RE: "Changes in Vertebral Artery and Cerebral Hemodynamics Following Various Head Positions & Cervical Manipulation in Patients with Chronic/Recurrent Neck Pain."

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To whom it may concern,

We thank you for you consideration of this work. Please find below 4 pages detailing the methods and procedures of the study.

Sincerely,

Nicholas Moser, BSc, DC

Methods

Participants

Twenty patients were recruited from the campus-teaching clinic located at the Canadian Memorial Chiropractic College. Included were adults aged 18 years and older with chronic grade II31 mechanical neck pain that had been prescribed cervical manipulation for treatment of their condition. Patients with a history of neck pain with associated arm pain within the last 6 months; any current or history of neurologic symptoms including facial or extremity weakness, abnormal sensation to the face, body, or extremities, uncontrolled movements, abnormal gait, dizziness, unexplained nausea/vomiting, difficulty with speaking or swallowing; history of new or severe (Visual Analogue Scale >6) headaches in the last 3 months; any contraindications to MRI; or any history of using drugs that affect blood flow such as Warfarin, or anti-coagulants were excluded. All participants refrained from vigorous physical activity, alcohol, and caffeine one day before commencement of the study.

The study was approved by the local ethics committees of St. Joseph's Healthcare Hamilton at McMaster University in Hamilton, Ontario, and Canadian Memorial Chiropractic College in Toronto, Ontario. Written informed consent was obtained from all participants before the study commenced. Data were collected at the Imaging Research Centre, Hamilton, Ontario. The study was registered with www.clinicaltrials.gov, NCT02667821.

Test Protocols

The study was conducted as a randomized controlled case-crossover trial. Prior to the MRI, baseline information on each participant was obtained and included age, gender, height, weight, hand dominance, NDI score neck pain intensity and headache intensity (see Table 1). On the day of the scan, prior to commencing, participants underwent a cervical spine examination, completed by the clinician performing the test maneuvers (SM), in order to identify the site of manipulation. Once participants provided verbal acknowledgement of being comfortable to proceed, they underwent three separate test maneuvers. Each began with neutral (0 degree rotation) neck position (condition 1) as a standard natural control, followed by either maximum voluntary rotation (conditions 2), and one dynamic high-velocity-low-amplitude (HVLA) cervical rotary manipulation targeted at C1-C2 (condition 3). Each procedure was performed to the side of clinical findings elicited during cervical examination by the clinician. Tasks were selected based on their use in evaluation or treatment during clinical encounters.

Neutral position of the head was defined by alignment in the Frankfort plane in a vertical orientation. For continuity of neutral alignment between protocols, the MRI laser land marking tool was used to cross triangulate three tape marks on the MRI head RF coil with three oil based markers (Vitamin E capsules) taped to the nasion (bridge of the nose) and immediately in front of the tragus of the ears, bilaterally. For each brain image obtained, the marker set provided quantitative orientation of the head position.

Participants were randomly assigned to condition 2 or condition 3 using a randomized table generator (GraphPad Software Inc, La Jolla, CA). Each head condition was applied in the MRI scanner room adjacent to the MRI bore (Fig 1) and for condition 2 the position of the head was held for 1 minute and then returned to neutral for MRI sequencing. For the manipulation, the head was repositioned to neutral immediately after

and the patient was retracted into the MRI bore. Condition 2 was independently measured by inclinometer.

After each condition, MRI of the cerebrum and upper neck for blood flow ensued. The total time elapsed for each participant testing protocol was 60 minutes. The total time elapsed for each condition was approximately 20 minutes. This included applying the condition (1 minute), replacing the participant back into the MRI bore (approximately 1 minute), and routine image sequencing (approximately 18 minutes). Estimates of repeatability of flow measures were made in preliminary work by quantifying VA flow in a single healthy participant twice over a 2-month interval.

A practitioner with more than 30 years of practice experience conducted the upper cervical manipulation.32-34 The manipulation procedure required no transfer of the participant; rather, it was performed on the adjustable and pivotal MRI bed in the MRI room with the participant in the supine position (Fig 1). The procedure was a high velocity, low amplitude (HVLA) impulse, designed to initially position the participants' heads in an axial rotation, flexion as well as lateral flexion postures. Variations of head positions between operators have been demonstrated to be relatively small.35,36 The clinician performed the procedures in representative manner, first establishing the end range of motion to determine appropriate preload position for the manipulation before applying a typical clinical force impulse in the coronal plane with minimal traction component. Before the delivery of each maneuver, the participants were queried on their comfort, condition, and willingness to continue.

A brief 5-item analogue scale was administered to each of the participants and clinician immediately after the test maneuvers. This scale provides quantitative estimates of the patient's and operator's judgment of the descriptive characteristics of the CSM procedure on a continuous scale of 0 to 10. The words "fast," "force," "comfort," "confidence," and "precise" were used as descriptors as validated and used in several other studies.37-39 The scale provided a means of contrasting the procedure characteristics within the context of the range of typical performance, as represented within the literature.

Magnetic Resonance Imaging Protocols

Four MRI series were performed on each participant (3-Telsa GE Signa Excite HD MRI scanner and 20 channel neurovascular array RF coil; GE Healthcare, Milwaukee WI), including anatomical images, arterial spin labeling (ASL), blood oxygen level dependent (rsMRI BOLD) and phase contract MRI. Following head positioning, immobilization with sponges and a localizer scan, anatomical images were collected using a 3D IR-prepped fast SPGR T1-weighted scan (TR/TE=11.4/4.3 ms, T1 = 450 ms, flip angle = 12 degrees, 512 x 256 matrix, 140 slices, 24 cm FOV, reconstructed to 1mm3 isotropic voxels). Microvasculature was assessed using a 3D spiral-based fast spin echo pseudo-continuous arterial spin labeling (pCASL). pCASL sequence was used to assess cerebrovascular perfusion (TE/TR/TI=10.5/4629/1525ms, FOV=24cm, 512x8spiral interleaves, 3NEX, reconstructed to 128x128matrix with 4mm thick slices, scan time =30s). The ASL sequence was immediately followed with the phase contrast MRI. At the level of C1-2, the contralateral and ipsilateral vertebral arteries (to the direction of head motion) were assessed and anatomical images were established to localize the VA circulation. As previously published by Ho et al the method for obtaining flow quantification of the VA was a 2-dimensional phase-contrast pulse sequence. To capture accurate VA flow, the imaging plane is ideally perpendicular to the central axis of the

blood vessel. This imaging plane was selected on the vessel of interest at the C1-2 intervertebral level based on arterial visualization on a maximum intensity projection of a 2-dimensional time-of flight MRI angiogram. Magnetic resonance acquisition parameters were as follows: fast gradient recalled echo; echo time/repeat time, 3.9/8.9 milliseconds; flip angle, 20o' 20 cm field of view; 512* 512 matrix; 244-Hz/pixel receiver bandwidth; 1 average; 4 mm thick; and velocity encoding of 50 cm/s encoded for 30 phases per cardiac cycle. Owing to anatomical variations, occasional slices could only approximate orthogonality. All image measurements were obtained by manually selecting the optimal anatomical site between the base of the odontoid process and approximately 1 cm above the tip of the dens. According to Lotz et al vessel obliquity is tolerable to above or equal to 15 degrees, above which will cause a deviation from the true flow.41 Data capture was triggered by prospective gating using a peripheral pulse (MRI scanner–pulsed oximeter). The image acquisition time for each flow measurement was approximately 1 minute 30 seconds depending on heart rate. 40 Flow analysis was performed using Segment v1.9 software 47 (Medviso, Lund, Sweden). Dynamic regions of interest were drawn on the left and right vertebral arteries to quantify mean, as well as peak velocities, and flows. Data in the trigger window portion of the cardiac cycle were derived by spline interpolation using Matlab (Mathworks, Natick, MA). The final data set for a complete cardiac cycle, therefore, contained measurements from all the cardiac cycles that elapsed during the scan time. A typical phase-contrast image is presented in Figure 2, with regions of interest (ie, vascular cross sections) labeled. Subsequently, rsMRI BOLD was performed. This was acquired using a gradient echo, echo planar imaging (GE-EPI) sequence (64x64 matrix, 28 axial slices (5mm thick, no skip), 24cm FOV, TE/TR/flip angle = 35ms/2000ms/90o, 180 temporal points, total scan time=6 minutes). During the rsMRI BOLD scan, subjects were asked to keep their eyes open, stay awake, and not think of anything in particular.

Following the 3D anatomical, rsMRI BOLD, ASL and phase contrast MRI the subject was removed from the MRI and condition 2 and 3 were applied separately in a randomized order. Each condition was immediately followed by realignment of the head, return to the MRI bore, scan of a 3-plane localizer and then ASL, phase contrast, rsMRI BOLD and 3D anatomical scans. The latter three were always performed with the 3D anatomical last seeing as microvascular changes (i.e. blood flow) were more likely to be detected early after the manipulation while structural changes (i.e. 3D scan) wouldn't be expected to occur.

Data Analysis

Data quantification was performed by an experienced analyst who remained blinded to the test maneuvers. ASL blood flow and rsMRI BOLD data were analyzed using analysis of functional neuroimaging (AFNI). Functional rsMRI BOLD data preparation first involved slice timing correction and 3D rigid body motion correction. For each subject all ASL and rsMRI BOLD data were spatially registered to the initial (neutral condition) position. Anatomical, blood flow and functional data were transformed automatically to the Colin27 atlas, using the AFNI command @auto_tlrc, with functional data resampled to a 2mm isometric grid.42 Temporal band-pass filtering with cutoffs of $0.009Hz < f < 0.08Hz$ was performed in order to suppress unwanted physiological signals and some hardware noise.43 Functional connectivity within the default mode network (DMN) was assessed using the AFNI plugin InstaCorr, a seed-based approach, which uses the Pearson

method of correlation to compare time signals.44 The DMN is the most dominant temporally correlated resting network in the awake brain, defined as regions positively correlated in time with the posterior cingulate cortex (PCC) seed voxel. The PCC was defined automatically using the AFNI Talairach method 'Talairach to' and selecting a single voxel from each the left and right PCC for one analysis (ColinN27 coordinates: 10, 54, 14, and -10, 54, 14). These were both subsequently fused in the post-processing. A 5mm FWHM Gaussian spatial smoothing filter was applied for maximize likelihood of overlap with inter-subject group analysis. Also, temporal outliers determined with the AFNI function 3dToutcount were censored out. The ASL cerebral blood flow data was analyzed similar to rsMRI BOLD in that following spatial co-registration to the neutral condition and spatial blurring with a 5mm FWHM Gaussian convolution kernel, ASL data was warped to the N27 atlas. Group analysis was accomplished using a 1-way within subject 3D-ANOVA, with the one factor being neck position. Post hoc testing included contrasts between neutral, maximum voluntary rotation and CSM, and also a contrast between maximum voluntary rotation and CSM. Statistical significance was defined as anything lower than an alpha value of 0.05.

Mean and SDs were calculated for VA blood velocity, flow, peak velocity, and peak flow for each of the head conditions and VA side. Differences between task maneuvers and VA flow and velocity were evaluated using a repeated-measures analysis of variance with factors of head position and VA side, and a level of significance was set at .05, using R-project version 2.12.1 (R Development Core Team, 2010. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/).

Ratings for the perception of performance of the HVLA cervical manipulation procedure were expressed quantitatively as the distance along the VAS line of intensity for each descriptive term as a percent of the total length. In this form, the results were expected to represent the typical experience at midpoint (e.g. 5 of 10). A composite rating was developed as the average across all descriptive terms, assuming equal weighting, as an index of the overall experience. As secondary variables, descriptive statistics were calculated and Student-tests were performed between the raters using Bonferroni correction for significance of p less than or equal to 0.01. ICCs were also calculated examining for correspondence between raters.