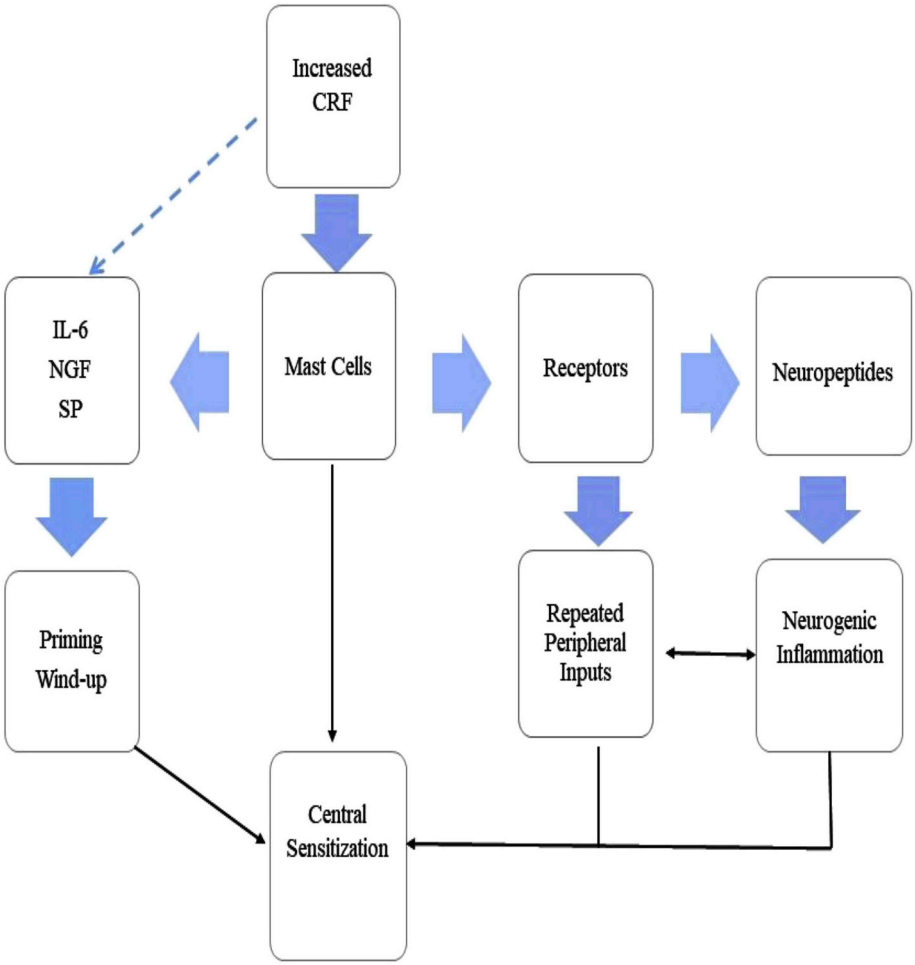

Fig. 1. Link between HPA axis and pain. CRF – Corticotropin Releasing Hormone, IL-6 – Interleukin-6, NGF – Nerve Growth Factor, SP – Substance-P.

hyperthyroidism, osteoporosis, atherosclerosis and rheumatoid arthritis [49]. Interestingly, females suffer from chronic pain more than their male counterparts. One proposed mechanism is that oestrogen increases the release of gamma interferon (a peripheral cytokine), which in turn increases cortisol levels [50]. On the other hand, males may suffer less from chronic pain as testosterone may inhibit cortisol through mechanisms explained previously. Either way, it can be seen that the HPA and the HPG axis interact with each other at various levels (functional cross-talk) with each axis mutually inhibiting the other [42]. This mutual inhibition may mean that multiple measures across these two systems (HPA-HPG for example) should be considered to provide an accurate representation of stress physiology.

Traditionally, studies have measured either ACTH or cortisol in body fluids (blood, saliva or urine) as an indicator of HPA axis activity [35,51]. Though this approach is still valid, the field of endocrinology has gradually moved towards a dual hormone approach, given the mutual inhibition between the HPA and HPG axis. The balance between testosterone and cortisol represented as a ratio termed T/C ratio may therefore provide a better estimation of the HPA axis activity (Fig. 2) [52]. Being hormones, both cortisol and testosterone exhibit circadian rhythmicity with peak concentrations in the morning with a gradual decline over the day and reaching a nadir around midnight [53,54]. An early morning increase in cortisol accelerates metabolism and stimulates gluconeogenesis, which in turn prepares the individual for the day. However, the increase in cortisol also results in increased skeletal protein degradation [55]. Hence, the increase in testosterone at around the same time may be an attempt to counterbalance the effects of cortisol on skeletal protein degradation [56]. This provides another example where the interaction between the two axes is apparent.

Discussion

This article reviewed the role of HPA axis in pain and inflammation. Secondly, evidence on manual therapy in influencing various biochemical markers of pain and/or inflammation was presented. Finally, recent developments in the field of endocrinology were provided and the use of T/C ratio as a marker of stress activity was discussed. These may have important implications for both osteopathic practice and research.

Manual therapy has been hypothesised to activate the liberation of various biochemical markers from neural tissue [57]. These biochemical markers include various neuropeptides such as neurotensin, oxytocin, substance P and hormones such as cortisol and oxytocin [58]. The interaction between the HPA axis and these biochemicals and its importance cannot be overstated. As explained previously, HPA axis may influence substance P through its actions on mast cells. Substance P in turn has been shown to modulate inhibitory transmission in lamina V of the spinal cord thoracic horn, thereby resulting in reduction of pain [59]. Further, neurotensin may play a role in stress induced analgesia. Therefore, the HPA axis may be an important therapeutic target for osteopaths (manual therapy in general).

Though some attempts have been made, the numbers of randomised controlled trials that have explored the effects of manual therapy on the HPA axis have been rather sporadic and disjointed. The evidence from available studies has shown that manual therapy can influence cortisol levels; however, the direction of change has been conflicting

Table 1
Studies included in the review.

Study/Design	Participant Characteristics	Intervention	Outcome Measure(s)	Findings
Brennan 1991	Randomised: 99 healthy volunteers.	Thoracic SM: 42 participants received a thoracic SMT (T1 to aT6). Sham: 38 participants received sham manipulation (low velocity, low amplitude thrust). Soft tissue: 19 participants received soft tissue manipulation to either the left or right gluteal area.	Plasma concentration SP 15 min pre and 15 min post-intervention	↑SP in SM group
Christian 1988 4 groups RCT.	Randomised: 40 males. 20 with pain and 20 pain-free.	Pain-free cervical SM: 10 asymptomatic participants received cervical SM. Pain cervical SM: 10 participants with pain received cervical SM. Pain-free sham: 10 asymptomatic participants received sham intervention where a very slight pressure was exerted on the neck. Pain sham: 10 participants with pain received sham intervention.	Plasma samples Cortisol ACTH β-endorphin Pre-intervention, 5 and 30 min post-intervention.	No changes in any outcome measures
Molina-Ortega 2014 3 groups RCT	Randomised: 30 healthy volunteers	Control: 10 participants received no intervention. Cervical SM: 10 participants received cervical manipulation. Thoracic SM: 10 participants received thoracic manipulation.	Serum samples NO2 SP Pre-intervention, immediately after and 2 h post-intervention.	↑SP in CSM group No effects on NO2
Padayachy et al., 2010 RCT Quasi-experimental.	Total of 30 participants with low back pain were randomised into group A or group B.	Group A: 15 participants received lumbar spinal manipulation. Group B: 15 participants received lumbar spinal manipulation	Day time serum cortisol levels.	An increase in the rate of change of daytime serum cortisol levels following SMT.
Plaza-Manzano 2014 3 groups RCT.	Randomised: 30 healthy participants.	Control: 10 participants received no intervention. Cervical SM: 10 participants received cervical manipulation. Thoracic SM: 10 participants received thoracic manipulation.	Serum samples neurotensin oxytocin orexin A cortisol. Samples were collected before, immediately after and 2 h after manipulation.	↑neurotensin, ↑oxytocin in CSM and TSM groups immediately. ↑cortisol in CSM group immediately.
Puhl 2012 2 group RCT.	Randomised: 56 healthy participants.	Thoracic SM: 18 participants received a thoracic SMT. Sham: 18 participants received sham manipulation (identical setup like SMT but without the thrust).	Plasma samples NE E Pre-intervention, immediately after and 15 min post-intervention.	No changes in E or NE levels.
Teodorczyk-Injeyan 2006 3 groups RCT.	Randomised: 64, healthy participants	Thoracic SM: 24 participants received a thoracic SMT. Sham: 20 participants received sham manipulation (identical setup like SMT but without the thrust). Control: participants (n = 20) did not receive any treatment.	Serum samples TNF-α SP IL-1 Pre-intervention, 20 min and 2 h post-intervention.	↓ IL-1$^{\beta}$ No effects on TNF-α or SP
Teodorczyk-Injeyan 2010 3 groups RCT.	Randomised: 74 healthy participants	Thoracic SM with cavitation: 27 participants received a thoracic SMT with an audible cavitation. Thoracic SM without cavitation: 25 participants received sham manipulation (identical setup like SMT but without cavitation). Control: participants (n = 22) in this group did not receive any treatment.	Serum samples PBMC IgG IgM Pre-intervention, 20 min and 2 h post-intervention.	↑IgG, ↑IgM in SM-C group at 20-min and 2 h post-intervention. Inconclusive.

(*continued on next page*)

Table 1 (*continued*)

Study/Design	Participant Characteristics	Intervention	Outcome Measure(s)	Findings
Tuchin 1998 Case series	Prospective case series, for six weeks duration set in adults (n = 9).	Chiropractic manipulation at spinal levels determined by practitioner (guided with X rays and palpation).	Primary: Changes in salivary cortisol, at baseline (2 weeks pre), 2 weeks treatment and 1 week post-treatment	
Whelan 2002 3 groups RCT.	Randomised: 30 healthy student volunteers	Control: 10 participants were just supine lying. No manipulation or vertebral positioning done. Sham: 10 participants were lying supine with their cervical spine positioned but without any manipulation. Cervical SM: An upper cervical manipulation was performed on 10 participants.	Salivary samples Cortisol 5 consecutive weeks. Week-1: 5 consecutive days. Week 2–5: pre-intervention, 5 and 60 min after intervention	No effects on basal cortisol levels.

Note. ACTH – Adreno-Corticotropic Hormone, CSM – Cervical Spinal Manipulation, fMRI- Functional magnetic Resonance Imaging, Ig – Immunoglobulin, IL – Interleukin, NO2 – Nitric Oxide, PBMC – Peripheral Blood Mononuclear Cells, PET – Positron Emission Tomography, 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.

[32,33,35,36]. While one study [35] reported an immediate increase in cortisol levels following intervention, another study [36] demonstrated an immediate decrease in cortisol levels. The studies differed in terms of intervention (thoracic vs cervical) and the methods of cortisol collection (salivary vs phlebotomy), which may explain the difference in findings. Being hormones, both cortisol and testosterone have a known circadian rhythm that could be influenced by a number of methodological factors including the timing of sampling; number and nature of study days; day of data collection; drinking habits; eating habits; dental hygiene/tooth brushing; smoking; and intense physical activity before data collection [14,60]. Therefore, adequate control of these factors is crucial for reliably measuring changes in hormone levels before and after an intervention.

A key recommendation of this review was the use of T/C ratio as a surrogate measure of HPA axis activity/response. This was based on findings that clearly show that the HPA and the HPG axes mutually inhibit each other. Hence, the T/C ratio (instead of just cortisol) may be a better representation of stress physiology. T/C ratio has been widely used in sports and exercise science research. This is because testosterone has both anabolic and anti-catabolic effects on muscle tissue [61]. On the other hand cortisol exerts catabolic effects on muscle tissue [62]. Therefore T/C ratio has been used as an indicator of overtraining [63]. In fact, a decrease in T/C ratio of more than 30% compared to baseline may indicate overtraining in athletes who are followed up over a long term [64,65]. This may be of interest to osteopaths or manual therapists who regularly treat people with overtraining injuries [66] (e.g. Achilles tendinopathy). T/C ratio has also been widely used to evaluate an individual's behaviour such as aggression, fear and catastrophizing [67,68]. It is well documented that manual therapy has non-specific responses such as placebo, patient expectation and alleviating fear [4]. However manual therapy study designs have not accounted for these non-specific effects adequately. Growing body of evidence indicates that hormones such as growth hormone and cortisol play a role in these non-specific responses [69,70]. Interestingly, a gender bias has also been noted in placebo response [71]. Hence it could be argued that future studies may make use of T/C ratio along with subjective questionnaires to measure these responses.

Recently, it has been shown that a thoracic spinal manipulation could influence the T/C ratio both immediately and at 6 h after the intervention in healthy men [36]. As T/C ratio has been established as a reliable measure to index general health status, increase in T/C ratio after intervention for example may clearly establish that the 'whole' person is addressed, which is the defining as well as the distinct philosophy of osteopathy. That is, it is more than just about biomechanical changes. An important limitation however revolves around the interpretation of hormonal changes following an intervention. Given there is a statistically significant change following an intervention, a pertinent question important to clinical practice may be 'what is the biological significance of these hormonal changes?' Biological significance or critical difference is defined as the 'least significant difference' thresholds considered to be clinically meaningful [2] and is to be discussed elsewhere.

Conclusion

When the body's homeostasis is under threat, the HPA axis provides the frontline of defence resulting in the production of cortisol. Measurement of cortisol has been long established as a reliable way to index HPA axis activity before and after an intervention. However, the stress response involves multiple other systems; of particular importance is the HPG axis, with cortisol and testosterone mutually inhibiting each other. For osteopathic clinicians, the functional cross-talk between the HPA and the HPG axis may be of interest given their implicit role in chronic pain and inflammation. For osteopathic researchers, it may be timely to adopt a dual hormone approach (testosterone and cortisol) while investigating the HPA axis. It has been

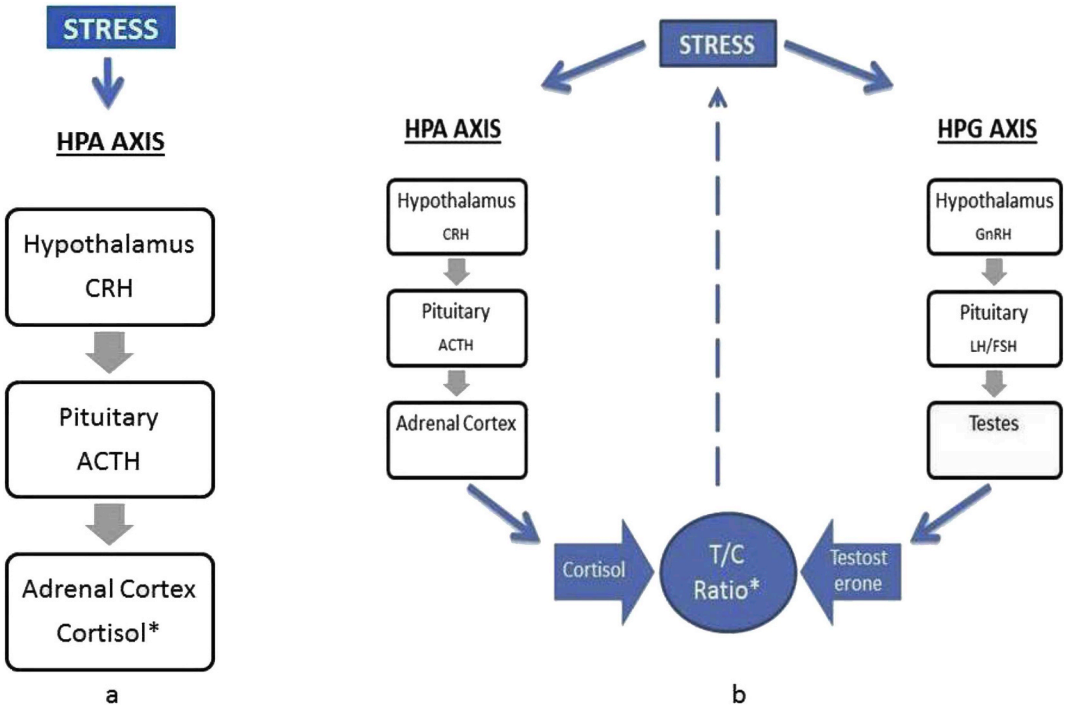

Fig. 2. Measurement of stress: (a) Traditional model; (b) Proposed model – a dual hormone approach. ACTH – Adrenocorticotropic Hormone, CRH – Corticotropin Releasing Hormone, FH – Follicle Stimulating Hormone, GnRH – Gonadotropin Releasing Hormone, LH- Luteinizing Hormone, * - Measure of Stress.

generally accepted that a higher T/C ratio is an indicator of good health and wellness. Hence, T/C ratio could be of interest for osteopathic research as it may enable us to capture the holistic effects of osteopathy. While preliminary findings have been positive, future longitudinal studies are required to shed more light in this least explored area of osteopathic research.

Conflicts of interest

The authors declare that they have no financial affiliation (including research funding) or involvement with any commercial organization that has a direct financial interest in any matter included in this manuscript. No other conflict of interest (ie, personal associations or involvement as a director, officer, or expert witness) are known.

Funding sources

Not applicable.

Ethical approval

Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijosm.2019.05.001.

References

[1] Ferreira M, Ferreira P, Latimer J. Does spinal manipulative therapy help people with chronic low back pain? Aust J Physiother 2002;48:277–84.
[2] Fraser CG, Fogarty Y. Interpreting laboratory results. BMJ Br Med J (Clin Res Ed) 1989;298(6689):1659–60.
[3] Sampath KK, Mani R, Miyamori T, Tumilty S. The effects of manual therapy or exercise therapy or both in people with hip osteoarthritis: a systematic review and meta-analysis. Clin Rehabil 2016;30(12):1141–55https://doi.org/10.1177/0269215515622670.
[4] Bialosky JE, Bishop MD, Price DD, Robinson ME, George SZ. The mechanisms of manual therapy in the treatment of musculoskeletal pain: a comprehensive model. Man Ther 2009;14:531–8https://doi.org/10.1016/j.math.2008.09.001.
[5] Flynn TW, Wainner RS, Fritz JM. Spinal Manipulation in physical therapist professional degree education: a model for teaching and integration into Ccinical practice. J Orthop Sport Phys Ther 2006;36(8):577–87https://doi.org/10.2519/jospt.2006.2159.
[6] Sueki DG, Cleland JA, Wainner RS. A regional interdependence model of musculoskeletal dysfunction: research, mechanisms, and clinical implications. J Man Manip Ther 2013;21(2):90–102.
[7] Bulbulian R, Burke J, Dishman JD. Spinal reflex excitability changes after lumbar spine passive flexion mobilization. J Manip Physiol Therapeut 2002;25(8):526–32.
[8] Dishman JD, Burke J. Spinal reflex excitability changes after cervical and lumbar spinal manipulation: a comparative study. Spine J 2003;3(3):204–12.
[9] Vicenzino B, Paungmali A, Buratowski S, Wright A. Specific manipulative therapy treatment for chronic lateral epicondylalgia produces uniquely characteristic hypoalgesia. Man Ther 2001;6(4):205–12.
[10] Sparks C, Cleland JA, Elliott JM, Zagardo M, Liu W-C. Using functional magnetic resonance imaging to determine if cerebral hemodynamic responses to pain change following thoracic spine thrust manipulation in healthy individuals. J Orthop Sport Phys Ther 2013;43(5):340–8.
[11] Vicenzino B, Cartwright T, Collins D, Wright A. Cardiovascular and respiratory changes produced by lateral glide mobilization of the cervical spine. Man Ther 1998;3(2):67–71https://doi.org/10.1016/S1356-689X(98)80020-9.
[12] Brennan P, Triano J, McGregor M. Enhanced neutrophil respiratory burst as a biological marker for manipulation forces: duration of the effect and association with substance P and tumor necrosis factor. J Manip Physiol Therapeut 1992;15(2):83–9.
[13] Brennan PC, Kokjohn K, Kaltinger CJ, Lohr GE, Glendening C, Hondras MA, et al. Enhanced phagocytic cell respiratory burst induced by spinal manipulation: potential role of substance P. J Manip Physiol Therapeut 1991;14(7):399–408.
[14] Chrousos GP, Gold PW. The concepts of stress and stress system disorders: overview of physical and behavioral homeostasis. J Am Med Assoc 1992;267(9):1244–52https://doi.org/10.1001/jama.1992.03480090092034.
[15] Canon WB. Organization for physiological homeostasis. Physiol Rev 1929;9(3):399–431.
[16] Hackett RA, Steptoe A. Type 2 diabetes mellitus and psychological stress — a modifiable risk factor. Nat Rev Endocrinol 2017;13:547–60https://doi.org/10.1038/nrendo.2017.64.
[17] Elhamdani A, Palfrey CH, Artalejo CR. Ageing changes the cellular basis of the "fight-or-flight" response in human adrenal chromaffin cells. Neurobiol Aging 2002;23(2):287–93.
[18] Horrocks P, Jones A, Ratcliffe W, Holder G, White A, Holder R, et al. Patterns of ACTH and cortisol pulsatility over twenty four hours in normal males and females. Clin Endocrinol 1990;32(1):127–34.
[19] Rashid S, Lewis GF. The mechanisms of differential glucocorticoid and mineralocorticoid action in the brain and peripheral tissues. Clin Biochem

2005;38(5):401–9.
[20] Woolf CJ. Pain: moving from symptom control toward mechanism-specific pharmacologic management. Ann Intern Med 2004;140(6):441–51https://doi.org/10.7326/0003-4819-140-8-200404200-00010.
[21] Meeus M, Nijs J. Central sensitization: a biopsychosocial explanation for chronic widespread pain in patients with fibromyalgia and chronic fatigue syndrome. Clin Rheumatol 2007 April 01;26(4):465–73https://doi.org/10.1007/s10067-006-0433-9.
[22] Reichling DB, Levine JD. Critical role of nociceptor plasticity in chronic pain. Trends Neurosci 2009;32(12):611–8. 2009/12/01/ https://doi.org/10.1016/j.tins.2009.07.007.
[23] Ikeda H, Stark J, Fischer H, Wagner M, Drdla R, Jäger T, et al. Synaptic amplifier of inflammatory pain in the spinal dorsal horn. Science 2006;312(5780):1659–62https://doi.org/10.1126/science.1127233.
[24] Theoharides TC, Cochrane DE. Critical role of mast cells in inflammatory diseases and the effect of acute stress. J Neuroimmunol 2004;146(1):1–12. 2004/01/01/ https://doi.org/10.1016/j.jneuroim.2003.10.041.
[25] Rozniecki JJ, Dimitriadou V, Lambracht-Hall M, Pang X, Theoharides TC. Morphological and functional demonstration of rat dura mater mast cell–neuron interactions in vitro and in vivo. Brain Res 1999;849(1):1–15. 1999/12/04/ https://doi.org/10.1016/S0006-8993(99)01855-7.
[26] Steinhoff M, Vergnolle N, Young SH, Tognetto M, Amadesi S, Ennes HS, et al. Agonists of proteinase-activated receptor 2 induce inflammation by a neurogenic mechanism. Nat Med 2000;6:151. [Article]. https://doi.org/10.1038/72247.
[27] Leon A, Buriani A, Dal Toso R, Fabris M, Romanello S, Aloe L, et al. Mast cells synthesize, store, and release nerve growth factor. Proc Natl Acad Sci Unit States Am 1994;91(9):3739–43https://doi.org/10.1073/pnas.91.9.3739.
[28] Chapman CR, Nakamura Y. A passion of the soul: an introduction to pain for consciousness researchers. Conscious Cognit 1999;8(4):391–422. 1999/12/01/ https://doi.org/10.1006/ccog.1999.0411.
[29] Peters A, McEwen BS, Friston K. Uncertainty and stress: why it causes diseases and how it is mastered by the brain. Prog Neurobiol 2017;156:164–88. 2017/09/01/ https://doi.org/10.1016/j.pneurobio.2017.05.004.
[30] McEwen BS. Physiology and neurobiology of stress and adaptation: central role of the brain. Physiol Rev 2007;87(3):873–904https://doi.org/10.1152/physrev.00041.2006.
[31] Bot I, Kuiper J. Stressed brain, stressed heart? The Lancet 2017;389(10071):770–1https://doi.org/10.1016/s0140-6736(17)30044-2.
[32] Padayachy K, Vawda GHM, Shaik J, McCarthy PW. The immediate effect of low back manipulation on serum cortisol levels in adult males with mechanical low back pain. Clin Chiropr 2010;13(4):246–52https://doi.org/10.1016/j.clch.2010.05.002.
[33] Tuchin PJ. The effect of chiropractic spinal manipulative therapy on salivary cortisol levels. Australas Chiropr Osteopathy 1998 Jul;7(2):86–92.
[34] Molina-Ortega F, Lomas-Vega R, Hita-Contreras F, Manzano GP, Achalandabaso A, Ramos-Morcillo AJ, et al. Immediate effects of spinal manipulation on nitric oxide, substance P and pain perception. Man Ther 2014 Oct;19(5):411–7https://doi.org/10.1016/j.math.2014.02.007.
[35] Plaza-Manzano G, Molina F, Lomas-Vega R, Martinez-Amat A, Achalandabaso A, Hita-Contreras F. Changes in biochemical markers of pain perception and stress response after spinal manipulation. J Orthop Sport Phys Ther 2014 Apr;44(4):231–9https://doi.org/10.2519/jospt.2014.4996.
[36] Sampath KK, Botnmark E, Mani R, Cotter JD, Katare R, Munasinghe PE, et al. Neuroendocrine response following a thoracic spinal manipulation in healthy men. J Orthop Sport Phys Ther 2017;47(9):617–27https://doi.org/10.2519/jospt.2017.7348.
[37] Vernon HT, Dhami MS, Howley TP, Annett R. Spinal manipulation and beta-endorphin: a controlled study of the effect of a spinal manipulation on plasma beta-endorphin levels in normal males. J Manip Physiol Therapeut 1986;9(2):115–23.
[38] Puhl AA, Injeyan HS. Short-term effects of manipulation to the upper thoracic spine of asymptomatic subjects on plasma concentrations of epinephrine and norepinephrine—a randomized and controlled observational study. J Manip Physiol Therapeut 2012;35(3):209–15https://doi.org/10.1016/j.jmpt.2012.01.012.
[39] Teodorczyk-Injeyan JA, Injeyan HS, Ruegg R. Spinal manipulative therapy reduces inflammatory cytokines but not substance P production in normal subjects. J Manip Physiol Therapeut 2006;29(1):14–21.
[40] Teodorczyk-Injeyan JA, McGregor M, Ruegg R, Injeyan HS. Interleukin 2-regulated in vitro antibody production following a single spinal manipulative treatment in normal subjects. Chiropr Osteopathy 2010;18(1):1.
[41] Kivlighan KT, Granger DA. Salivary α-amylase response to competition: relation to gender, previous experience, and attitudes. Psychoneuroendocrinology 2006;31(6):703–14https://doi.org/10.1016/j.psyneuen.2006.01.007.
[42] Bedgood D, Boggiano MM, Turan B. Testosterone and social evaluative stress: the moderating role of basal cortisol. Psychoneuroendocrinology 2014;47:107–15https://doi.org/10.1016/j.psyneuen.2014.05.007.
[43] Rivier C, Rivest S. Effect of stress on the activity of the hypothalamic-pituitary-gonadal Axis: peripheral and central Mechanisms1. Biol Reprod 1991;45(4):523–32https://doi.org/10.1095/biolreprod45.4.523.
[44] Porter DWF, Lincoln DW, Naylor AM. Plasma cortisol is increased during the inhibition of LH secretion by central LHRH in the Ewe. Neuroendocrinology 1990;51(6):705–12https://doi.org/10.1159/000125414.
[45] Viau V. Functional cross-talk between the hypothalamic-pituitary-gonadal and -adrenal axes. J Neuroendocrinol 2002;14(6):506–13https://doi.org/10.1046/j.1365-2826.2002.00798.x.
[46] Handa RJ, Burgess LH, Kerr JE, O'Keefe JA. Gonadal steroid hormone receptors and sex differences in the hypothalamo-pituitary-adrenal Axis. Horm Behav 1994;28(4):464–76. 1994/12/01/ https://doi.org/10.1006/hbeh.1994.1044.
[47] Viau V, Meaney M. The inhibitory effect of testosterone on hypothalamic-pituitary-adrenal responses to stress is mediated by the medial preoptic area. J Neurosci 1996;16(5):1866–76https://doi.org/10.1523/jneurosci.16-05-01866.1996.
[48] Handa RJ, Nunley KM, Lorens SA, Louie JP, McGivern RF, Bollnow MR. Androgen regulation of adrenocorticotropin and corticosterone secretion in the male rat following novelty and foot shock stressors. Physiol Behav 1994;55(1):117–24. 1994/01/01/ https://doi.org/10.1016/0031-9384(94)90018-3.
[49] Tsigos C, Chrousos GP. Hypothalamic–pituitary–adrenal axis, neuroendocrine factors and stress. J Psychosom Res 2002;53(4):865–71.
[50] Truchon M. Determinants of chronic disability related to low back pain: towards an integrative biopsychosocial model. Disabil Rehabil 2001;23(17):758–67. 2001/01/01 https://doi.org/10.1080/09638280110061744.
[51] Christian GF, Stanton GJ, Sissons D, How HY, Jamison J, Alder B, et al. Immunoreactive ACTH,[beta]-Endorphin, and cortisol levels in plasma following spinal manipulative therapy. Spine 1988;13(12):1411–7.
[52] Filaire E, Bernain X, Sagnol M, Lac G. Preliminary results on mood state, salivary testosterone: cortisol ratio and team performance in a professional soccer team. Eur J Appl Physiol 2001;86(2):179–84https://doi.org/10.1007/s004210100512.
[53] Adam EK, Kumari M. Assessing salivary cortisol in large-scale, epidemiological research. Psychoneuroendocrinology 2009;34(10):1423–36. 2009/11/01/ https://doi.org/10.1016/j.psyneuen.2009.06.011.
[54] Touitou Y, Haus E. Alterations with aging of the endocrine and neuroendocrine circadian system in humans. Chronobiol Int 2000;17(3):369–90. 2000/01/01 https://doi.org/10.1081/cbi-100101052.
[55] Dinneen S, Alzaid A, Miles J, Rizza R. Metabolic effects of the nocturnal rise in cortisol on carbohydrate metabolism in normal humans. J Clin Investig 1993;92(5):2283–90https://doi/org/10.1172/jci116832.
[56] Kraemer WJ, Häkkinen K, Newton RU, McCormick M, Nindl BC, Volek JS, et al. Acute hormonal responses to heavy resistance exercise in younger and older men. Eur J Appl Physiol Occup Physiol 1998 February 01;77(3):206–11https://doi.org/10.1007/s004210050323.
[57] Skyba DA, Radhakrishnan R, Rohlwing JJ, Wright A, Sluka KA. Joint manipulation reduces hyperalgesia by activation of monoamine receptors but not opioid or GABA receptors in the spinal cord. Pain 2003;106(1):159–68https://doi.org/10.1016/S0304-3959(03)00320-8.
[58] Dobner PR. Neurotensin and pain modulation. Peptides 2006;27(10):2405–14https://doi.org/10.1016/j.peptides.2006.04.025.
[59] Nakatsuka T, Chen M, Takeda D, King C, Ling J, Xing H, et al. Substance P-driven feed-forward inhibitory activity in the mammalian spinal cord. Mol Pain 2005;1(1):1https://doi.org/10.1186/1744-8069-1-20.
[60] Stalder T, Kirschbaum C, Kudielka BM, Adam EK, Pruessner JC, Wüst S, et al. Assessment of the cortisol awakening response: expert consensus guidelines. Psychoneuroendocrinology 2016;63:414–32https://doi.org/10.1016/j.psyneuen.2015.10.010.
[61] Hayes LD, Grace FM, Kilgore J, Young J, Baker J. Diurnal variation of cortisol, testosterone, and their ratio in apparently healthy males. Sport SPA 2012;9(1):5–13.
[62] Florini JR. Hormonal control of muscle growth. Muscle Nerve 1987;10(7):577–98.
[63] Hug M, Mullis PE, Vogt M, Ventura N, Hoppeler H. Training modalities: over-reaching and over-training in athletes, including a study of the role of hormones. Best Pract Res Clin Endocrinol Metabol 2003;17(2):191–209https://doi.org/10.1016/S1521-690X(02)00104-5.
[64] Adlercreutz H, Härkönen M, Kuoppasalmi K, Näveri H, Huhtaniemi I, Tikkanen H, et al. Effect of training on plasma anabolic and catabolic steroid hormones and their response during physical exercise. Int J Sports Med 1986;7(Suppl 1):27–8. 1986/06//.
[65] Maso F, Lac G, Filaire E, Michaux O, Robert A. Salivary testosterone and cortisol in rugby players: correlation with psychological overtraining items. Br J Sports Med 2004;38(3):260–3https://doi.org/10.1136/bjsm.2002.000254.
[66] Hayes LD, Bickerstaff GF, Baker JS. Interactions of cortisol, testosterone, and resistance training: influence of circadian rhythms. Chronobiol Int 2010;27(4):675–705https://doi.org/10.3109/07420521003778773.
[67] Carré JM, Mehta PH. Importance of considering testosterone–cortisol interactions in predicting human aggression and dominance. Aggress Behav 2011;37(6):489–91https://doi.org/10.1002/ab.20407.
[68] Terburg D, Morgan B, van Honk J. The testosterone–cortisol ratio: a hormonal marker for proneness to social aggression. Int J Law Psychiatry 2009;32(4):216–23https://doi.org/10.1016/j.ijlp.2009.04.008.
[69] Enck P, Benedetti F, Schedlowski M. New insights into the placebo and nocebo responses. Neuron 2008;59(2):195–206. 2008/07/31/ https://doi.org/10.1016/j.neuron.2008.06.030.
[70] Benedetti F, Pollo A, Lopiano L, Lanotte M, Vighetti S, Rainero I. Conscious expectation and unconscious conditioning in analgesic, motor, and hormonal placebo/nocebo responses. J Neurosci 2003;23(10):4315–23https://doi.org/10.1523/jneurosci.23-10-04315.2003.
[71] Aslaksen PM, Bystad M, Vambheim SM, Flaten MA. Gender differences in placebo analgesia: event-related potentials and emotional modulation. Psychosom Med 2011;73(2):193–9https://doi.org/10.1097/PSY.0b013e3182080d73.