THE EFFECT OF CHIROPRACTIC MANIPULATION ON SALIVARY CORTISOL LEVELS

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ABSTRACT

Background: The stress response in humans is a healthy response and is necessary for life. The effects of chiropractic manipulation (CM), if any, on stress are ill-defined. Cortisol has been used as an accurate measure of the stress response system in humans. Salivary cortisol is a noninvasive technique to accurately quantify biologically active cortisol.

Objective: To determine whether basal salivary cortisol levels can be properly detected and whether CM has any direct effect on basal salivary cortisol levels in humans.

Methods: Subjects were adult male students attending a chiropractic college. Salivary samples were collected for 5 weeks. During Week 1, samples were collected by the students at home upon waking. During Weeks 2 through 5, home samples were collected upon waking and were followed by an additional time course of samples collected in a laboratory setting before and after CM. Salivary cortisol was measured by enzyme-linked immunoassay.

Results: Chiropractic manipulative therapy did not significantly change basal salivary cortisol levels. The time course of acute changes to cortisol levels was independent of testing week and group. A decrease in salivary cortisol was detected over time on each trial testing day. Overall, cortisol levels significantly decreased from the time of the home samples until the pretreatment laboratory measurement ($P < .05$). Cortisol levels subsequently decreased from pretreatment to 15 minutes after treatment ($P < .05$). After treatment, there were progressive decreases in cortisol levels from the 15- and 30-minute time points to the 60-minute time point ($P < .05$).

Conclusion: The results of this pilot study suggest that there is no effect of CM on salivary cortisol levels in asymptomatic subjects. As such, we conclude that neither the anticipation of CM nor the spinal manipulative procedure itself induces a state of stress or anxiety. (J Manipulative Physiol Ther 2002;25:149-53)

Key Indexing Terms: *Chiropractic; Salivary Cortisol; Stress*

INTRODUCTION

Chiropractic manipulation (CM), with its hallmark high-velocity, low-amplitude (HVLA) impulsive thrust, has been documented to be efficacious in the treatment of neck and back pain.[1] However, the literature is scarce with regard to the effects of CM on physiologic stress.[2-4] CM may indeed result in some degree of physiologic stress, as a result of paraphysiologic joint space excursion and moderate forces being applied to paraspinal soft tissues. Alternatively, CM may actually attenuate stress-induced physiologic changes to the spine that might otherwise contribute to back pain. Methodologic limitations of previous research warrant further investigation into the relationship between CM and physiologic stress.

The level of stress an individual has can be positively correlated with secreted cortisol levels.[5,6] Cortisol circulates in plasma primarily bound to plasma proteins and is physiologically active in its unbound form. Salivary cortisol has been shown to be a valid and reliable measure of unbound "free" cortisol levels, compared with the unbound hormone levels in blood.[5] Salivary cortisol concentration is also independent of salivary flow rate. Because saliva can be collected in a noninvasive manner, this method of measuring cortisol levels is particularly useful when conducting stress research with humans.

A more complete understanding of the effects of CM on cortisol levels may provide insight on the interrelationships among CM, neuroendocrine mechanisms, and physiologic stress for the following reasons. Cortisol, a steroid hormone secreted by the adrenal cortex, is essential for life. Its effects on cell metabolism are diverse and global in nature. Cortisol plays an integral role in glucose, fat, and protein metabolism. Cortisol acts to decrease local edema and pain by blocking early stages of inflammation. In addition to its anti-inflammatory actions, basal cortisol is also believed to increase the rate of healing by stimulating gluconeogenesis. Specifically, increased basal cortisol levels activate the precursor molecules of gluconeogenesis that can be used as building blocks of tissue repair.

In general, neuroendocrine mechanisms increase the rate of cortisol secretion during different types of stressful situations to increase metabolism and/or maintain cellular homeostasis. However, not all stress-induced mediators of augmented cortisol release are beneficial for health. Supraphysiologic increases in circulating cortisol for extended periods of time invariably give rise to Cushing's Syndrome, a serious endocrine abnormality characterized by hypertension, hyperglycemia, osteoporosis, infection, and poor wound healing. It is currently unknown whether CM has beneficial effects on the alleviation of stress and/or whether the manipulation itself is physiologic stressor. The goal of this research was to begin to address the latter issue.

Our purpose was to collect preliminary data to assist in establishing the feasibility for additional studies designed to investigate the reactivity of the stress response and CM. Specifically, the study was performed to determine whether basal salivary cortisol levels can be properly detected and to determine whether CM has any direct effect on basal salivary cortisol levels in humans. By monitoring salivary cortisol levels before and after CM, useful information regarding the magnitude of physiologic stress that may be induced as a result of the manipulative procedures may be elicited.

METHODS

Subjects

Subjects were 30 asymptomatic male students enrolled at a chiropractic college. Students from only one class were recruited to control for the variability of daily stressors on individual basal salivary cortisol levels. The student subjects were voluntary participants and were recruited by class announcements. Candidates were excluded from the study if they had a history of endocrine or psychiatric disorders. Subjects were not included in this study if they had any contraindications to CM therapy, or if they were currently on any medication.

Eligible candidates were educated on the procedure, the possible benefits, and the possible risks of the study. Both verbal and written informed consents were obtained at least 24 hours before the commencement of the study. The pro-

tocol used was approved by the institutional review board for experimentation involving human subjects. On inclusion into the study, the students were randomly assigned to 1 of 3 experimental groups.

Experimental Design

The experimental groups were the control group (CTL, n = 10), the sham group (SHAM, n = 10), and the CM group (CM, n = 10). One chiropractor performed all of the sham and chiropractic manipulations throughout the study. The study took place over a 5-week period. Subjects agreed not to receive chiropractic treatment during Week 1 of the study and for 24 hours before the test sessions in Weeks 2 through 5 of the study.

During Week 1, all subjects collected salivary samples at home upon waking for 5 consecutive days (Monday-Friday). The 4 consecutive Mondays during Weeks 2 through 5 were chosen as trial testing days. On testing days during Weeks 2 through 5 (Mondays), subjects also provided home samples upon waking. Each subject was provided with written sampling instructions, labeled collection tubes, and storage materials. Because of the rapid decrease in basal cortisol levels after waking, subjects were also instructed to record the time of sampling each morning to evaluate the population for uniform home sampling times.[6]

On the trial testing days, a time course of samples (t_{-5}-t_{60}) was collected in the laboratory from each subject in addition to a home sample upon waking (t_{-65}). A pretreatment sample (t_{-5}) was collected 5 minutes before the delivery of the appropriate treatment. After treatment (t0), samples were collected at 5 (t_5), 15 (t_{15}), 30 (t_{30}), and 60 (t_{60}) minutes. All subjects remained in a supine position during the time between the t_{-5} sample and the t_5 sample. Between the time points t_5 and t_{60}, all subjects were allowed to move about freely within the laboratory.

All laboratory sampling took place in 2 technique classrooms at a chiropractic college. Testing occurred between 8 AM and 10 AM each test day. Five subjects from each experimental group were tested in each room.

Sample Collection

Subjects were instructed to refrain from eating, exercising, using tobacco, and consuming any drinks other than water for 1 hour before all sample collection. Plain cotton Salivettes (Sarstedt, Germany) were used for the quick and hygienic collection of saliva. Saliva was collected by chewing on a cotton-wool swab for 30 to 60 seconds. Subjects collected home samples and stored them in their personal freezers until the final day of testing. Home samples were transported to the laboratory on ice on the final day of testing. Laboratory samples were collected on ice and stored at −80° C until biochemical analysis.

Manipulative Procedures

All CM and sham manipulation procedures were performed by 1 clinician with 15 years of clinical experience.
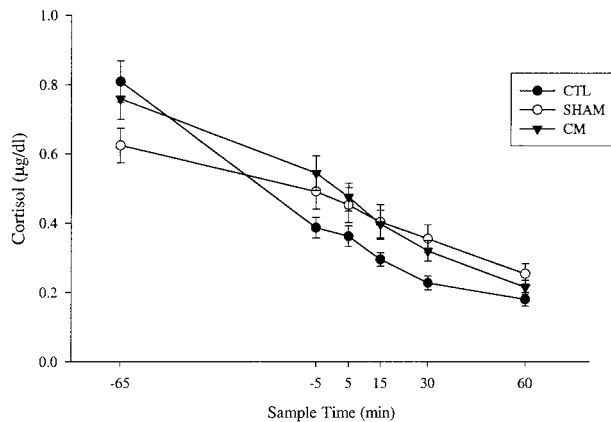
Fig 1. *Short-term time course of salivary cortisol levels collapsed across testing trial days as a function of experimental group. Data are expressed as mean +/− SE.*
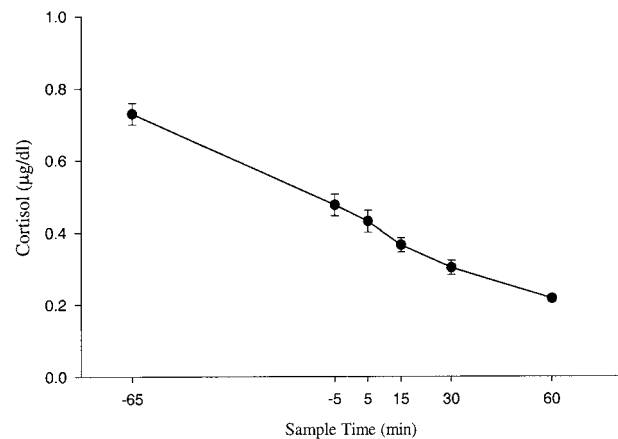
Fig 2. *Overall time course of salivary cortisol for all trial testing days collapsed across all experimental groups. Data are expressed as mean +/− SE.*

The CM procedures consisted of HVLA manipulation, as commonly performed by practitioners of chiropractic,[7-10] specifically, a supine, coupled lateral flexion-rotational manipulation for the upper cervical region. The CM procedures were delivered unilaterally to the right side of the spine. The force applied to the spine in these types of procedures has been previously reported to be delivered in approximately 200 ms with linear vertebral displacements of less than 10 mm.[7,8] The manual force, or thrusts, to the zygapophysial joint are applied at the end of physiologic range of joint motion and extend into the so-called "paraphysiologic zone" of joint motion.[11] The paraphysiologic zone is defined as the endpoint of range of motion to which a joint can be passively forced without any deleterious effects.[11]

By using the right-handed Cartesian orthogonal coordinate system of movement as a reference, manual tension was slightly increased by providing +Y-axis translation (axial distraction) to the spine, coupled with a +θY-axis rotation force, thereby increasing the mechanical load on the soft tissues.[12] Once tissue tension was maximized, a high-velocity, low-amplitude impulsive force was applied. The primary force vector applied to the zygapophysial joint was +Z-axis translation (posterior-anterior), with a secondary vector consisting of +θY-axis rotation (left axial rotation). These CM procedures have been previously described.[13-16] On completion of the CM procedure, the subject was immediately returned to the supine neutral position.

Sham procedures consisted of manual contact and spinal positioning, as described previously; however, the zygapophyseal joint was not passively taken to the so-called "end-range" of motion. Additionally, the manipulative operator did not apply any HVLA thrusts to the spine. In effect, the vertebral positioning replicated that for spinal manipulation, but no thrust into the paraphysiologic zone was applied. The subject's head was returned to the neutral position after the sham procedure was performed.

Subjects in the CTL group were instructed to remain in a supine position between the t_{-5} sample and the t_5 sample. No manipulation or vertebral positioning was delivered; the subjects' heads remained in the neutral position for the same duration as those of subjects receiving CM.

Assay Procedure

Free, unbound cortisol from saliva samples was measured with a commercially available enzyme linked immunoassay, according to manufacturer's instructions (Salimetrics, LLC, University Park, Penn).[17,18] Samples were thawed to room temperature and centrifuged at 1500 g (3000 rpm) for 15 minutes before the beginning of the assay. Samples were run in duplicate. Any individual samples with duplicate tests that varied by more than 10% were repeated in subsequent assays. Cortisol levels were recorded in μg/dL.

Statistical Analysis

The 1-way analysis of variance (ANOVA) intraclass reliability coefficient was calculated for the home samples taken during Week 1 (Monday-Friday). This statistical procedure was also applied to all the home samples and all the pretreatment laboratory samples taken on of the trial testing days (Mondays, Weeks 2-5) to verify the reliability of the cortisol measurements. Group by Day and Group by Week ANOVA models were used to reveal any differences in these basal cortisol measurements.

A group × trial week × sample time ANOVA model was used to reveal differences in the short-term and long-term changes to cortisol levels as a function of the experimental treatment groups. The Student-Newman-Keuls test was used to detect pairwise differences in cortisol levels. Significance for all statistics tests was accepted at $P < .05$.

RESULTS

Figure 1 depicts the short-term course of cortisol levels collapsed across test weeks as a function of group. Salivary

cortisol levels decreased across the sample times during the 4 trial testing days in a similar manner ($P < .05$). This overall time course profile for decreases in cortisol levels was not different among the experimental groups throughout the study ($P > .05$). The consistent decreases in cortisol levels over the 4 trial testing days indicate that there was no long-term effect of CM on physiologic stress levels. The lack of group differences in salivary cortisol levels at each sample time during the 4 trial testing days indicate that there was no short-term effect of CM on physiologic stress levels. Collectively, these data indicate that CM does not alter basal salivary cortisol levels over the short or long term. These data conclusions are supported by the fact that there was no significant group $\times$ trial week $\times$ sample time interaction.

Figure 2 depicts the overall salivary cortisol time course for all trial testing days collapsed across all experimental groups. Decreases in cortisol levels across sample times were significant ($P < .05$, sample time main effect). Cortisol levels significantly decreased from the time of the home samples to the pretreatment laboratory measurement ($P < .05$). Cortisol levels subsequently decreased from pretreatment to 15 minutes after treatment ($P < .05$). After treatment, there were progressive decreases in cortisol levels from the 15- and 30-minute time points to the 60-minute time point ($P < .05$). These data indicate that there is a diurnal rhythm in basal salivary cortisol levels.

The following data indicate that there was adequate day-to-day reliability in the basal salivary cortisol measurements. Intraclass reliability coefficients were 0.67, 0.70, and 0.70 for the home samples taken during Week 1 and the home samples and pretreatment laboratory samples taken on each of the trial testing days during the project, respectively. In addition, the home basal cortisol measurements were stable for the duration of the project. No significant differences were detected in the salivary cortisol levels on any day during Week 1 (Monday-Friday). The salivary cortisol levels were similar among the experimental groups during Week 1. Similar salivary cortisol levels were measured from the home samples (t_{-65}) on each trial testing day (Mondays, Weeks 2-5). These salivary cortisol levels (t_{-65} samples) were similar among the experimental groups on each trial testing day. The pretreatment laboratory samples (t_{-5}) were also stable across the trial testing days and among the experimental groups.

DISCUSSION

The results of this research study suggest that CM has no effect on salivary cortisol levels in asymptomatic subjects. Therefore, we conclude that in a population of subjects familiar with chiropractic technique, neither the anticipation of CM manipulation nor the CM procedure itself induces a state of stress or anxiety.

The time course of decreases in salivary cortisol between samples taken upon waking and samples taken 1 hour after treatment demonstrates the natural circadian drop in basal cortisol levels upon waking. Kirchbaum and Hellhammer[5] report a marked and rapid decrease in basal cortisol levels over the early morning hours. The data express physiologic change, with respect to time, that is independent of treatment. Although CM was found to have no effect on basal salivary cortisol levels, this study positively measured a changing diurnal rhythm in asymptomatic male subjects.

It should be noted that women were not included in the current study. Hypothalamic-pituitary-adrenal axis response patterns differ between men and women, which is evident from both animal and human studies. Kirschbaum et al[19] report a significant sex difference and menstrual cycle phase difference in the availability of free, biologically active cortisol. Men were also shown to have consistently enhanced salivary cortisol responses when demonstrating similar subjective or emotional responses to women. To control for gender bias on cortisol measurements, only men were recruited for our initial investigation.

Our results are in agreement with Vicenzino et al,[2] who carried out a double-blinded, placebo controlled study in which stress perceived by an individual was measured with a rating scale and a stress Visual Analog Scale. They report no effect of treatment on perceived stress and, in fact, a reduction in stress levels over the course of the experiment. The subjective outcomes reported by the investigators are in corroboration with the objective findings of our study, in which cortisol levels were used to assess stress in subjects.

Further indications that CM is not a stressor have been reported by Christian et al.[3] In a study designed to examine the effect of CM on the possibility of a humorally mediated analgesic response, plasma cortisol levels were measured, as well as plasma ACTH and B-endorphin. Asymptomatic and symptomatic subjects were grouped into either a CM group or a sham treatment group. Cortisol levels were measured before treatment and at 5 minutes and 30 minutes after treatment. Significant decreases in cortisol levels between pretreatment and 30 minutes after treatment were reported for all groups except the asymptomatic CM group. The researchers noted a similar trend within that group. It must be noted that this study differed from the current study in that total plasma cortisol was measured by more invasive methods with intravenous butterfly catheters. Basal total plasma cortisol levels (bound and unbound) were also much higher than the basal unbound salivary cortisol levels reported in the current study. It is possible that the drop in cortisol levels reported over 30 minutes by Christian et al[3] is a function of subject recovery from the stress of introducing an intravenous butterfly catheter into an antecubital vein.

Tuchin[4] also examined the effect of CM on salivary cortisol levels. However, the subjects involved in the study were not blinded, and a sham-adjustment group was not included in the research design. The study also did not control for gender bias on cortisol measurement, because

both men and women were used as subjects. We deemed the results of the study inconclusive because of reporting problems with subject attrition and outlying data.

CONCLUSION

Saliva collection is a safe, noninvasive method of collecting cortisol samples in a clinical chiropractic setting. The results of our study suggest that CM does not alter the profile of basal salivary cortisol when compared with sham or control groups. With respect to the specific population used in this study, our results definitively suggest that the physical component of CM is not a potent enough stressor to disrupt homeostatic mechanisms and activate the hypothalamic-pituitary-adrenal axis.

Future studies will be conducted in our laboratory to investigate the effects of CM on cortisol levels of symptomatic patients. In addition, studies will be designed to investigate the effects of CM on cortisol levels in subjects who have been exposed to physiologic and/or psychologic stressors.

ACKNOWLEDGMENTS

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