JAOA ORIGINAL CONTRIBUTION

Role of Osteopathic Manipulative Treatment in Altering Pain Biomarkers: A Pilot Study

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Context: Underlying mechanisms explaining the effects of osteopathic manipulative treatment (OMT) are poorly defined. The authors evaluate various nociceptive (pain) biomarkers that have been suggested as important mediators in this process.
Objective: To determine if OMT influences levels of circulatory pain biomarkers.
Methods: In a prospective, blinded assessment, blood was collected from 20 subjects (10 with chronic low back pain [LBP], 10 controls without chronic LBP) for 5 consecutive days. On day 4, OMT was administered to subjects 1 hour before blood collection. Blood was analyzed for levels of β-endorphin (βE), serotonin (5-hydroxytryptamine [5-HT]), 5-hydroxyindoleacetic acid (5-HIAA), anandamide (arachidonoylethanolamide [AEA]), and N-palmitoylethanolamide (PEA). A daily questionnaire was used to monitor confounding factors, including pain and stress levels, sleep patterns, and substance use.
Results: Increases from baseline in βE and PEA levels and a decrease in AEA levels occurred immediately posttreatment. At 24 hours posttreatment, similar biomarker changes from baseline were observed. A decrease in stress occurred from baseline to day 5. The change in PEA from baseline to 24 hours posttreatment correlated with the corresponding changes in stress. Subgroup analysis showed that subjects with chronic LBP had significantly reduced 5-HIAA levels at 30 minutes posttreatment (P=.05) and 5-HT levels at 24 hours posttreatment (P=.02) when compared with baseline concentrations. The increase in PEA in subjects with chronic LBP at 30 minutes posttreatment was two times greater than the increase in control subjects.
Conclusion: Concentrations of several circulatory pain biomarkers were altered after OMT. The degree and duration of these changes were greater in subjects with chronic LBP than in control subjects without the disorder.

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The goal of osteopathic manipulative medicine is to evaluate biomechanical (somatic) dysfunction and, through the application of osteopathic manipulative treatment (OMT), to promote healing in patients with these disorders. Objectively evaluating somatic dysfunction and how it changes after OMT has been challenging to researchers and clinicians alike. Thus, an osteopathic physician's information regarding the efficacy of OMT is usually based on patients' subjective evaluations of changes in pain levels.

Persistent pain is associated with the production and release of multiple nociceptive (pain) and inflammatory mediators. Although the complexity of the pain-inflammatory process is not fully understood, important roles in this process have previously been suggested for circulatory neurochemical biomarkers, including endocannabinoids, endogenous opioids, and serotonin. We hypothesized that the concentrations of circulatory biomarkers are influenced by OMT, thus providing objective measures that can be used in future research to better define the underlying mechanisms of OMT.

The analgesic properties of plant-derived opiates have been known since ancient times.[1] Endogenous opioids (dynorphins, endorphins, enkephalins) have also been implicated in pain modulation, both directly and through the placebo response.[2,3] Opioids act via central and peripheral opiate μ, κ, and δ receptors to produce analgesic effects.[2,3] In addition, endogenous opioids regulate inflammation through opioid receptors found on immune cells at the site of inflammation.[2,3] Pilot studies[4-9] have been performed to assess a variety of manual treatments on β-endorphin (βE) levels. Although two studies[6,9] demonstrated a positive correlation between elevated βE and manual treatments (connective tissue massage and spinal manipulation), other researchers have failed to find such a correlation. As a result of variable experimental methodologies, small sample sizes, and inconsistent outcomes in these studies, firm conclusions cannot be drawn regarding the relationship between manual treatments and endogenous opioid levels.

Serotonin (5-hydroxytryptamine [5-HT]) is a major neurotransmitter component of the inflammatory chemical milieu

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and a potent stimulant for nociceptive nerve endings in the peripheral nervous system.[10,11] Serotonin is found in platelets and basophils, where it can be released under conditions of injury, and it acts on more than 15 receptors, of which 5-HT_{1A}, 5-HT_{2A}, 5-HT_3, and 5-HT_4 have the greatest relevance in nociception.[12,13] Some studies[12,13] have shown that serotonin is found at higher concentrations in the blood products of individuals with chronic painful inflammatory conditions, such as fibromyalgia and rheumatoid arthritis. Similar studies involving individuals with chronic low back pain (LBP) have previously not been performed. Furthermore, there have been no published studies evaluating the effects of OMT on serotonin or its metabolic derivative, 5-hydroxyindoleacetic acid (5-HIAA), in human subjects. However, Skyba et al[14] have shown in an animal model that mobilization of the knee can induce a release of 5-HT in the spinal cord.

Cannabinoids, such as Δ^9-tetrahydrocannabinol, act via cannabinoid-1 (CB_1) receptors within peripheral, spinal, and central pathways to produce analgesic effects.[15-17] Cannabinoid-2 (CB_2) receptors are sparse in the central nervous system but are prominent in cells of the immune system, playing an important role in the mediation of inflammatory pain.[17] The endocannabinoid anandamide (arachidonoylethanolamide [AEA]) causes strong analgesic and anti-inflammatory effects in animal models.[15] Although not an endocannabinoid, an endogenous analog of AEA called *N-palmitoylethanolamide* (PEA) also possesses potent analgesic and anti-inflammatory properties.[18]

Little is known about the role of endocannabinoids in human pathophysiologic processes. In a compilation of three original studies published by several authors of the present article (N.A.D., B.F.D., V.D.),[19] two studies were presented showing elevated PEA levels in humans with inflammatory and neuropathic conditions. In one study, a 1.89-fold increase in PEA levels was found in colon biopsy tissue in individuals with ulcerative colitis. In a second study—the preliminary report of the present pilot study, in fact—PEA levels in patients with chronic LBP increased significantly, 1.6-fold (P=.05), immediately after OMT. In other research, McPartland et al[20] found a 168% increase (P=.14) in AEA levels after OMT, an increase that correlated with symptom changes typically associated with cannabinoid effects in humans.

Although pilot studies have been conducted to assess the effects of manual treatment on other biochemicals—such as adrenocorticotropic hormone,[4] cortisol,[4] prostaglandins,[21] substance P,[22,23] and tumor necrosis factor[21]—results from these studies remain preliminary and inconsistent.

Low back pain is a major healthcare concern, with an incidence rate of 60% to 80% in industrialized countries and etiologic factors that, in approximately 85% of those cases, are considered nonspecific or biomechanical.[24] Osteopathic manipulative treatment,[25,26] as well as certain other forms of manual therapy,[27-30] have previously been shown to be beneficial in the treatment of patients with LBP. In the present investigation, we assessed the effects of OMT on five pain biomarkers—βE, 5-HT, 5-HIAA, AEA, and PEA—in volunteer subjects with chronic LBP. Because chronic LBP can be explained by pathophysiologic mechanisms involving mechanical and inflammatory mediator-induced abnormalities,[24] we hypothesized that subgroup analysis would allow the consideration of important nuances that are often raised in OMT research, such as the placebo response.

Methods

Subjects

Twenty white participants from a Midwestern rural community in the United States were enrolled in the present study. Enrolled subjects had a mean (SD) age of 38 (9) years, with an age range of 24 to 53 years. Ten subjects had chronic LBP; 10 age- and gender-matched control subjects did not have chronic LBP. There were 3 men and 7 women in each study group. Low back pain was defined as pain, muscle tension, or stiffness localized below the posterior costal margin and above the inferior gluteal folds. Subjects in the chronic LBP group had pain in the low back for a minimum of 5 days a week for at least 3 months. Control subjects had no self-reported incidents of persistent LBP (>3 d/wk) during the previous 3 months.

Subjects were excluded from the study if they had received any form of manual treatment of the spine within the 8 weeks preceding study entry; if they were currently taking anticonvulsants, antidepressants, muscle relaxants, opioids, or steroids; if they were experiencing current acute back pain; if they were diagnosed with an autoimmune disease; or if they had infections or inflammatory conditions at study initiation. Informed consent was obtained from each subject, with all procedures being approved by the institutional review board at A.T. Still University-Kirksville College of Osteopathic Medicine in Missouri.

Procedures

All 20 subjects participated in the present study during a consecutive 5-week period, with 4 subjects participating each week. Each subject had blood drawn for analysis at the same time of day for 5 consecutive days. On days 1, 2, 3, and 5, subjects completed an environmental factors questionnaire before blood was drawn. The questionnaire addressed subjects' current perceived pain level, stress level, amount of sleep, diet quality, and confounding substance use.

On day 4 of the study protocol, participants reported to the clinic 1 hour before the scheduled blood draw. All subjects completed the environmental factors questionnaire before physical evaluation and treatment by the primary investigator (B.F.D.), an osteopathic physician who is board-certified in neuromusculoskeletal medicine. The physician was blinded to subjects' group assignments. For the first 5 to 10 minutes of each physical examination, this physician conducted a routine osteopathic palpatory examination of the subject's muscu-

articulatory treatment system	Using a gentle springing or thrust, a joint is carried through its full motion with the therapeutic goal of increased freedom and range of movement
muscle energy	The patient is placed in a specific position by the physician and instructed to voluntarily move the body against a defined resistance
soft tissue technique	The physician uses lateral stretching, linear stretching, deep pressure, traction, or compression while monitoring tissue response and motion changes by palpation
Strain-Counterstrain	The physician places the patient in a position that relieves point tenderness and maintains that position until the tenderness does not return when the patient resumes a neutral position

Figure 1. *Descriptions of the osteopathic manipulative procedures used (B.F.D.) in the present pilot study of pain biomarkers.*

loskeletal system. Areas of somatic dysfunction (ie, sites of muscle hypertonicity, tenderness, and joint restriction) were identified, and the severity level of each finding was recorded on a 3-point scale, where 0 indicated no dysfunction; 1, mild to moderate dysfunction; and 2, severe dysfunction.

For the next 20 to 25 minutes, subjects in both study groups received OMT directed to specific sites of somatic dysfunction. The OMT consisted of the commonly performed techniques listed in *Figure 1*.[31] High-velocity/low-amplitude (HVLA) techniques were not used in treatment because we determined that it was unlikely that these techniques could be used safely with all of the subjects in the study. Compared with other osteopathic manipulative (OM) procedures, HVLA techniques are more likely to cause nociceptive input that could stimulate biomarkers secondary to a pain response/reaction, rather than as a primary therapeutic response—especially in cases of traumatic contracture, advanced degenerative joint disease, and ankylosis.[31] The OM procedures chosen for the present study were selected because we believed they could be applied safely to all study participants.

After receiving treatment on day 4, subjects rested approximately 30 minutes, reported their current perceived pain levels, and had a blood sample drawn. Concentrations of βE, 5-HT, 5-HIAA, AEA, and PEA were measured in each of the five blood samples drawn from subjects.

Determination of Environmental Factors

Subjects were asked to indicate their current perceived pain level on a well-established, 11-point pain-intensity numerical rating scale that ranged from 0, which indicated an absence of pain, to 10, which indicated the subject had the most severe pain.[32,33] Similarly, subjects were asked to provide information concerning their current stress levels on another 11-point numerical rating scale (0, no stress, to 10, extreme stress). Hours of sleep during the previous night were assessed on a 7-point numerical rating scale, where 1 indicated less than 4 hours of sleep and 7 indicated 9 or more hours of sleep).

On days 2 though 5, subjects were asked to indicate if they had used alcohol (>2 drinks), anticonvulsants, antidepressants, muscle relaxants, pain medications (including acetaminophen and nonsteroidal anti-inflammatory drugs [NSAIDs]), steroids, or stimulants during the previous 24 hours. Caffeinated beverage consumption was also recorded.

Blood Extraction and Quantification

On each day of the study, blood samples (5-day total=50 mL/subject) were collected in 10 mL tubes by antecubital venipuncture in the presence (5-day total=40 mL/subject) or absence (5-day total=10 mL/subject) of potassium salt of ethylenediaminetetraacetic acid (final concentration=5 mmol/L). Technicians who were masked to group assignments processed the samples within 1 to 2 hours after blood withdrawal.

For βE quantification, blood was allowed to clot for 2 hours before the serum was separated by centrifugation at 400 xg for 5 minutes. Isolated serum was promptly frozen at −80°C (−112°F) for as long as 1 week before concentrations were analyzed using a commercially available, competitive enzyme-linked immunosorbent assay (ELISA) kit (Model S-1240; Peninsula Laboratories Inc, San Carlos, Calif). For 5-HT and 5-HIAA quantification, the method used for separating plasma from whole blood was based upon a modification of procedures published by Cubeddu et al[34] and Schinelli et al.[35] Serotonin and 5-HIAA were extracted separately from 2 mL plasma samples by a modification of methods used by Oishi et al[36] and Ishida et al.[37] The extracted 5-HT and 5-HIAA samples were then analyzed by high-performance liquid chromatography with electrochemical detection (HPLC-ECD), based on a modification of the method described by Chaurasia et al.[38]

Separation of serotonin and 5-HIAA from other electrochemical compounds was achieved on a 10 cm × 3.2 mm RP-C18 column (ODS, 3 μm packing; BAS, West Lafayette, Ind) via an electrochemical detector (L-ECD-6A; Shimadzu Corp, Kyoto, Japan) connected to a syringe pump (500D; Teledyne

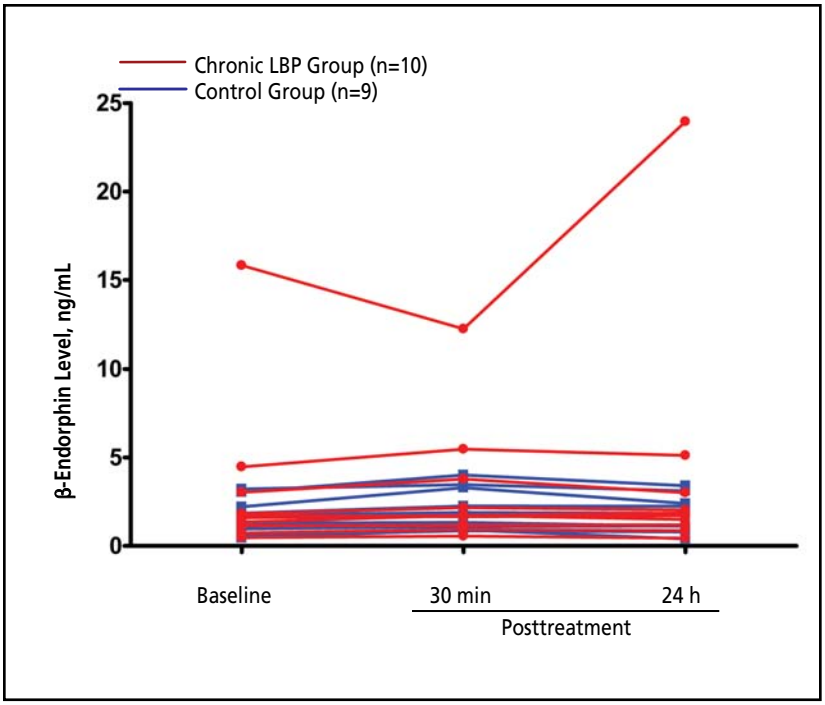

Figure 2. *Profile of β-endorphin levels for study subjects at baseline, at 30 minutes after osteopathic manipulative treatment, and at 24 hours posttreatment.*

Isco Inc, Lincoln, Neb) and a chromopac integrator (C-R7Ae; Shimadzu Corp, Kyoto, Japan). The glassy carbon working electrode was set at a potential of 650 mV relative to a reference electrode (Ag/AgCl). Anandamide and PEA quasimolecular ions were quantified by isotope dilution based on the methods of Darmani et al.[19]

Statistical Analysis

For each biomarker parameter, the pretreatment measurements for days 1, 2, and 3 were averaged to establish a baseline value for each subject. The stability of the three pretreatment values for pain and for each biomarker was determined using intraclass correlation coefficients (ICCs) and 95% confidence intervals (CIs). Because the distributions for several measures were skewed, nonparametric statistics were used to analyze all study data. For continuous measures (biomarker concentrations, sleep, stress), median baseline values were compared with the values obtained on day 4 at 30 minutes posttreatment as well as with values recorded on day 5 at 24 hours after OMT. These comparisons were made using the Wilcoxon signed rank test for all subjects within each subgroup. The Mann-Whitney test was used to compare biomarker concentrations in the chronic LBP group with those in the control group on median baseline and changes from baseline.

In order to determine whether subjects' use of confounding substances changed from baseline, the McNemar test was used. The correlation of changes in self-reported pain levels and environmental factors with changes in biomarkers was measured using Spearman rank correlation coefficients (ρ). Statistical significance was defined as $P \leq .05$.

Results

A summary of the biomarker characteristics in subjects with chronic LBP and control subjects at baseline, 30 minutes posttreatment, and 24 hours posttreatment is presented in *Table 1*. Data were analyzed to examine biomarker changes for subjects within and between subgroups. Data are reported as medians with 25th to 75th percentiles or as frequencies and percentages, as appropriate.

β-Endorphin

The βE data from 1 subject in the control group were not obtained because that individual's serum concentrations were below detectable levels on all 5 days. For the remaining 19 subjects within both subgroups, the three pretreatment serum βE measurements were found to be stable (ICC 0.92; 95% CI, 0.86-0.98). At 30 minutes posttreatment, there was a statistically significant increase in βE concentrations when compared with baseline measures for all subjects—a median increase of 19% (P=.002) (*Figure 2*). At 24 hours posttreatment, there was a median increase of 11% in βE concentrations for all subjects (P=.003) .

The baseline βE concentrations between the two study groups were not significantly different (P=.81). β-endorphin concentrations in the control group had a statistically significant increase over baseline at 30 minutes posttreatment—a median increase of 21% (P=.004). In the chronic LBP group, the increase above baseline βE was statistically significant at 24 hours posttreatment—a median increase of 11% (P=.01). In the control group, by contrast, βE levels did not differ significantly from baseline at 24 hours posttreatment, undergoing a median change of only 8% (P=.10).

Serotonin (5-Hydroxytryptamine)

Plasma 5-HT concentrations were stable during the 3-day pretreatment measurement period (ICC 0.84; 95% CI, 0.72-0.96). Serotonin concentrations for all subjects in both study groups did not change significantly at 30 minutes posttreatment (P=.67) or at 24 hours posttreatment (P=.45) (*Figure 3*).

Although baseline 5-HT plasma concentrations were higher in control subjects, subgroup analysis indicated that the difference between study groups was not statistically significant (P=.55). The 5-HT concentrations did not change significantly in subjects with chronic LBP (P=.19) or in control sub-

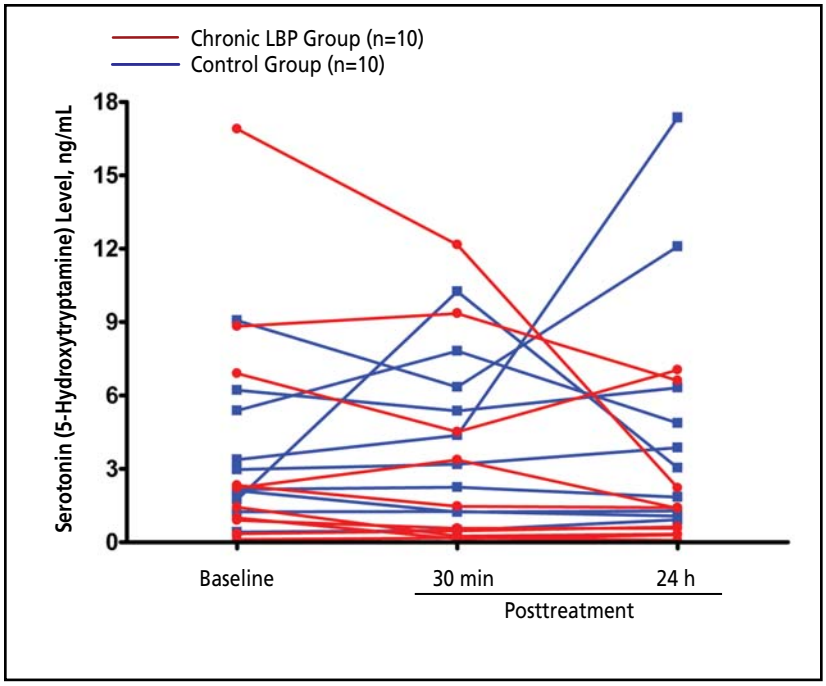

Figure 3. *Profile of serotonin (5-hydroxytryptamine [5-HT]) levels for study subjects at baseline, at 30 minutes after osteopathic manipulative treatment, and at 24 hours posttreatment.*

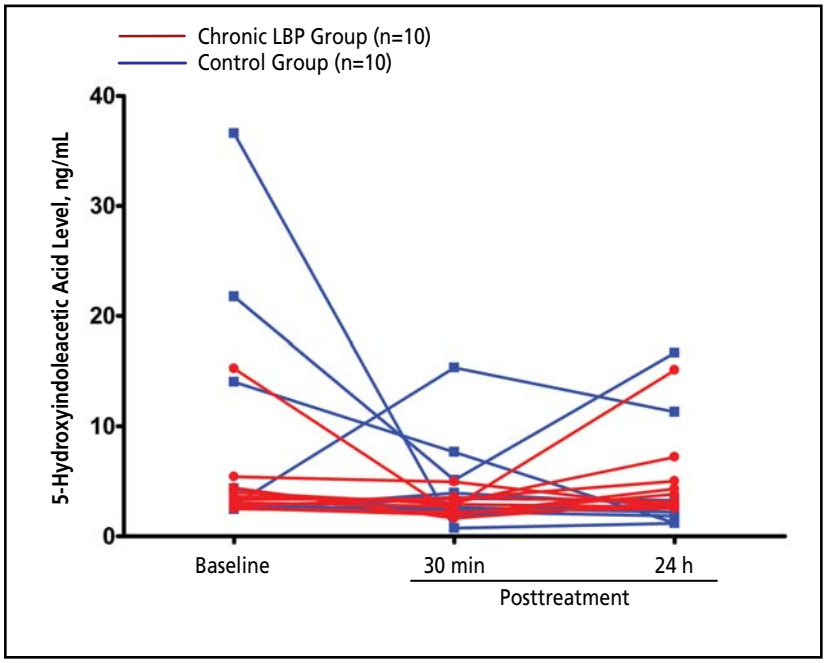

Figure 4. *Profile of 5-hydroxyindoleacetic acid (5-HIAA) levels for study subjects at baseline, at 30 minutes after osteopathic manipulative treatment, and at 24 hours posttreatment.*

jects (P=.49) at 30 minutes posttreatment. However, 5-HT concentrations decreased significantly at 24 hours posttreatment in the chronic LBP group—relative to baseline measures (median decrease of 37% [P=.02]) and to the control group (P=.01).

5-Hydroxyindoleacetic Acid

Concentrations of the 5-HT metabolite 5-HIAA were not measurable in one of the three pretreatment samples from 2 subjects with chronic LBP and from 3 subjects in the control group. Plasma concentrations of 5-HIAA were stable during the pretreatment measurement period (ICC 0.86; 95% CI, 0.76-0.96). The 5-HIAA concentrations for all subjects in both study groups did not change significantly at 30 minutes posttreatment (P=.06) or at 24 hours posttreatment (P=.57) (*Figure 4*).

The baseline plasma 5-HIAA concentrations in control subjects were not significantly different from baseline values in subjects with chronic LBP (P=.71). For the chronic LBP group only, 5-HIAA concentrations declined significantly at 30 minutes posttreatment—a median decrease of 13% (P=.05). No significant change within the chronic LBP group (P=.70) or the control group (P=.32) was observed at 24 hours posttreatment.

5-Hydroxyindoleacetic Acid/5-Hydroxytryptamine Turnover

The 5-HIAA/5-HT turnover (ie, the concentrations of 5-HIAA/5-HT[39]) for all subjects did not change significantly from baseline at either 30 minutes or 24 hours posttreatment (*Figure 5*). Although the 5-HIAA/5-HT turnover increased in subjects with chronic LBP and declined in control subjects at 30 minutes and at 24 hours posttreatment, these differences did not attain statistical significance.

Anandamide

For 1 subject with chronic LBP, the anandamide concentration was not detectable in the plasma samples taken on day 3. Pretreatment AEA concentrations demonstrated poor consistency (ICC −0.08; 95% CI, −0.31-0.15). Significant decreases in AEA for all subjects occurred at 30 minutes and 24 hours posttreatment—median decreases of 17%

Table 1
Baseline and Posttreatment Biomarker Characteristics of Study Participants by Chronic Pain Status (N=20)

Biomarker/ Subject Group	Baseline Median (Q1-Q3)	30 min Median (Q1-Q3) Δ Median (Q1-Q3)*	*P* Value†	24 h Median (Q1-Q3) Δ Median (Q1-Q3)*	*P* Value†
■ β-Endorphin, ng/mL					
□ All Subjects (n=19)	1.7 (1.0-3.0)	1.7 (1.1-3.5)		1.9 (1.1-3.0)	
		0.3 (0.1-0.5)	.002	0.1 (0-0.3)	.003
□ Chronic LBP					
– Yes (n=10)	1.7 (1.1-3.0)	1.7 (1.2-3.8)		1.8 (1.2-3.0)	
		0.2 (0-0.4)	.13	0.1 (0-0.3)	.01
– No (n=9)‡	1.7 (1.0-2.2)	1.9 (1.1-3.3)		1.9 (1.1-2.4)	
		0.3 (0.2-0.5)	.004	0.2 (−0.1-0.2)	.10
□ *P*§	.81	.25		.57	
■ Serotonin (5-HT), ng/mL					
□ All Subjects (N=20)	2.2 (1.1-5.8)	2.7 (0.5-5.9)		1.6 (0.8-5.6)	
		0 (−0.9-0.4)	.67	−0.2 (−0.8-0.4)	.45
□ Chronic LBP					
– Yes (n=10)	1.8 (0.9-6.9)	1.0 (0.3-4.5)		1.0 (0.3-2.2)	
		−0.6 (−1.2-0.1)	.19	−0.8 (−1.1-0)	.02
– No (n=10)	2.6 (1.8-5.4)	3.8 (1.3-6.4)		3.5 (1.3-6.3)	
		0.1 (−0.8-1.0)	.49	0.3 (−0.3-1.3)	.28
□ *P*§	.55	.26		.01	
■ Serotonin Metabolite 5-HIAA, ng/mL					
□ All Subjects (N=20)	3.2 (2.6-4.9)	2.8 (2.2-3.8)		3.1 (2.6-4.7)	
		−0.4 (−2.6-0.1)	.06	−0.2 (−2.0-1.2)	.57
□ Chronic LBP					
– Yes (n=10)	3.5 (2.8-4.4)	2.8 (2.1-3.4)		3.5 (2.7-5.0)	
		−0.5 (−1.1-−0.2)	.05	0.2 (−1.3-1.8)	.70
– No (n=10)	2.7 (2.5-14.0)	3.0 (2.3-5.1)		2.8 (1.9-3.3)	
		−0.2 (−6.4-0.9)	.38	−0.6 (−5.1-0.4)	.32
□ *P*§	.71	.71			.41
■ 5-HIAA/5-HT Turnover					
□ All Subjects (N=20)	1.7 (0.9-5.0)	1.6 (0.8-5.3)		2.3 (0.7-4.2)	
		−0.1 (−2.0-0.9)	.55	0.3 (−1.6-1.2)	.84
□ Chronic LBP					
– Yes (n=10)	1.7 (0.9-4.5)	3.8 (1.0-8.9)		4.2 (1.7-11.6)	
		0.1 (−0.7-1.2)	.92	0.9 (0.2-2.0)	.11
– No (n=10)	2.3 (0.9-5.5)	1.4 (0.7-2.8)		1.7 (0.4-2.5)	
		−0.5 (−2.4-0.3)	.38	−0.6 (−3.3-0.4)	.19
□ *P*§	.88	.45			.07

* Median change from baseline.
† Within-group *P* values based on Wilcoxon signed rank test comparing posttreatment biomarker levels with baseline biomarker levels.
‡ β-endorphin data from 1 subject were not obtained because the subject's serum concentrations were below detectable levels on all days.
§ Between-group *P* values based on Mann-Whitney test comparing biomarker levels of subjects with chronic LBP and those without at baseline and at posttreatment.

Abbreviations: 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, 5-hydroxytryptamine; LBP, low back pain; Q1, 25th percentile; Q3, 75th percentile.

(continued)

Table 1 (continued)
Baseline and Posttreatment Biomarker Characteristics of Study Participants by Chronic Pain Status (N=20)

Biomarker/ Subject Group	Baseline Median (Q1-Q3)	30 min Median (Q1-Q3) Δ Median (Q1-Q3)*	*P* Value†	24 h Median (Q1-Q3) Δ Median (Q1-Q3)*	*P* Value†
■ **Anandamide, pmol/mL**					
□ All Subjects (N=20)	1.7 (1.5-2.0)	1.6 (1.0-2.0)		1.5 (0.9-1.9)	
		−0.3 (−0.9-0.2)	.05	−0.1 (−0.9-0.1)	.04
□ Chronic LBP					
– Yes (n=10)	1.7 (1.3-1.9)	1.7 (0.9-2.1)		1.6 (0.8-1.9)	
		0.1 (−0.7-0.4)	.95	0 (−0.7-0.2)	.61
– No (n=10)	1.9 (1.7-2.7)	1.5 (1.0-1.6)		1.3 (1.0-1.6)	
		−0.7 (−1.2-−0.3)	.02	−0.7 (−1.1-0)	.04
□ *P*§	.11	.05			.11
■ **N-Palmitoylethanolamide, pmol/mL**					
□ All Subjects (N=20)	13.5 (11.7-15.9)	19.2 (13.1-23.3)		16.9 (14.2-22.5)	
		4.0 (0.6-9.8)	<.001	5.2 (−2.5-10.4)	.03
□ Chronic LBP					
– Yes (n=10)	14.2 (11.9-15.9)	22.5 (18.0-28.3)		19.5 (13.3-25.7)	
		8.4 (3.9-12.1)	.006	5.9 (−2.7-11.4)	.19
– No (n=10)	12.8 (11.6-15.7)	16.6 (12.7-20.1)		16.3 (15.0-19.5)	
		1.4 (0.1-5.7)	.03	4.6 (−2.4-7.1)	.08
□ *P*§	.76	.05			.71

* Median change from baseline.
† Within-group *P* values based on Wilcoxon signed rank test comparing posttreatment biomarker levels with baseline biomarker levels.
‡ β-endorphin data from 1 subject were not obtained because the subject's serum concentrations were below detectable levels on all days.
§ Between-group *P* values based on Mann-Whitney test comparing biomarker levels of subjects with chronic LBP and those without at baseline and at posttreatment.

Abbreviations: 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, 5-hydroxytryptamine; LBP, low back pain; Q1, 25th percentile; Q3, 75th percentile.

Table 2
Spearman Rank Correlation Coefficientsof Change in Self-Reported Pain*by Biomarker Levels for Study Participants With Chronic Low Back Pain (n=10)

Biomarker	Baseline to Posttreatment Change, ρ 30 min	24 h
β-Endorphin	0.10	0.38
Serotonin (5-HT)	0.20	-0.26
Serotonin Metabolite 5-HIAA	0.60	-0.67†
5-HIAA/5-HT Turnover	0.07	-0.46
Anandamide	-0.07	-0.34
N-Palmitoylethanolamide	-0.26	-0.47

* Subjects reported pain on an 11-point numerical rating scale (0=no pain, 10=most severe pain).
† $P \leq .05$

Abbreviations: 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, 5-hydroxytryptamine.

(*P*=.05) and 5% (*P*=.04), respectively (*Figure 6*).

Baseline AEA levels in subjects with chronic LBP were not significantly different from those in subjects without chronic LBP (*P*=.11). Significant decreases in AEA occurred in the control group at 30 minutes and 24 hours posttreatment—median decreases of 34% (*P*=.02) and 42% (*P*=.04), respectively. However, AEA concentrations in the chronic LBP group did not change significantly at either 30 minutes (*P*=.95) or 24 hours posttreatment (*P*=.61). When the two subgroups were compared with each other, there was a significant difference (*P*=.05) in the change in AEA levels at 30 minutes posttreatment.

N-Palmitoylethanolamide

On day 3, the laboratory was unable to detect PEA concentration in the same subject for which AEA concentration was undetectable. Although the blood PEA concentrations were not significantly different during the three pretreatment measurements, measured values showed only fair consistency

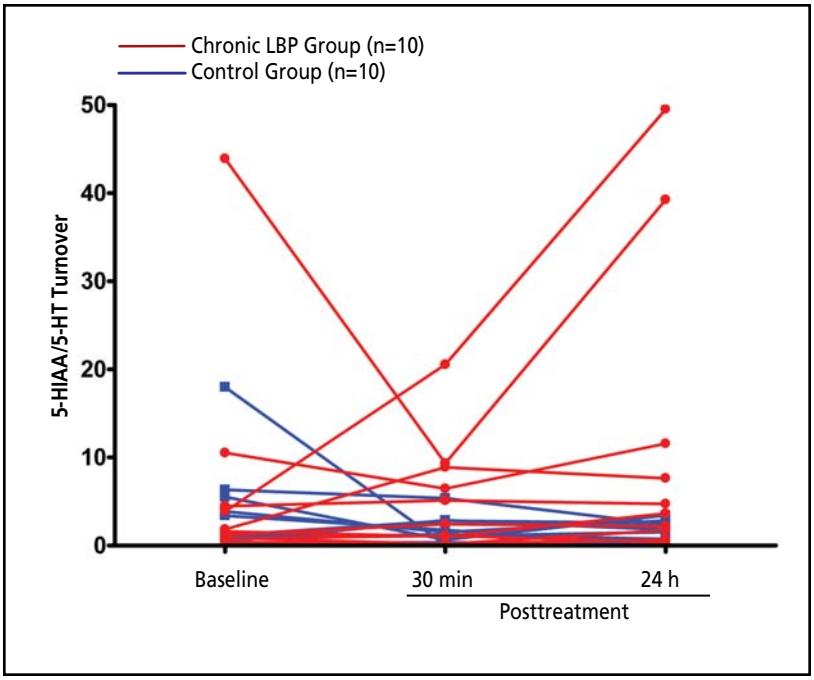

Figure 5. *Profile of 5-hydroxyindoleacetic acid (5-HIAA)/5-hydroxytryptamine (5-HT) turnover for study subjects at baseline, at 30 minutes after osteopathic manipulative treatment, and at 24 hours posttreatment.*

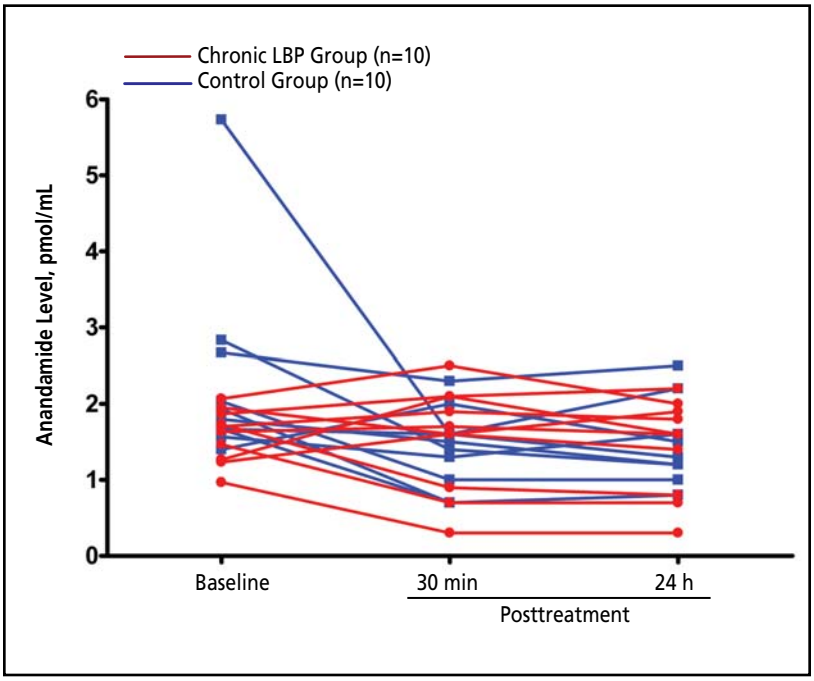

Figure 6. *Profile of anandamide (arachidonoylethanolamide) levels for study subjects at baseline, at 30 minutes after osteopathic manipulative treatment, and at 24 hours posttreatment.*

(ICC 0.29; 95% CI, 0.00-0.58). Median increases in PEA for all subjects of 27% ($P<.001$) at 30 minutes posttreatment and 37% ($P=.03$) at 24 hours posttreatment were statistically significant (*Figure 7*).

Baseline PEA levels in subjects with chronic LBP did not differ significantly from control subjects ($P=.76$). Median increases of 51% ($P=.006$) and 12% ($P=.03$) in PEA for subjects with and without chronic LBP, respectively, at 30 minutes posttreatment demonstrated a significantly larger increase in the chronic LBP group relative to the control group ($P=.05$). However, PEA at 24 hours posttreatment was not significantly different from baseline levels for either the chronic LBP group ($P=.19$) or the control group ($P=.08$).

Perceived Pain, Stress, Sleep, Substance Use

Considering only the subjects with chronic LBP, changes in perceived pain did not correlate with changes in concentrations of βE, 5-HT, AEA, or PEA at 30 minutes or 24 hours posttreatment (*Table 2*). The correlation between the change in self-reported pain and 5-HIAA was statistically significant at 24 hours posttreatment ($\rho=-0.67$, $P=.03$). However, the relationship between the change in perceived pain and 5-HIAA at 30 minutes posttreatment was the opposite of the 24-hour posttreatment change ($\rho=0.60$, $P=.06$).

Data on self-reported stress levels, sleep patterns (*Table 3*), and confounding substance use (*Table 4*) were collected and analyzed. Stress level was significantly decreased at 24 hours posttreatment ($P=.001$). There were no statistically significant changes in sleep patterns or confounding substance use during the study. Increased NSAID use on day 4—the day of OMT—was significantly correlated with increased AEA concentration at 30 minutes posttreatment ($\rho=0.44$, $P=.05$) (*Table 5*). Increased stress levels at 24 hours posttreatment were significantly correlated with decreased PEA concentration ($\rho=-0.49$, $P=.03$). In addition, increased allergy medication use at 24 hours posttreatment was associated with decreased PEA levels ($\rho=-0.49$, $P=.03$).

Comment

The present pilot study was designed to investigate if OMT affects circulatory biomarker

Table 3
Self-Reported Environmental Factors for Study Participants (N=20)

Environmental Factor*	Baseline Median (Q1-Q3)	Day of OMT Median (Q1-Q3) Δ Median (Q1-Q3)†	*P* Value‡	Day After OMT Median (Q1-Q3) Δ Median (Q1-Q3)†	*P* Value‡
Sleep	3.0 (2.3-4.3)	3.0 (2.0-4.0)		3.0 (2.0-4.0)	
		0 (−0.3-0)	.51	0 (−1.0-0)	.11
Stress	4.0 (2.4-6.2)	4.3 (2.8-6.5)		3.5 (1.5-6.0)	
		0 (−0.2-0.2)	.81	−0.3 (−1.0-0)	.001

* Subjects rated sleep on a 7-point numerical rating scale. Subjects rated stress on an 11-point numerical rating scale (0=no stress, 10=extreme stress).
† Median change from baseline.
‡ *P* values based on Wilcoxon signed rank test comparing posttreatment ratings with baseline ratings.

Abbreviations: OMT, osteopathic manipulative treatment; Q1, 25th percentile; Q3, 75th percentile.

levels. The protocol was meticulously performed during a seasonally stable 4-week period; blood samples were collected at exactly the same time of day for all subjects to minimize potential diurnal variability.[40] Blood samples were processed within 2 hours to minimize sample degradation. Blood levels from 3 consecutive days were used to establish baseline readings. All subjects received the same OM procedures for the same treatment duration, administered by the same osteopathic physician (B.F.D.).

It was hypothesized that, if no changes were detected in subjects' biomarkers, OMT likely had no effect. However, if changes in biomarkers were noted and were the same in age- and gender-matched chronic LBP group versus the control group, these changes would support the hypothesis that the effect of OMT may be secondary to touch alone and mediated by the placebo response. Furthermore, if there was a correlation between unique changes in circulatory biomarkers in the chronic LBP group, this finding would provide support for more comprehensive research to determine possible underlying nonplacebo, pain-modulating mechanisms for OMT. Potential confounding variables (sleep, stress, substance use) were monitored throughout the 5-day period.

The results of the present study show statistically significant biomarker changes in the overall study population, as well as statistically significant differences between the two subgroups—even though the sample size was small. These find-

Table 4
Self-Reported Confounding Substance Use for Study Participants (N=20)

Confounding Substance	Baseline No. (%)	Day of OMT No. (%)	*P* Value*	Day After OMT No. (%)	*P* Value*
■ **Overall†**	12 (60)	10 (50)	>.99	12 (60)	>.99
□ Acetaminophen	3 (15)	2 (10)	>.99	4 (20)	.38
□ Allergy Medication	3 (15)	4 (20)	.25	3 (15)	.50
□ Caffeine	12 (60)	10 (50)	>.99	10 (50)	.63
□ Hormone Replacement Therapy	9 (45)	8 (40)	>.99	8 (40)	>.99
□ NSAIDs	5 (25)	3 (15)	>.99	2 (10)	.38
□ Stomach Medication	3 (15)	3 (15)	>.99	3 (15)	>.99
□ Vitamins	6 (30)	6 (30)	>.99	6 (30)	>.99

* *P* values based on McNemar test comparing posttreatment use with baseline use.
† Includes alcohol (>2 drinks), allergy medication, anticonvulsants, antidepressants, caffeine, hormone replacement therapy, muscle relaxants, pain medication, steroids, stimulants, stomach medication, or vitamins within previous 24 hours.

Abbreviations: NSAIDs, nonsteroidal anti-inflammatory drugs; OMT, osteopathic manipulative treatment.

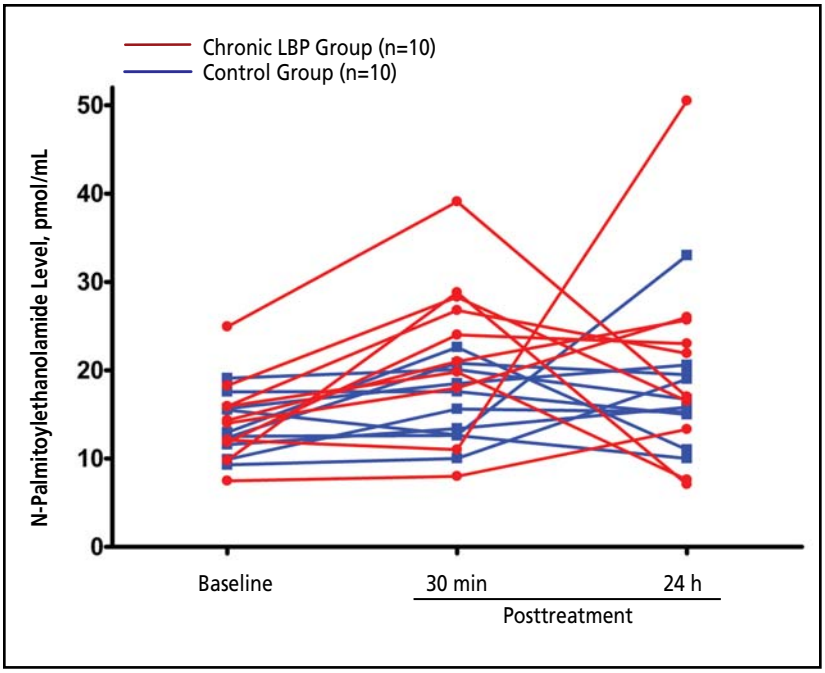

Figure 7. *Profile of N-palmitoylethanolamide levels for study subjects at baseline, at 30 minutes after osteopathic manipulative treatment, and at 24 hours posttreatment.*

ings support more rigorous research on the mechanisms of OMT, using a more standardized treatment protocol involving control and light-touch sham treatment groups.

Because the data in the present study were skewed, nonparametric statistical analyses were used. For readers not familiar with such statistics, it may appear odd that small changes in the median value can be statistically significant. However, by examining how data for each subject change over time, such variations can illustrate consistent trends of a population or subgroup. Because it is unclear where the biomarkers were formed or how the mechanisms of OMT affected biomarker concentrations, it is possible that small changes in serum biomarker concentrations may reflect larger changes in other tissues.

Overall, the results of the current study demonstrated that the biomarkers βE, AEA, and PEA significantly changed at 30 minutes and 24 hours posttreatment. No statistically significant overall changes occurred for 5-HT or its derivative, 5-HIAA. This finding implies that effects secondary to OMT may be mediated by endogenous opioid and endocannabinoid pathways, but not by serotonergic pathways.

Subgroup analysis allowed further interpretation of the overall results. Baseline serum concentrations of βE did not differ between subjects with LBP and control subjects. Although substantial clinical data suggest that βE concentrations increase with acute pain,[41,42] only scant data is available regarding the effects of chronic pain on the circulating concentrations of endogenous opioids. In an animal model of chronic arthritis, βE was elevated in the spleen.[43] Thus, one could speculate that, in cases of chronic pain in humans, increases in βE concentrations would reduce perceived pain. However, low βE concentrations have been found to occur in women with a history of back pain[44] and endometriosis.[45] These low concentrations possibly reflect an exhausted release of endogenous opioids. Additional studies are needed to clarify the effect of chronic pain on circulating βE concentrations.

Findings at 30 minutes posttreatment suggest that OMT activates the endogenous opioid system by releasing βE, a conclusion that is consistent with two studies that showed statistically significant increases of βE at 5 to 30 minutes after manual treatment (connective tissue massage, spinal manipulation).[6,9] However, this conclusion stands in contrast to various other studies of tissue massage and chiropractic therapies, which showed no such changes in βE concentrations.[4,5,7,8]

In the present study, the change in βE concentration immediately after OMT was most significant in the control group. A change in βE concentration was noted in the chronic LBP group at 24 hours posttreatment. Whether the increased βE was secondary to a progressive effect of OMT or secondary to environmental changes is unclear. However, because the decrease in pain in the chronic LBP group was most apparent at 30 minutes posttreatment (rather than at 24 hours posttreatment), the βE findings are unlikely to be related to pain modulation. In addition, because most research has shown that the placebo response can be mediated through the opioid system,[46] it is possible that, based on the present study's data, the underlying mechanism of OMT could be the result of a placebo-mediated response. By including a touch-placebo group in future studies and expanding the sample size, this association could be further delineated.

The central and peripheral bases for the involvement of cannabinoid receptors and the endocannabinoid system in pain and inflammation are well established in animal models,[15,17] but little is known about this system in humans. Cannabinoids have been shown to be active in animal models of acute and persistent inflammatory pain and nerve-injury pain.[15,17] Furthermore, pain triggered by subcutaneous injection of formalin increases the release of AEA in the periaqueductal grey, a pain-modulatory site in the midbrain.[47] Preliminary data from the present study, focusing on the change in PEA at 30 minutes posttreatment, were included in our first published report,[19] which analyzed endocannabinoids in humans.

Table 5
Spearman Rank Correlation Coefficients of Change in Self-Reported Environmental Factors and Confounding Substance Usage With Change in Biomarker Levels for Study Participants (N=20)

Biomarker and Factor/Substance*	Baseline to Posttreatment Change, ρ	
	30 min	24 h
■ β-Endorphin†		
□ Environmental Factor		
– Sleep	-0.03	0.45
– Stress	-0.25	0.05
□ Confounding Substance	−0.43	0
– Acetaminophen	-0.04	-0.03
– Allergy Medication	−0.10	-0.09
– Caffeine	-0.43	-0.09
– NSAIDs	-0.37	0.12
■ Serotonin (5-HT)		
□ Environmental Factor		
– Sleep	0.32	-0.38
– Stress	-0.08	-0.07
□ Confounding Substance	-0.14	-0.43
– Acetaminophen	-0.35	-0.15
– Allergy Medication	-0.16	-0.40
– Caffeine	-0.14	-0.38
– NSAIDs	-0.08	-0.41
■ Serotonin Metabolite 5-HIAA		
□ Environmental Factor		
– Sleep	-0.11	0.11
– Stress	0.01	0.01
□ Confounding Substance	0.40	-0.04
– Acetaminophen	0.24	-0.04
– Allergy Medication	0.30	0.09
– Caffeine	0.40	0.14
– NSAIDs	0.06	0.09
■ 5-HIAA/5-HT Turnover		
□ Environmental Factor		
– Sleep	-0.09	0.20
– Stress	0.09	0.16
□ Confounding Substance	0.37	0.15
– Acetaminophen	0.31	-0.07
– Allergy Medication	0.31	0.35
– Caffeine	0.37	0.24
– NSAIDs	0.19	0.18

(continued)

Table 5 (continued)
Spearman Rank Correlation Coefficients of Change in Self-Reported Environmental Factors and Confounding Substance Usage With Change in Biomarker Levels for Study Participants (N=20)

Biomarker and Factor/Substance*	Baseline to Posttreatment Change, ρ	
	30 min	24 h
■ Anandamide		
□ Environmental Factor		
– Sleep	0.15	0.28
– Stress	0.40	-0.01
□ Confounding Substance	0.03	0.18
– Acetaminophen	0.37	-0.35
– Allergy Medication	0.39	0.38
– Caffeine	0.03	0.35
– NSAIDs	0.44‡	0.10
■ N-Palmitoylethanolamide		
□ Environmental Factor		
– Sleep	-0.07	-0.24
– Stress	0.03	-0.49‡
□ Confounding Substance	-0.01	-0.06
– Acetaminophen	-0.05	-0.17
– Allergy Medication	-0.30	-0.49‡
– Caffeine	-0.01	0.12
– NSAIDs	0.28	-0.35

* Includes alcohol (>2 drinks), allergy medication, anticonvulsants, antidepressants, caffeine, hormone replacement therapy, muscle relaxants, pain medication, steroids, stimulants, stomach medication, or vitamins within previous 24 hours.

† β-endorphin data from 1 subject in the non-chronic LBP group were not obtained because the subject's serum concentrations were below detectable levels on all days.

‡ $P \leqslant .05$

Abbreviations: 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, 5-hydroxytryptamine; NSAIDs, nonsteroidal anti-inflammatory drugs.

Although derived from different precursors, AEA and PEA are synthesized and hydrolyzed by the same enzymes.[18] However, PEA is not a putative endocannabinoid, because it does not bind cannabinoid receptors efficiently. Instead, PEA has cannabimimetic properties, including analgesic and anti-inflammatory effects seen in several animal models of inflammation and pain.[18] The present study demonstrated that, though daily PEA blood concentrations can be variable, baseline PEA concentrations were not significantly different between the chronic LBP and control groups. Osteopathic manipulative treatment increased PEA concentrations in both study groups at 30 minutes posttreatment, with significantly greater changes observed in the chronic LBP group at that time interval. This change persisted in the overall study population, but it did not persist after 24 hours for either group independently. These findings suggest that OMT causes a short-lived but greater increase in PEA concentrations in subjects with chronic LBP, relative to the increase in subjects without chronic LBP.

In the present study, no significant relationship between OMT and AEA was demonstrated in subjects with chronic LBP. However, there were significant reductions in

AEA in the control group at 30 minutes and at 24 hours posttreatment, suggesting a link between OMT and circulating AEA levels. McPartland et al[20] analyzed the pre- and postintervention levels of AEA in two groups of healthy subjects: an OMT group and a sham OMT group. The similarity between the McPartland et al[20] study groups and the control group in the present study allows direct comparison between outcomes. Our study showed a lower level of AEA at baseline, with less variability, compared with that of McPartland et al[20] (our median=1.9 pmol/mL; McPartland et al means=2.99 pmol/mL [treatment], 2.26 pmol/mL [sham treatment]). Although our study demonstrated a small yet statistically significant decrease in the AEA level for the control group (P=.02), McPartland et al[20] reported a large increase in AEA that did not demonstrate statistical significance (P=.14).

Because McPartland et al[20] used parametric statistics to analyze their data instead of the nonparametric statistics used in the present study, there are possible limitations in the comparison of these two studies. The different statistical analyses may also explain the apparently contradictory results. We believe that the reported data of McPartland et al[20] indicates that their data were significantly skewed and that nonparametric statistical analysis would be more appropriate for that data set.

Published results investigating the relationship between changes in 5-HT and painful inflammatory musculoskeletal conditions, such as fibromyalgia and arthritic joint pain, are complicated. Depending on the study, the 5-HT concentrations in blood products have been shown to increase,[12,13] decrease,[48-50] or remain unchanged,[51,52] compared with controls. By contrast, joint manipulation in animal studies has been shown to lead to increases in central 5-HT concentrations, which may produce analgesia via spinal 5-HT receptors in descending inhibitory pathways.[14] In addition, low concentrations of the 5-HT metabolite 5-HIAA have been correlated with high pain scores in humans.[52]

In the present study, baseline 5-HT concentrations tended to be lower, while 5-HIAA concentrations were higher, in the chronic LBP group relative to the control group. However, these differences between the subgroups did not attain statistical significance, probably because of large intersubject variability and limited sample size. Relative to baseline and to control levels, levels of 5-HT were reduced at 30 minutes and 24 hours posttreatment in subjects with chronic LBP. Concentrations of 5-HIAA in subjects with chronic LBP were significantly reduced compared with baseline measures and control subjects at 30 minutes posttreatment, but not at 24 hours posttreatment. The 5-HIAA/5-HT turnover tended to increase in subjects with chronic LBP and decrease in control subjects.

Overall, because of the small sample size and large intersubject variability, trends in 5-HT and 5-HIAA levels were not statistically significant. Still, these findings suggest that OMT may reduce peripheral analgesic effects of 5-HT in subjects with chronic LBP by increasing 5-HIAA/5-HT turnover and, thus, decreasing serum 5-HT concentrations. Further studies are necessary to determine if such a relationship exists.

One of the monitored potential confounding factors—stress level—changed significantly during the course of the present study. There was evidence that change in stress level and change in use of allergy medication may be related to changes in PEA levels. In addition, the present study provided evidence that changes in NSAID use may be related to changes in AEA levels. To better analyze the effects of potentially confounding medications, especially NSAIDs, future studies should collect data for quantitative, not just a qualitative, analysis. The conclusions of our study are based on relatively small sample sizes, low statistical power (particularly for the McNemar test), and limited variability of findings.

By using 3 days of tightly controlled measurements of circulatory biomarkers to establish baselines and by monitoring potential confounding factors, we can have reasonable confidence that changes in the measured biomarkers correlated with subjects receiving OMT. However, any direct association or significance of these changes to a therapeutic effect from OMT remains speculative. It is well known that measured biomarkers interact with each other and can produce substantial additive or synergistic analgesic effects.[16] For example, noneffective doses of Δ^9-tetrahydrocannabinol and precursors of 5-HT enhance the potency of opioids, such as morphine, at different anatomic levels in animal models of pain.[10,53] Similar interactions may contribute to the therapeutic effect commonly observed after OMT. In future studies, the use of a larger and more homogeneous population—including a light-touch sham treatment group—will help determine the importance of biomarker changes in relation to OMT.

Conclusion

Changes from baseline levels of βE, AEA, and PEA occurred immediately after, as well as 24 hours after, OMT. The data suggest that alterations in levels of circulatory biomarkers were most likely caused by OMT, rather than by changes in potential confounding factors. However, these results are based on small sample sizes and tests with low statistical power. The observed alterations in blood concentration were present, but variable, in both study groups. While encouraging, these results are correlational rather than mechanistic. More rigorously controlled research into the mechanisms of OMT is required before these mechanisms can be adequately hypothesized and tested.

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