Effects of Rib Raising on the Autonomic Nervous System: A Pilot Study Using Noninvasive Biomarkers

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Context: Rib raising is an osteopathic manipulative treatment technique used to address restricted excursion of the rib cage and modulate sympathetic nervous system (SNS) activity. However, the physiologic effects of this technique have not been well documented.

Objective: To investigate the effects of rib raising on the autonomic nervous system and the hypothalamic-pituitary-adrenal axis using noninvasive biomarkers.

Methods: Changes in salivary biomarkers after rib raising were investigated using a pretest-posttest, placebo-controlled design. Healthy adult participants were recruited and randomly assigned to rib raising or placebo (light touch) groups. All participants provided baseline saliva samples and samples immediately and 10 minutes after receiving the rib raising or placebo procedure. Salivary flow rate, α-amylase activity, and cortisol levels were measured for each sample.

Results: Twenty-three participants were recruited, of whom 14 completed the study (7 in each group). Subjects who received rib raising had a statistically significant decrease in α-amylase activity both immediately after (P=.014) and 10 minutes after (P=.008) the procedure. A statistically significant change in α-amylase activity was not seen in the placebo group at either time point. Changes in salivary cortisol levels and flow rate were not statistically significant in either group.

Conclusions: The results of the present pilot study suggest that SNS activity may decrease immediately after rib raising, but the hypothalamic-pituitary-adrenal axis and parasympathetic activity are not altered by this technique. Salivary α-amylase may be a useful biomarker for investigating manipulative treatments targeting the SNS. Additional studies with a greater number of subjects are needed to expand on these results.


Based on the idea that disruptions in autonomic tone can compound the disease process, osteopathic manipulative treatment (OMT) has been used extensively in clinical practice for nonmusculoskeletal indications such as asthma, chronic obstructive pulmonary disease (COPD), and irritable bowel syndrome.1-4 A number of OMT techniques are used presumably to modulate autonomic nervous system (ANS) activity.5 However, the quantitative relationship between manipulation and the ANS is not well understood, and designing studies to investigate this relationship has been challenging.

In a 2008 study, cervical myofascial release was shown to modulate vagal tone in healthy subjects using heart rate variability as a surrogate measure of autonomic activity.6 Other studies have explored the effects of OMT on the ANS using changes in blood pressure and Traube-Hering-Mayer oscillations as indirect measures.7,8 However, additional translational studies and tools for measuring the impact of specific OMT techniques on both branches of the ANS are needed.

Although microneurography can be used to directly investigate sympathetic nervous system (SNS) activity, this technique is invasive and requires specialized equipment. Surrogate measures such as heart rate variability and skin conductance have also been used to study the ANS, but there are questions surrounding the specificity of these techniques.9

The use of salivary biomarkers to study ANS activity is a noninvasive approach that has become widely accepted in recent years. Salivary α-amylase is a particularly well-established marker of SNS activity. Unlike cortisol, which is used as a measure of the hypothalamic-pituitary-adrenal (HPA) axis, amylase does not diffuse into saliva from the blood but is produced in the acinar cells of the salivary glands. In rodents, α-amylase secretion by these glands increases after direct sympathetic stimulation.10,11 Studies in human subjects have reported an increase in salivary α-amylase levels in response to physical or psychological stress.12-14 This response is inhibited by the β-adrenergic blocker propranolol.14 It has also been

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shown that intravenous yohimbine induces peripheral norepinephrine release and secretion of α-amylase into the saliva.\textsuperscript{14} Amylase secretion is independent of flow rate and does not correlate to changes in cortisol.\textsuperscript{15} However, there is a correlation between salivary amylase and SNS activity as measured using spectral analysis.\textsuperscript{17,18} Therefore, salivary α-amylase is considered to be a specific measure of sympathetic activity and is a potential biomarker of the response to OMT techniques that affect the ANS.

The goal of the present pilot study was to investigate the physiologic response to rib raising using salivary biomarkers. Rib raising is used to enhance the range of motion of the rib cage, thereby minimizing the amount of work required for thoracic expansion during respiration.\textsuperscript{19} Raising the rib heads at the costotransverse articulation is also believed to modulate ANS activity by activating the thoracic sympathetic chain ganglia that are proximal to this articulation.\textsuperscript{19} It has been proposed that this OMT technique initially stimulates sympathetic efferent activity but results in a prolonged reduction of sympathetic outflow.\textsuperscript{19} To investigate this hypothesis, salivary α-amylase was measured in healthy subjects before and after receiving either rib raising or placebo (light touch). Based on the proposed effects of this technique on SNS activity,\textsuperscript{19} an initial increase in salivary α-amylase followed by a more prolonged decrease was expected.

Salivary flow rate, an indicator of parasympathetic activity, and cortisol levels were also measured in this study. Salivary cortisol is commonly used as an indicator of HPA-axis activity and was tested because some studies have found an increase in endogenous cannabinoid levels after OMT.\textsuperscript{20,21} Because cannabinoids are believed to suppress HPA activity,\textsuperscript{22,23} a decrease in salivary cortisol after rib raising was expected.

In addition to providing mechanistic information, biomarkers of the response to rib raising could be used to determine how the response to this technique varies in symptomatic patients and how it is altered by the addition of other techniques. These biomarkers would also facilitate the standardization of treatment protocols, training of practitioners, and validation of sham therapy procedures (eg, light touch), thus enabling the design of high quality therapeutic efficacy studies.

Methods
The study protocol was approved by the institutional review board at the West Virginia School of Osteopathic Medicine in Lewisburg. Written informed consent was obtained from all participants.

Participants
From November 2007 through June 2008, we recruited healthy volunteers by placing flyers at various locations on the WVSOM campus. Participants were required to be between the ages of 21 and 60 years. Volunteers were excluded if they met any of the following exclusion criteria:

\begin{itemize}
  \item chewing gum or consuming anything other than water within an hour of the appointment
  \item rib fracture
  \item history of unstable cardiac arrhythmia or symptoms related to the chest cavity (eg, difficulty breathing, chest pain)
  \item symptoms suggestive of bowel obstruction (eg, abdominal bloating with pain, nausea and vomiting, diarrhea)
  \item pregnancy
\end{itemize}

Because previous knowledge of the technique could potentially affect the response, osteopathic physicians and osteopathic medical students past the first year of study were also excluded.

Subjects who responded to the flyer via e-mail or telephone were scheduled for a 1-hour appointment and reminded not to eat or drink anything but water for at least 1 hour before the appointment. Upon arrival, all volunteers were randomly assigned to either the OMT or the placebo group and provided written informed consent. Next, volunteers completed a form regarding their age, sex, previous OMT experience, medications, and time since their last meal and drink. Once study eligibility was confirmed, participants were asked to provide a baseline saliva sample. Immediately after providing this sample, participants underwent either a rib raising or light touch placebo procedure as described below. The participants provided two additional saliva samples: one immediately after the procedure and one 10 minutes after the procedure. Participants were also asked to state if they believed they had received OMT or placebo.

All appointments were scheduled in the afternoon hours with the same osteopathic physician (DO) (T.S.L.) in order to reduce variability. Participants were not compensated.

OMT and Placebo Protocols
The application of the rib raising and placebo protocols occurred in an examination room setting, but no diagnostic procedures were performed.

Rib raising—the participant laid in the supine position on an OMT table and the DO sat on the left side. The DO then reached under the participant’s arm to contact the paravertebral muscles on that side. The first four ribs were contacted and the DO’s fingertips were placed on the area where the ribs meet the transverse processes. Using a rocking motion, and using the table as a leverage to facilitate the lift, the DO lifted the rib angles anteriorly and released pressure for 5 seconds. The DO used sufficient pressure during the lifting phase to achieve anterior movement of the contacted tissues and ribs such that minimal to no tissue movement was appreciated at the end of the lift.

The lifting motion was performed for five cycles, and the process was repeated two more times on that side for the remaining ribs (with T5-T8 being raised together followed by T9-T12). The process described above was repeated on the
other side and then for each side starting again with the left. The entire process lasted approximately 300 seconds.

**Light touch placebo**—Hand placing and timing for the placebo protocol was identical to that of the rib raising protocol except no lifting was done. The physician’s hands were kept in place for 25 seconds instead of five repeats of a 5-second lift. The placebo protocol also lasted approximately 300 seconds. Contact with the ribs was maintained for 25 seconds.

**Saliva Collection**

Methods for the collection of saliva have been described. Special devices designed for the collection of saliva are available, but several studies have suggested that these devices may interfere with some assays. Oral stimulants can cause salivation, but they may lead to increased parasympathetic activity. Therefore, saliva collection through passive drool yields the most reliable results. Although it is possible for individuals to express saliva directly into cryotubes through a small straw, this process requires training and can be unpleasant for participants.

We used the passive drool method to collect saliva samples. Participants were in the seated position and were told to allow saliva to pool in their mouths and allow it to gently flow into the 50 mL tubes periodically for 3 minutes. Participants were instructed not to spit. Salivary flow rate was calculated by determining the volume expressed per minute.

Aliquots of 500 μL were transferred into 1 mL tubes and the tubes were immediately placed on ice. At the end of the session, the tubes were placed in a freezer at the storage facility for less than 2 months. Freezing ensured stability and allowed for precipitation of the mucins. We found amylase and cortisol to be stable at -20°C for more than 6 months.

**Assays**

Immediately before performing the assays, saliva aliquots were thawed on ice and centrifuged for 15 minutes at 3000 revolutions per minute at 4°C to pellet the mucins. The supernatant was transferred to a fresh tube and amylase assays were performed immediately. These assays were performed with assay kits purchased from Salimetrics LLC (State College, Pennsylvania) according to the manufacturer’s instructions.

The kit for salivary amylase (Salimetrics Alpha-amylase Salivary Assay Kit) uses a maltotriose substrate linked to chloro-p-nitrophenol to measure amylase activity by detecting the change in absorbance at two time points after the addition of substrate. Samples were initially run at a 100-fold dilution. If amylase activity was above or below detection limits, the experiments were repeated using different dilutions. Standards included with the kit were used to confirm the accuracy of the assay. All assays were performed at least three times, with each data point done in triplicate. The intra- and inter-assay variabilities for amylase assays were less than 5% and less than 10%, respectively (coefficient of variation [%CV] = standard deviation/mean × 100).

Cortisol assays were performed with a competitive immunoassay kit (Salimetrics Salivary Cortisol Kit) from Salimetrics LLC according to the manufacturer’s instructions. Standard curves were generated and used to determine the amount of cortisol in undiluted saliva samples. If cortisol levels were outside of the standard curve, the experiments were repeated using diluted samples. Accuracy was confirmed using provided known samples. All cortisol assays were performed at least three times with each data point done in triplicate. The intra- and interassay variabilities for cortisol assays were less than 10% and less than 20%, respectively.

**Data Analysis**

All data analyses were performed using SPSS statistical software (version 170; SPSS Inc, Chicago, Illinois). Baseline values for each group were compared using unpaired t tests. Postprocedure values were compared to baseline values using paired t tests. Correlation with other factors such as age and time since eating was investigated by calculating Pearson product-moment correlation coefficients for each variable measured. Because this was a pretest-posttest study with more than one posttest data point that also had between-subjects factors, generalized linear modeling for repeated measures analysis of variance (ANOVA) was performed to investigate the effect of treatment on the change in biomarker levels over time. A P value less than .05 was considered to indicate a statistically significant difference.

**Results**

A total of 23 volunteers were recruited, but 6 were subsequently excluded for reasons including eating or chewing gum before the appointment and the presence of blood in the saliva sample. Data collected from the first 3 participants were used for refinement of protocols and excluded from the final analysis. These participants are further described in the “Comment” section. Therefore, 14 participants were included in the present study. The average age of the remaining 14 participants was 30.9 years, and 9 were women.

Seven participants received rib raising and 7 received the placebo procedure. Four of the 7 subjects who received OMT thought they had received the placebo. Only 1 participant who received placebo thought he or she had received OMT.

**Flow Rate and α-Amylase Activity**

Salivary flow rate and α-amylase levels were determined for each sample. The results of these assays are summarized in the Table and Figure 1.

Salivary flow rate, which is highly dependent on parasympathetic activation, was also measured and did not change significantly in either the OMT or placebo group (Table).
Repeated measures ANOVA showed no effect of treatment on flow rate over time \( (P=.75) \). No correlation was seen between flow rate and amylase activity.

The mean values of OMT and placebo groups at baseline were not significantly different for either of the parameters measured. A decrease in salivary α-amylase activity was seen in all participants who received OMT, including those who thought they had received placebo. The change from baseline was statistically significant for samples taken immediately after OMT \( (P=.014) \) and 10 minutes after OMT \( (P=.008) \), but there was a more pronounced decrease at the later time point. The extent of the decrease in α-amylase activity ranged from 14% to 61% in the OMT group at the 10-minute time point (average decrease of 39%). For the placebo group, α-amylase activity increased in 2 participants and remained the same or decreased slightly in 3 participants. However, 2 participants in the placebo group had a decrease in amylase activity of greater than 20%. Neither of these participants believed they had received OMT, but one of them was an extreme outlier for baseline amylase activity (greater than three times the interquartile range above the third quartile). Even including these participants, the change from baseline was not significant within the placebo group at either posttest time point.

Generalized linear modeling for repeated measures ANOVA was also performed to further investigate the effect of treatment on the repeated measurements over time. The extreme outlier for baseline amylase in the placebo group was identified as an influential outlier (standardized residuals and Cook distance) and was therefore removed from this analysis. A significant interaction between treatment and the change in amylase levels over time was observed \( (P=.046) \). However, this analysis is limited by the small number of participants (observed power of 59%). Amylase activity did not correlate significantly to age or time since eating or drinking.

These results are consistent with previous studies indicating that salivary α-amylase levels are independent of flow rate and that having subjects refrain from eating and drinking 1 hour before the experiment is a sufficient cutoff point.

### Cortisol Assays

Levels of salivary cortisol were measured for each subject who generated enough saliva to perform all of the assays (7 participants in the OMT group, 6 in the placebo group). The results of the cortisol assays are summarized in Figure 2. A slight but statistically insignificant decrease in salivary cortisol was seen in both groups at both time points, suggesting that rib raising has no immediate effect on HPA-axis activity. Repeated measures ANOVA found no interaction between treatment and cortisol levels over time \( (P=.59) \).

**Comment**

Rib raising is an OMT technique used for many indications, including rib restriction and conditions associated with sympathetic hypertension. This procedure is believed to initially stimulate sympathetic efferent activity but results in a prolonged reduction in sympathetic outflow from the treated region. However, there is a limited amount of information on the physiologic effects of this technique and the mechanisms through which it exerts these effects. Although the small number of participants limits the power of the study reported here, the findings support the hypothesis that rib raising decreases SNS activity immediately following the procedure. No statistically significant changes were seen in flow rate or cortisol levels, suggesting that rib raising has little immediate effect on parasympathetic or HPA-axis activity.

Because rib raising is believed to initially stimulate SNS activity, an increase in salivary α-amylase had been predicted. However, this effect was not seen. The first 3 study participants provided saliva samples at 5 minutes and 10 minutes after the procedure and a decrease in α-amylase was seen at both time points in those who received OMT. To ensure that a rapid, transient increase was not being missed, the protocol was changed to collect samples immediately after and 10 minutes after the procedure. Therefore, results from the first 3 subjects were excluded from further analysis. However, even when samples were collected immediately following the procedure, no increase in α-amylase was seen.

### Table

<table>
<thead>
<tr>
<th>Assay</th>
<th>OMT Group (n=7), mean (SD)</th>
<th>P Value*</th>
<th>Placebo Group (n=7), mean (SD)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Flow Rate, mL/min</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.54 (0.25)</td>
<td>NA</td>
<td>0.73 (0.40)</td>
<td>NA</td>
</tr>
<tr>
<td>Immediately after</td>
<td>0.67 (0.30)</td>
<td>.20</td>
<td>0.84 (0.59)</td>
<td>.52</td>
</tr>
<tr>
<td>10 min after</td>
<td>0.63 (0.17)</td>
<td>.37</td>
<td>0.73 (0.31)</td>
<td>.99</td>
</tr>
<tr>
<td><strong>Salivary Amylase, U/mL</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>76.2 (35.5)</td>
<td>NA</td>
<td>83.4 (54.6)</td>
<td>NA</td>
</tr>
<tr>
<td>Immediately after</td>
<td>57.3 (24.6)</td>
<td>.014†</td>
<td>76.4 (49.8)</td>
<td>.22</td>
</tr>
<tr>
<td>10 min after</td>
<td>46.0 (20.8)</td>
<td>.008†</td>
<td>71.5 (43.9)</td>
<td>.29</td>
</tr>
</tbody>
</table>

* The mean differences between the osteopathic manipulative treatment (OMT) and placebo groups at baseline were not statistically significant for either of the parameters measured (unpaired t tests). Paired t tests were performed to identify statistically significant changes from baseline.
† Statistically significant \( (P<.05) \).

**Abbreviation:** NA, not applicable; SD, standard deviation.
These results may reflect the fact there are several different ways in which rib raising can be performed. In this study, the ribs were lifted repetitively in a rhythmic fashion. It has been suggested that applying constant pressure to the rib angles may be more sympathoexcitatory than the rhythmic method. This hypothesis can be tested in subsequent studies.

It is also possible that an initial increase in α-amylase activity occurred during the procedure but was very transient. Because collection of saliva in the course of a procedure is not feasible, other techniques for measuring SNS activity would need to be used to investigate this hypothesis. Experiments examining the mechanisms through which manual techniques modulate the ANS would also facilitate interpretation of these results. The costovertebral joints do contain mechanoreceptors that mediate the effects of rib motion on afferent activity in the thoracic dorsal root. Manipulation of these joints has been shown to alter phrenic nerve efferent activity, and visceral mechanoreceptors have been shown to modulate sympathetic efferent firing. However, a link between costovertebral mechanoreceptors and SNS activity has not been investigated to our knowledge.

Another limitation of this study was that the impact of baseline α-amylase levels on the response to manipulation could not be fully investigated as a result of the limited number of participants. Rib raising may have different effects in patients with high or low sympathetic tone and may modulate activity in either direction depending on the individual characteristics of each patient. Because diagnosis was not a component of this study, the impact of somatic dysfunction or other conditions on the change in salivary α-amylase could not be investigated. Because all of the procedures in the present study were performed by the same DO, the possibility that minor differences in technique between practitioners may elicit a different response also was not addressed. These questions, along with other parameters such as racial or ethnic differences, can be investigated in follow-up studies.

The results of the present pilot study suggest that salivary α-amylase may be a viable noninvasive biomarker for investigating the effects of rib raising. A decrease in salivary α-amylase was seen in all participants who received OMT, but the extent of the decrease varied. In addition to the factors described above, this variability could also reflect differences in the amount of pressure applied during the procedure. The protocol called for the use of sufficient pressure to achieve anterior movement of the contacted tissues and ribs such that minimal to no tissue movement was appreciated at the end of the lift. However, the amount of pressure needed to achieve this endpoint is likely to differ between participants.

A decrease in α-amylase level was also seen in 2 participants who received placebo. Although no lifting was performed in the placebo procedure, it is possible that sufficient pressure to elicit a response was used for participants in whom a decrease in amylase was seen. The relationship between the amount of pressure applied and the change in salivary α-amylase level can be addressed in follow-up experiments by using tactile pressure sensors.

If validated, a noninvasive biomarker of the response to rib raising would be a valuable tool for exploring other issues...
not addressed in the present study, such as the duration of the response to this technique and how the response may be altered in symptomatic patients. It would also facilitate the design of therapeutic efficacy studies. Rib raising has been used as a treatment component in OMT trials. Two studies examined OMT techniques in patients with COPD. The development of tools that can be used to validate sham procedures and determine how the addition of other techniques affects the response to rib raising will be a critical step forward in optimizing treatment protocols for subsequent efficacy trials.

Conclusion
The results in the present pilot study suggest that rhythmic rib raising decreases sympathetic nervous system activity immediately following the procedure but has little effect on HPA-axis or parasympathetic activity. Additional studies are needed to investigate the impact of baseline sympathetic tone and disease status on the response to rib raising and to validate the use of salivary α-amylase as a biomarker of this response. The development of noninvasive tools for measuring the physiologic response to OMT will aid in the design of translational studies and clinical efficacy trials needed to advance the field.

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