Single Osteopathic Manipulative Therapy Session Dampens Acute Autonomic and Neuroendocrine Responses to Mental Stress in Healthy Male Participants

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Financial Disclosures: None reported.

Support: Funding for this study was provided by Collegio Italiano di Osteopatia.

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Submitted September 19, 2016; final revision received March 9, 2017; accepted March 14, 2017.

Context: The efficacy of osteopathic manipulative therapy (OMTh; manipulative care provided by foreign-trained osteopaths) is supported by observational data and patient feedback, but there is still a need for objective, quantitative biomarkers that allow measurement of the underlying mechanisms. No study exploring the protective potential of OMTh for mental stress has been published, to the authors' knowledge.

Objectives: To explore the modulating effect of OMTh on autonomic neural regulation of the heart and verifiy its ability to influence the activity of the hypothalmic-pituitary-adrenocortical axis.

Methods: Healthy young adult men who had never received OMTh were exposed to either a brief protocol using craniosacral techniques or sham therapy (control) involving the same anatomical areas. A laboratory stress episode consisting of a 5-minute arithmetic task participants were required to perform in front of a committee preceded the therapy sessions. Continuous electrocardiograph recordings were done before, during, and after the stress episode. Heart rate and frequency-domain parameters of heart rate variability (specifically, high-frequency component power in normalized units and the ratio of low-frequency to high-frequency power) were measured to quantify the activity of the parasympathetic nervous system and the state of sympathovagal balance at the level of the heart, respectively. Saliva samples were also collected at points throughout the study to determine cortisol levels.

Results: Osteopathic manipulative therapy reduced the overall chronotropic effect of the stressor (t=−2.9, P<.05) and counteracted the vagal withdrawal and the shift of autonomic balance toward sympathetic prevalence (t=−2.8, P<.05) that were observed in control participants. Moreover, OMTh participants had a much lower overall cortisol level during the mental stressor compared with control participants (t=−2.3, P<.05). Participants in the OMTh group did not show the statistically significant reduction in the amplitude of the cortisol awakening response observed in their control counterparts after the stress episode (control: t=2.7, P<.05; OMT: P=.83).

Conclusion: The application of a single OMTh session to healthy participants induced a faster recovery of heart rate and sympathovagal balance after an acute mental stressor by substantially dampening parasympathetic withdrawal and sympathetic prevalence. The OMTh session also prevented the typical increase in cortisol levels observed immediately after a brief mental challenge.

J Am Osteopath Assoc. 2017;117(9):559-567
doi:10.7556/jaoa.2017.110

Keywords: cortisol, hypothalmic-pituitary-adrenocortical axis, mental stress, osteopathic manipulation

The diagnosis and therapeutic approach of osteopathy (manipulative care provided by foreign-trained osteopaths) emphasizes the relationship between structure and function in the body and the ways it can be affected through osteopathic manipulative therapy (OMTh).[1] The efficacy evidence of OMTh techniques mostly relies on observational data and feedback from patients, involving pain scale and motion range improvements, as well as other empiric measurements.[2,3] Nonetheless, the practice of OMTh has long recognized a link between health and autonomic nervous system functioning. Notwithstanding, only sporadic studies that support the favorable effects of OMTh on the autonomic neural modulation of peripheral target organs are available. Since the late 1970s, this relative paucity of data was the consequence of the difficulty to obtain accurate measurements of sympathovagal balance in a noninvasive manner. Over the past 4 decades, indirect methods that provide noninvasive and reliable information on the autonomic neural input to the heart have been developed and validated.[4-6] Among them, heart rate variability (HRV) analysis is by far the most popular and powerful.[4-6]

In more recent years, by means of HRV measurement techniques, the impact of OMT (osteopathic manipulative treatment) on cardiac sympathovagal balance has been assessed.[7-10] For example, a single cervical OMT protocol (upper cervical spine manipulation) was applied to healthy normotensive participants under resting conditions, and its effects were compared with sham therapy (placement of fingers with no pressure exerted).[9] The treatment resulted in an increase in the spectral power in the high-frequency (HF) band, indicative of parasympathetic modulation of heart rate (HR) and a decrease in the ratio of low-frequency (LF) to HF power (LF:HF), indicating sympathovagal balance, compared with the sham treatment.[9] In a study by Henley et al,[7] the immediate effects of OMT were explored not only at rest but also after a 50° head-up tilt challenge, a postural change that determines sympathetic excitation, concomitant vagal withdrawal, and a shift in sympathovagal balance toward sympathetic prevalence.[11] The cervical myofascial release technique dampened tilt-induced sympathetic prevalence significantly, as shown by a lower LF:HF ratio compared with that found after sham manipulation.[7] In a 2015 study, Ruffini et al[10] reported a significant increase in HF power as well as a reduction in LF:HF ratio in resting participants undergoing OMT, thus supporting the view that OMT influences autonomic neural modulation of HR by increasing parasympathetic activity and shifting sympathovagal balance toward vagal prevalence.[10] This experimental evidence suggests that OMT is a potentially powerful approach to enhance the parasympathetic input to the heart and hence prevent exaggerated stress-induced sympathetically driven cardiac activations. However, we are not aware of any study that has explored the protective potential of OMTh in challenging contexts involving mental stress, which has been reported to have large effects on cardiac autonomic modulation.[12]

There seems to be scant information available regarding the effects of OMTh on the major neuroendocrine axis involved in the stress response via the hypothalamic-pituitary-adrenocortivcal (HPA) axis. We are aware of only 1 study that investigated adrenocortical function (together with cardiac autonomic reactivity) after an OMT technique.[8] This study showed that rhythmic rib raising decreased sympathetic efferent activity as grossly measured by α-amylase level in saliva, but it had little effect on parasympathetic activity as grossly determined via salivary flow rate and HPA-axis function as indicated by salivary cortisol levels.

To contribute to the understanding of the potentially favorable effects of OMTh on both cardiac autonomic neural modulation and HPA axis activity regulation under challenging conditions, we performed a pilot study on healthy adult male participants, in which an acute psychological stressor was immediately followed by a brief OMTh or sham therapy session, with HRV and salivary cortisol levels used as biomarkers. We hypothesized that participants receiving OMTh would experience a faster recovery of autonomic and neuroendocrine stress activation.

Methods

The study protocol was approved by the institutional review board of the Collegio Italiano di Osteopatia in Parma. Written informed consent was obtained from all participants.

Participants

From April 2015 through April 2016, we recruited healthy male university students aged 20 to 30 years. Participants confirmed that: (1) they were not under any kind of long-term pharmacologic treatment, (2) they were not smokers, (3) they had no history of cardiovascular disease, (4) they were free from chronic pain or acute symptoms during the 72 hours before data collection, and (5) they had never received OMTh. Participants were instructed not to consume alcoholic drinks or perform sustained physical activity in the 24 hours preceding their laboratory session.

Experimental Protocol

Participants were randomly assigned to 1 of 2 experimental groups: OMTh, which entailed undergoing a single OMTh craniosacral technique session, and control, which comprised a single sham therapy session. Participants were allowed to have a light lunch at least 1 hour before the laboratory session, which was performed between 2:30 pm to 5:30 pm in a quiet room at a comfortable temperature (approximately 22°C).

The procedure began with a 15-minute adaptation phase (−30 to −15 minutes), during which 2 electrocardiograph (ECG) electrodes were secured to the right and left parasternal regions. A third electrode was secured on the right side of the groin area for reference. Respiratory rate was also recorded.

The ECG and the respiratory activity were continuously recorded with the participant in a supine position during the following phases (**Figure 1**): (1) baseline (−15 to −5 minutes); (2) stress (−5 to 0 minutes); (3) intervention (0-20 minutes); and (4) recovery (20-50 minutes). Saliva was collected 5 times during this period, at −15, −5, 20, 35, and 50 minutes.

The stressor consisted of a 5-minute arithmetic (subtraction) task, described elsewhere,[13,14] performed in front of a committee of 3 people (2 men [L.C. and A.S.] and 1 woman).

The day before (day −1) and after (day +1) the laboratory session, participants collected 2 saliva samples at home: on awakening and 30 minutes after awakening to allow the computation of the cortisol awakening response (CAR; ie, the Δ value calculated by subtracting the level obatined on awakening from the value obtained 30 minutes after awakening). Participants were instructed to abstain from eating, drinking stimulant beverages (eg, tea, coffee, alcohol), brushing their teeth, or smoking before taking the saliva samples.

Interventions

Immediately after the arithmetic task, participants received either OMTh or sham therapy, with only 2 operators in the experimental room: the osteopath (M.F.) and a researcher collecting physiologic data (L.C.).

The OMTh protocol lasted 20 minutes. The techniques used were at the discretion of the osteopath, although they were limited to craniosacral areas.[10,15] The sham therapy also lasted 20 minutes and consisted of light touch to the same craniosacral areas involved in the OMTh protocol.

For the sake of protocol homogeneity, all interventions were performed by the same osteopath.

ECG Data Acquisition and Processing

Continuous ECG recordings at a sampling frequency of 250 Hz were obtained by means of the BT16Plus System (version 1.9.0; Francesco Marazza, Hardware & Software) in 13 consecutive recording epochs, each lasting 5 minutes: 2 during baseline, 1 during the arithmetic task, 4 during the intervention, and 6 during the recovery phase (**Figure 1**).

BT16Plus is a system for real-time acquisition of physiologic signals. The main unit is a small device that collects ECG, respiration, and gross physical activity. The signals captured by transducers are amplified,

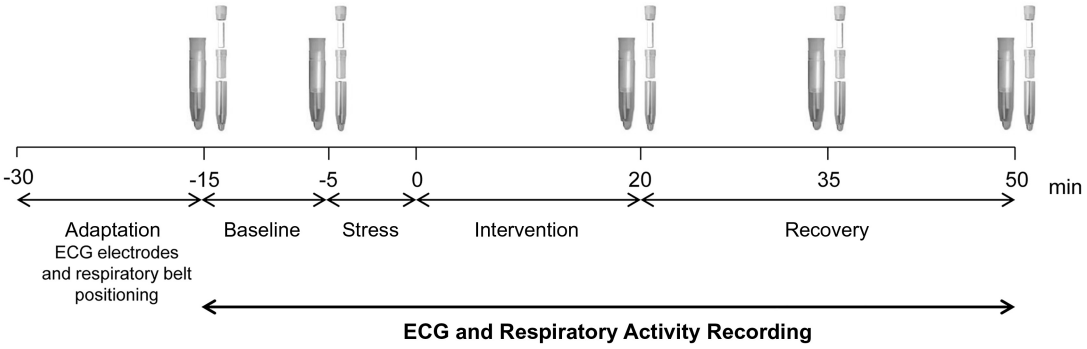

Figure 1.
Outline of the procedures used in the laboratory session of the current study in which osteopathic manipulative therapy (OMTh; manipulative care provided by foreign-trained osteopaths) was proposed to dampen acute autonomic and neuroendocrine responses to mental stress. Saliva was collected at −15, −5, 20, 35, and 50 minutes. *Abbreviation:* ECG, electrocardiogram.

converted to digital format, and transferred to a laptop computer by means of a bluetooth connection. The software also performs real-time wave form visualization.

Analyses of ECG recordings were performed by means of Chart5 software (ADInstruments). Initially, each raw ECG signal was manually inspected to ensure that all R waves were correctly detected. We then calculated HR by plotting the number of R waves per unit time (beats/min). Subsequently, we quantified frequency domain parameters of HRV. The power spectrum was obtained with a fast Fourier transform-based method (Welch periodogram: 256 points, 50% overlap, and Hamming window). We quantified the power of the LF (0.04-0.15 Hz) and HF (0.15-0.4 Hz) bands in normalized unit values. The power of an LF band is a nonspecific index, as it contains contributions of both the sympathetic and parasympathetic influences[16]; the power of the HF band results from the activity of the parasympathetic nervous system and includes respiration-linked oscillations of HR.[17] The LF:HF ratio estimates the fractional distribution of power and is taken as a synthetic measure of sympathovagal balance.[5] The parts of ECG recordings that were nonstationary or exhibited recording artifacts were excluded from the analysis in accordance with an automatic test checking stationarity of the mean and variance of HR.[5,18]

Saliva Collection and Cortisol Determinations

Oral swabs and swab storage tubes were used to collect saliva samples. The samples were frozen at −20°C until analysis by radioimmunoassay. At that time, samples were thawed, brought to room temperature, and centrifuged (1500 g × 10 minutes), resulting in a clear supernatant of low viscosity. Cortisol levels were assayed in duplicate (all those belonging to the same participant were included in the same assay) following kit instructions with a 96-well plate high-sensitivity salivary cortisol enzyme immunoassay kit (Salimetrics, Inc) using an Infinite F50 plate reader and Magellan software (Tecan Group Ltd).

Statistical Analysis

All statistical analyses were performed using SPSS software (version 23; IBM). Statistical significance was set at $P<.05$.

Baseline values for ECG parameters (HR, high-frequency component power in normalized units [Hfnu], and LF:HF) and saliva cortisol levels on day 0 were calculated as a mean of the 2 values obtained during the resting phase.

Two-way analyses of variance for repeated measures were applied to ECG data on day 0, with time as the within-participant factor (12 levels: baseline, stress,

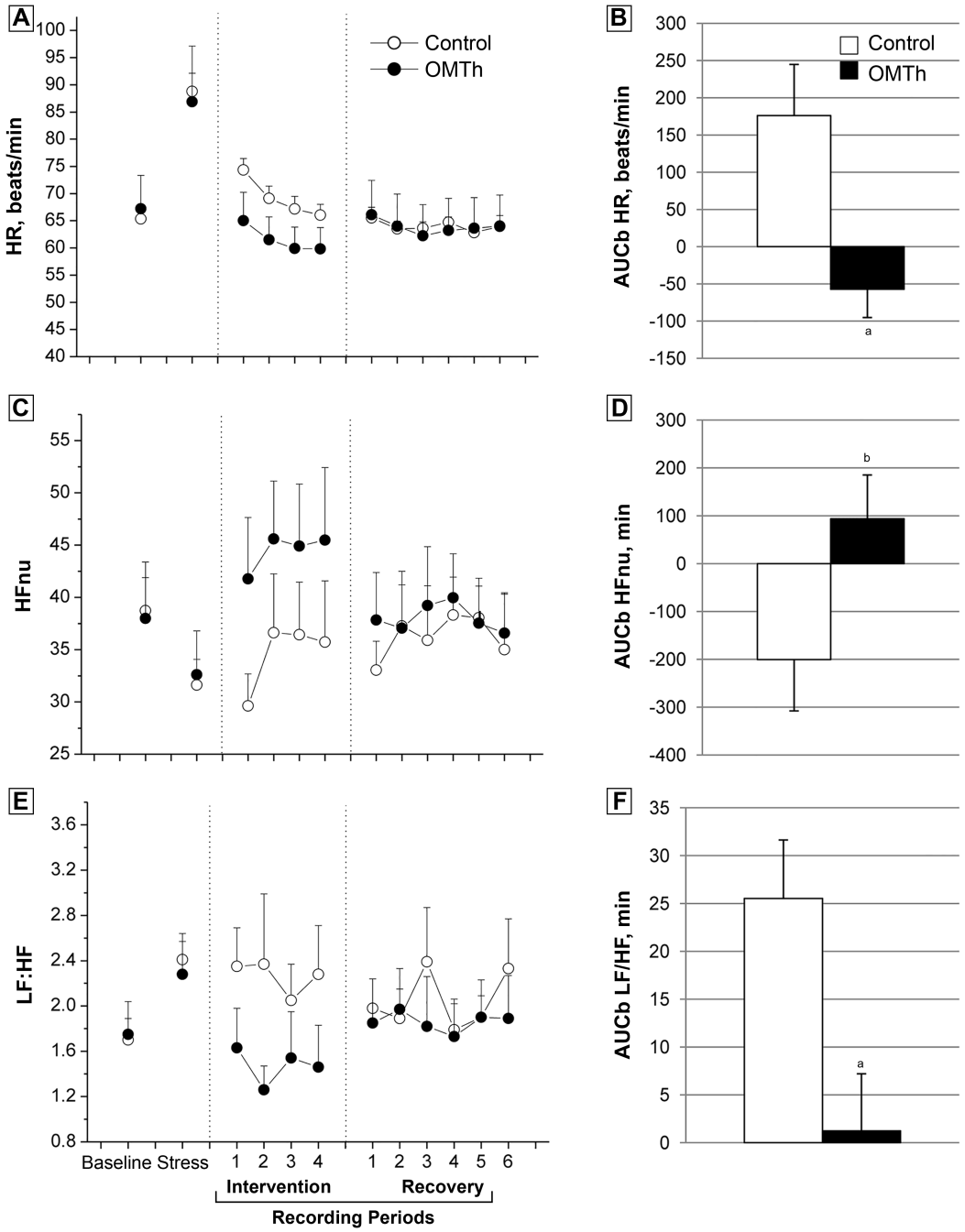

Figure 2.

Time course of changes in (A) heart rate (HR), (C) high frequency component power in normalized units (Hfnu), and (E) the ratio of low frequency power to high frequency power (LF:HF) across the consecutive recording periods (each lasting 5 minutes) of the laboratory session, in participants exposed to either osteopathic manipulative therapy (OMTh; manipulative care provided by foreign-trained osteopaths) (n=10) or sham therapy (control) (n=10). Area under the response time curve above the baseline (AUCb) for (B) HR, (D) Hfnu, and (F) LF:HF. [a]*P*<.05; [b]*P*=.056.

4 during manipulation, and 6 during recovery) and therapy as the between-participant factor (2 levels: OMTh and sham); cortisol data on day 0, with time as within participant factor (4 levels: baseline and 20, 35, and 50 minutes) and therapy as between-participant factor (2 levels: OMTh and sham); and CAR values, with time as within-participant factor (2 levels: day −1 and day +1) and therapy as between-participant factor (2 levels: OMTh and sham).

Follow-up analyses were conducted using *t* tests, with a Bonferroni correction for multiple comparisons for each outcome variable separately. However, to have a quantification of the individual, overall autonomic and neuroendocrine responsivity to the stressor +manipulation, HR, HRV, and cortisol data were also quantified as the area under the response time curve above baseline (AUCb).[19] Possible differences in AUCb values between OMTh and sham therapy participants were assessed by means of a *t* test.

Results

Twenty participants were included in the study—10 in the experimental group and 10 in the control group. No significant differences were found between control and OMTh participants for age (mean [SE], 24.1 [0.6] vs 24.2 [0.8] years), body weight (80.8 [3.2] vs 75.8 [2.6] kg), height (182.9 [2.8] vs 179.7 [2.0] m), or body mass index (24.1 [0.6] vs 23.5 [0.8]).

HR and HRV

The temporal dynamics of the values of HR and HRV indexes (HFnu and LF:HF) are reported in **Figure 2**. Two-way analysis of variance yielded a significant effect of time for HR (F=40.7, P<.01) but no statistically significant effects for HFnu and LF:HF.

Figure 2B, **D**, and **F** report the values of the AUCb for HR and HRV parameters. The AUCb values of HR were significantly lower in the OMTh group compared with corresponding control values (t=−2.9, P<.05), AUCb values of HFnu were tendentially higher (t=2.08, P=.056), and LF:HF values were significantly lower (t=−2.8, P<.05). Therefore, although absolute HR and HRV values did not significantly differ between the 2 groups in any recording phase (**Figure 2A**, **C**, and **E**), OMTh reduced the overall chronotropic effect and counteracted the vagal withdrawal and the shift of autonomic balance toward sympathetic prevalence that were observed in control participants.

Salivary Cortisol

The salivary cortisol levels are shown in **Figure 3**. Two-way analysis of variance revealed a significant effect of therapy (F=4.8, P<.05). The OMTh group had significantly lower values of salivary cortisol levels at 20 minutes (t=−2.4, P<.05) and 35 minutes (t=−2.3, P<.05) (**Figure 3**).

For cortisol levels, AUCb values were significantly lower in the OMTh group compared with the control group (−0.73 [0.93] vs 2.43 [0.99] $\mu g/dL^{-1} \times min^{-1}$; t=−2.3, P<.05).

Both groups had similar CAR values on day −1 (0.21 [0.08] vs 0.18 [0.08] μg/dL), as depicted in **Figure 4**. On day +1, CAR values were still not statistically different between groups (0.22 [0.06] vs 0.09 [0.03] μg/dL; P=.06). However, OMTh participants

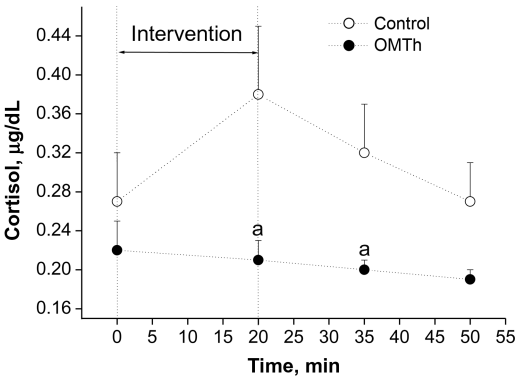

Figure 3.
Time course of changes in salivary cortisol concentration during the laboratory session, in participants exposed to osteopathic manipulative therapy (OMTh; manipulative care provided by foreign-trained osteopaths) (n=10) and sham therapy (control) (n=10). Time point 0 refers to the mean value obtained from −15 and −5 baseline. [a]P<.05.

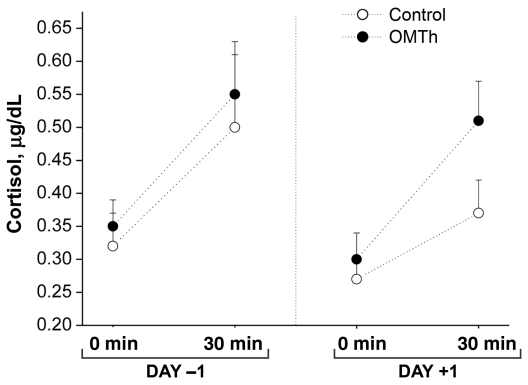

Figure 4.
Salivary cortisol levels on awakening (0 minutes) and 30 minutes after awakening on the day before (day −1) and the day after (day +1) the laboratory session, in participants exposed to osteopathic manipulative therapy (OMTh; manipulative care provided by foreign-trained osteopaths) (n=10) and sham therapy (control) (n=10).

did not show the significant reduction in CAR amplitude between day +1 and day −1 that was observed in control participants (control: 0.18 [0.08] vs 0.09 [0.03] µg/dL, t=2.7, P<.05; OMT: 0.21 [0.08] vs 0.22 [0.06] µg/dL, P=.83).

Discussion

The present study examined the potential dampening effect of OMTh on short-term cardiac and adrenocortical responses to a mental stressor consisting of a brief arithmetic task.

The major and novel findings of this study were that a single OMTh session (1) favored cardiac chronotropic return to baseline after an acute mental stressor by reducing parasympathetic withdrawal and sympathetic prevalence and (2) prevented the typical cortisol rise observed immediately after a brief mental challenge. In addition, OMTh seemed to abolish the day-after stress reduction in the amplitude of CAR. Overall, the autonomic cardiac results confirm previous findings obtained both in resting conditions[9,10] and during a tilt maneuver.[7] Osteopathic manipulative therapy techniques seem to positively influence autonomic neural modulation of HR by potentiating parasympathetic activity (increased HF index) and shifting sympathovagal balance toward a relative vagal prevalence (reduced LF:HF ratio).

In the current study, such "protective" properties of OMTh were found when the acute stressor was a mental rather than physical challenge. The test we used to induce sympathetic activation and sympathovagal balance change was partially derived from Kirschbaum's Trier Social Stress Test.[12] This task consistently produced rather large physiologic effects in most participants; nonetheless, it was mild enough to be approved by the ethical committee. A single craniosacral OMTh session immediately after the stressor counteracted the overall stress-induced positive chronotropic effects, vagal withdrawal, and shift of autonomic balance toward sympathetic prevalence compared with sham manipulation.

The other novel aspect of the present study is that the quantification of cardiac autonomic responsivity was coupled with a noninvasive measurement of HPA axis reactivity. Salivary cortisol concentrations indicated that the application of an acute OMTh protocol completely blocked the typical activation of the HPA axis that is usually seen after a brief mental challenge.[20] In addition, when checking CAR values the morning after the stress episode, we found that the OMTh group did not show CAR amplitude reduction exhibited by the control group. These neuroendocrine results are not consistent with data provided by Henderson et al,[8] who found no effects of OMT on the HPA axis function. However, at least 2 main features differentiate the present study from that of Henderson et al[8]: (1) the osteopathic technique used was different, ie, craniosacral technique vs rib raising, respectively, and (2) the participants in the current study received OMTh immeditely after the application of a mental stressor, whereas participants rested in the latter study.

The faster recovery of cardiovascular function and the block of adrenocortical stress response that we observed in the participants receiving OMTh represent intriguing health-related outcomes. The general idea is that a sluggish return to baseline might be a risk factor for cardiovascular morbidity[21] and that sustained elevations in HPA outflow represent a mechanism through

which prolonged stress exerts its pathogenic effects on the cardiovascular system.[22-24]

The function of CAR is still not clear, but it has been proposed to be associated with preparation for the upcoming day by the hippocampus.[25] In other words, an increase in cortisol level after awakening would favor the activation of prospective memory representations, thus enabling a person's orientation about oneself in time and space, as well as anticipation of possible demands of the upcoming day. The hippocampus, besides its established role in long-term memory consolidation, is involved in forming a cohesive representation of the outside world within the central nervous system and processing information about space, time, and relationships of environmental cues.[26]

A few large epidemiologic studies proved that decreased HRV is a risk factor for all-cause morbidity and mortality.[27] Moreover, several physiologic systems that are important for stress, health, and disease have been linked to vagal function and HRV, including glucose regulation, HPA axis function, and a number of allostatic systems.[28] Therefore, through its capacity to regulate HRV and potentiate the vagal component, OMTh might play an important role in preventing or reversing stress-related psychosomatic disorders. In addition to providing mechanistic information on the response to different OMTh techniques in healthy participants, the autonomic and neuroendocrine measurements collected in the present study could be further applied to determine how the response to different techniques varies in symptomatic patients and to facilitate the standardization of treatment protocols and practitioner training.

This pilot study was performed on a relatively small sample of healthy male participants and, therefore, one has to be cautious about generalizing its results to female and patient populations. One might also argue that the HRV parameters used to assess cardiac symapthovagal balance were calculated only in the time and frequency domains. Complex techniques have been developed based on nonlinear dynamics and chaos theory that are able to evaluate in greater detail the intrinsic complexity of HRV.[29,30] However, these techniques are mathematically complicated, they require more powerful computing, and they are still under development and evaluation.[31]

Conclusion

A single OMTh session may induce a faster recovery of HR and sympathovagal balance after an acute mental stressor by substantially dampening parasympathetic withdrawal and sympathetic prevalence; prevent the typical increase in cortisol immediately after a brief mental challenge; and block the stress-induced, day-after reduction in the amplitude of CAR. These preliminary data should be followed up by research testing whether similar changes are observed in both healthy participants and in those with disease or dysfunction; applying repeated OMTh and verifying the persistence of favorable effects; and validating the potential effectiveness of other OMTh techniques. The autonomic and endocrine biomarkers proposed in the current study represent a potentially useful tool to help select the most appropriate OMTh technique in patients with different disease states, including psychological and psychosomatic stress-related states.

Acknowledgments

We thank Angelo Azzoni and Sara Delmonte for their excellent technical assistance.

Author Contributions

All authors provided substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; Mr Fornari and Dr Sgoifo drafted the article or revised it critically for important intellectual content; Mr Fornari and Dr Sgoifo gave final approval of the version of the article to be published; and Mr Fornari and Dr Sgoifo agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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