STUDY TITLE: The effect of jugular vein compression on cerebral hemodynamics in healthy subjects.

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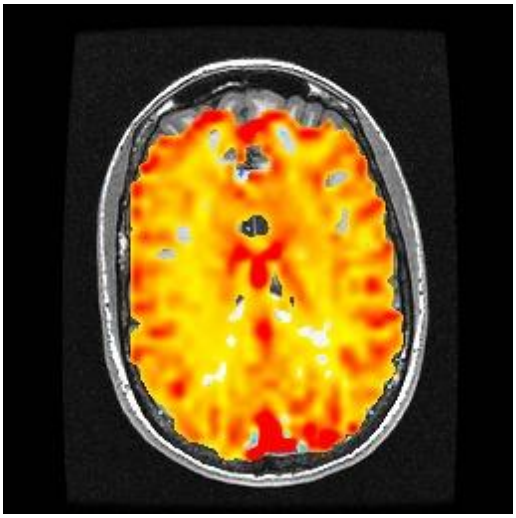

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List of short forms

ASL	arterial spin labeling
BOLD	blood oxygen level dependent
CBF	cerebral blood flow
CVR	cerebrovascular reactivity
dOHb	deoxyhemoglobin
GM	gray matter
IJV	internal jugular vein
MCA	middle cerebral artery
MRI	magnetic resonance imaging
O_2Hb	oxyhemoglobin
$PaCO_2$	partial pressure of carbon dioxide in arterial blood
$P_{ET}CO_2$	end-tidal partial pressure of carbon dioxide
ROI	region of interest
WM	white matter

Background

Introduction

As upright animals, the human brain is high above the level of the heart resulting in low intracranial venous pressures. Indeed, air can be sucked into the venous sinuses during neurosurgery. This low pressure also indicates that there is room for the vessels to expand,

or the presence of a capacitance for volume. It has been recently proposed that intracranial volume being less than skull volume allows the brain to be to be mobile inside the skull. In the presence of head trauma the brain may move relative to, and collide with, the skull ("rattle") or be internally deformed by pressure waves ("slosh"), resulting in traumatic brain injury. Both mechanisms can be mitigated, or eliminated by increasing the intra-cranial compartment volume, forcing all parts of the brain and skull to move as a unit. In addition, if the intracranial volume fills the skull, the brain will conduct blast energy waves through with minimal energy absorption by the brain, avoiding tissue displacement and shear. It has been proposed that a way to expand the intracranial volume is to fill it with venous blood. Since the brain blood flow is large, a small degree of resistance to drainage will quickly fill the cerebrovascular compliance. The major route of venous drainage in humans is via the internal jugular veins (IJV). In contrast, most quadrupeds have their head at near heart level and the vertebral venous plexi are the main venous outflow conduits (Lavoie *et al.*, 2008). Therefore, studies of the effects of jugular venous compression on brain blood flow and intracranial blood distribution must be performed in humans.

Aims of the study

Our aim was to study the effects of venous backpressure implemented via jugular vein compression, on

1. The distribution of blood (volume) in the brain
2. The resting blood flow in the brain
3. Re-distribution of blood flow in the brain at rest
4. The ability of the brain to increase its blood flow in response to a vasodilatory stimulus
5. The ability of the brain vasculature to sustain increased blood flow

Summary of findings

Compression of the internal jugular veins in supine healthy volunteers…

1. …increases in blood volume in the skull with venous blood distributed particularly to the large venous sinuses. A small increase in BOLD signal

seen in the middle cerebral artery and cerebellum indicate the possibility of a slightly increased cerebral blood flow or the accumulation of arterial blood diffusely as well.

2. …does not change the level or distribution of the resting blood flow in the brain.
3. …does not affect the response of the brain blood flow to hypercapnia. This implies that it would not affect the brain blood flow in response to an increase in metabolic demand.
4. …does not reduce the CBF if applied during an increase in demand, as occurs in this study during hypercapnia. This finding provides confidence that the brain would be able to maintain CBF if the collar is applied under other high flow demand states such as exercise.

Venous anatomy and physiology of the brain

Anatomy

There are 3 main drainage systems into the internal jugular vein (IJV) (Wilson *et al.*, 2013).

a) Cortical venous drainage via bridging veins that cover the brain surface and converge on the superior sagittal sinus (Figure 1.1). From there, blood flows to the transverse sinus, through the sigmoidal sinus and into the IJV. The right IJV is dominant in about 80% of people.

b) Deep anterior venous drainage is directed to the cavernous sinus which drains via superior and inferior petrosal sinuses into the jugular bulb.

c) Central thalamic areas drain into the great vein of Galen which also meets at the torcula (confluence of the sinuses), emptying into the transverse sinus and the IJV.

The IJV is the common final pathway for blood drainage. Backpressure on the IJV is transmitted directly back inside the skull. Additional drainage occurs from orbital veins and the vertebral venous plexi. The vertebral plexi are the main outflow for most horizontal posture animals.

Sinuses composed of dura mater lined with endothelium and are not vasoactive. The sympathetic innervation of the cavernous sinus and trigeminal innervation of other sinuses are sensory (distention) only.

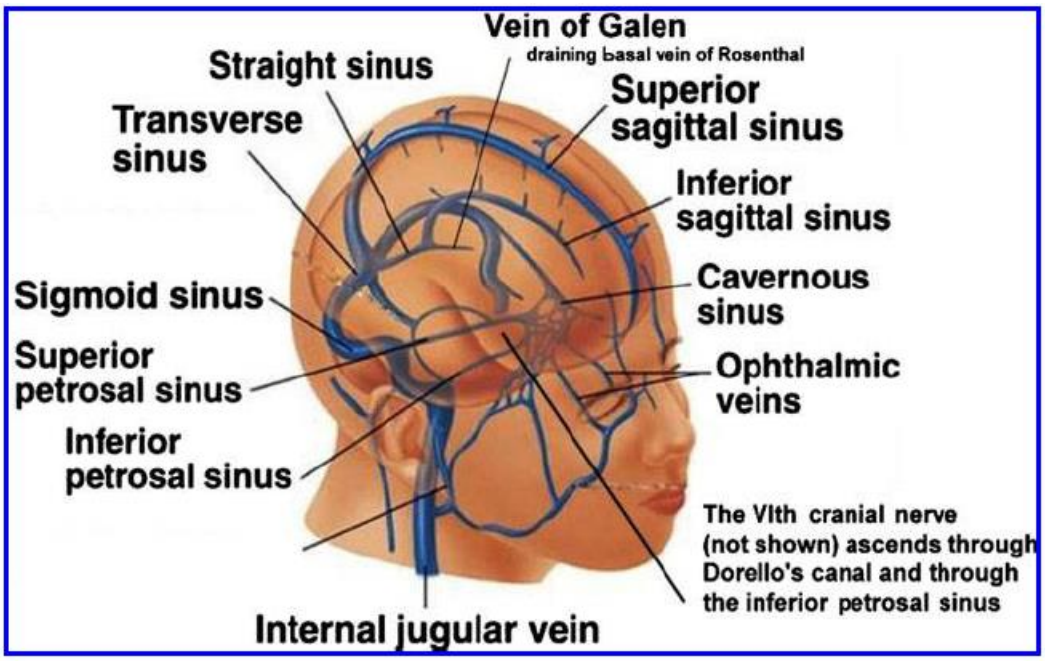


Figure 1.1: Simplified diagram of venous drainage (From (Wilson *et al*., 2013)).

Physiology

The brain is contained in a closed space which contains cerebrospinal fluid (CSF) and about 200 ml of blood. (KITANO *et al*., 1964) The balance of blood entering and leaving the skull is crucial in maintaining normal intracranial pressure (Figure 1.2). After acute reduction in venous outflow, the cerebral blood flow of about 12-15 m/s increases the intracerebral volume at a single exponential rate (KITANO *et al*., 1964) to the limit of its capacity. This increases intracranial venous pressure until the balance between inflow and outflow is restored. Figure 1.3 shows the effects on intracranial volume of compressing individual IJV's and compressing both. Moderate IJV compression at about 20 mmHg increase the intracranial pool by about 5-10%, or 10-20 ml (KITANO *et*

al., 1964). This is the same volume change as occurs with a 10 mmHg increase in PCO_2 (KITANO *et al.*, 1964) which occurs as part of daily living in most people.

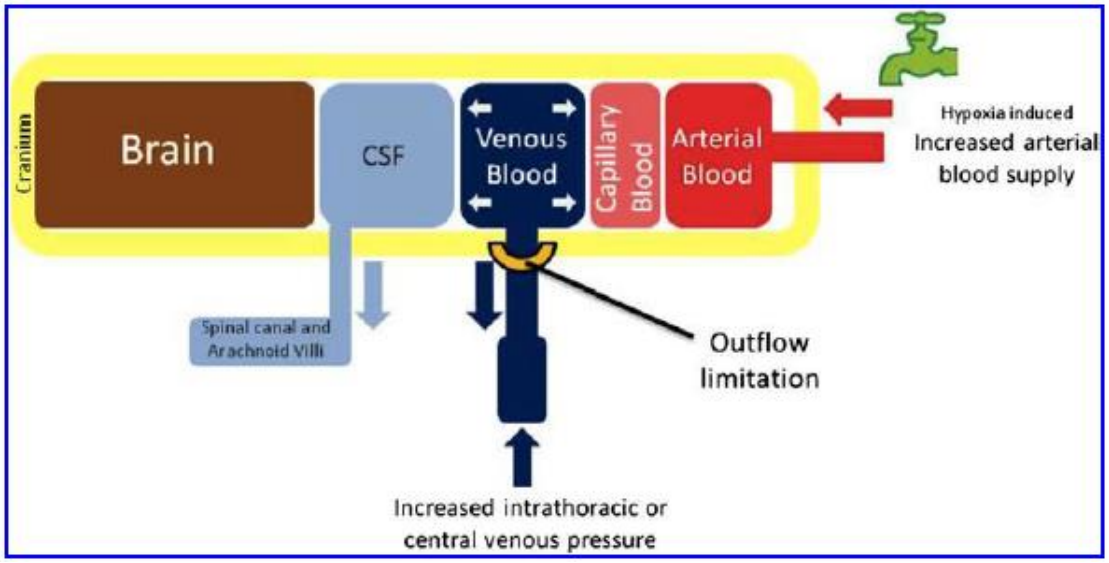


Figure 1.2: Diagram of venous hypertension mechanism. CSF = cerebrospinal fluid. (From (Wilson *et al.*, 2013))

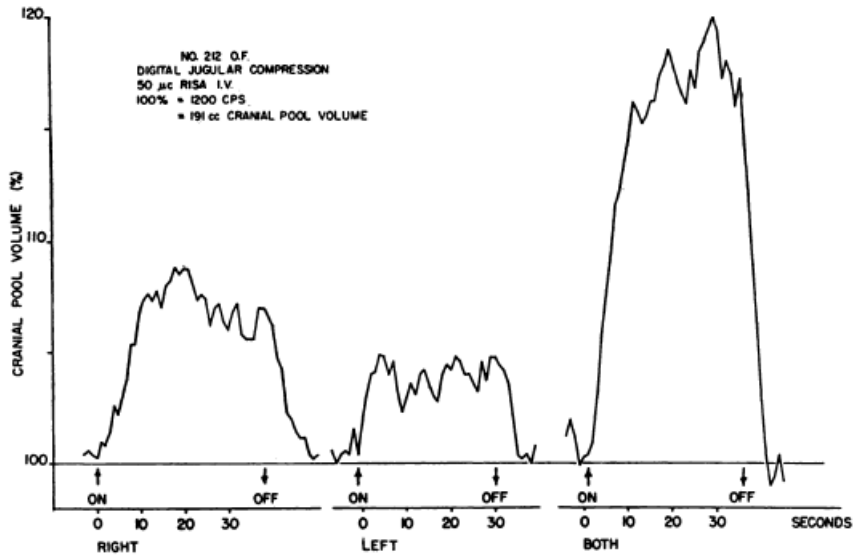


Fig. 3. The count rate increase of cranial pool volume following compression of the right, left, and both jugular veins. The increase of cranial blood volume following the right compression is more prominent than upon left neck compression.

Figure 1.3: Change in cranial blood pool volume measured as counts of radioactively tagged albumin in humans following internal jugular vein compression [from (KITANO *et al.*, 1964))

Hypercapnia as a vasoactive stimulus

CO_2 has many advantages as a vasodilatory stimulus. Increases in $PaCO_2$ raise CBF by about 3% (Fortune *et al*., 1992; Battisti-Charbonney *et al*., 2011) or more under hypoxic conditions (Poulin *et al*., 2002;Mardimae et al., 2012), and in contrast to intravenously injected drugs such acetazolamide, the administration of CO_2 is non-invasive and easily terminated. Cerebral blood flow (CBF) closely follows changes in the arterial partial pressure of CO_2 ($PaCO_2$) (Poulin *et al*., 1996; Mardimae et al., 2012). The physiological mechanisms of vasodilation with CO_2 have been reviewed (Brian, 1998). Moderate hypercapnia with end-tidal PCO_2 ($P_{ET}CO_2$) controlled at tensions between 40 and 50 mmHg is well tolerated by conscious humans (Steinback *et al*., 2009; Spano *et al*., 2012).

Prospective end-tidal targeting:

We employed an automated gas blender that administers gas to a sequential rebreathing circuit (RespirAct™, Thornhill Research Inc., Toronto, Canada) to implement a repeatable change in $PaCO_2$, while maintaining isoxia, with minimal subject cooperation. The core feature of the method is the capability of controlling the amount and content of gas entering the lung gas exchange region independent of the tidal volume and pattern of breathing (Slessarev *et al*., 2007). (Also see (Slessarev *et al*., 2007)for detailed discussion of this method.)

An important feature of this system is that there is obligatory rebreathing of previously exhaled gas which reduces the inhomogeneities of gas concentration in the lungs and thereby eliminates the gradient between the end-tidal PCO_2 and $PaCO_2$ (Ito *et al*., 2008; Fierstra *et al*., 2011) under steady conditions. Thus end-tidal PCO_2 values quoted herein are considered to be equivalent to $PaCO_2$.

Until now it has not been possible to accurately specify the $PaCO_2$ stimulus, and so it was not known how precisely the BOLD MRI signal follows the change in $PaCO_2$. We found

that after synchronizing the phases of the $P_{ET}CO_2$ and BOLD signals, the waveforms track precisely, voxel-by-voxel as shown in Fig. 1.4.

Blood Oxygen Level Dependent (BOLD) signal as a surrogate of cerebral blood flow

Despite its name, BOLD is not strictly oxygen dependent. Instead it is dependent on amount of dOHb present in the blood. Arterial blood is normally near full saturation with oxyhemoglobin (O_2Hb) and contains minimal concentrations of deoxyhemoglobin (dOHb). The arterial vessels branch repeatedly and end up as capillaries in tissues. Tissues are metabolically active using up O_2 and producing CO_2. Thus, a partial pressure gradient of O_2 from the blood to the tissues develops and for CO_2 from the tissues to the blood. O_2 dissociates from the O_2Hb and diffuses into the tissues leaving behind dOHb as the blood flows towards the veins. The CO_2 diffuses out of the tissues into the blood reducing the affinity of O_2Hb for the O_2, assisting O_2 availability for the tissues. If blood flow increases relative to the tissue O_2 demand, then the dOHb concentration and amount in the venous blood will decrease. The reverse occurs with a reduction in blood flow relative to tissue O_2 demand.

The BOLD method exploits the paramagnetic properties of dOHb. Paramagnetic substances are weakly magnetic and therefore they distort the homogeneity of the magnetic field in the scanner, reducing the regional signal strength. The signal is obtained via a T2*-weighted sequence using echo-planar imaging (EPI). As the BOLD signal is affected by total dOHb present in a voxel, it would be affected by the total blood volume in the voxel. For example, if blood volume increases even without a change in proportion of dOHb, the total volume of dOHb is increased and thus the BOLD signal is reduced. BOLD has a similar spatial resolution to PET and better temporal resolution.

We have previously shown that BOLD is a good surrogate for changes in CBF (Kassner *et al.*, 2010;Mandell *et al.*, 2011). This property is illustrated in a single subject in Figure 1.4.

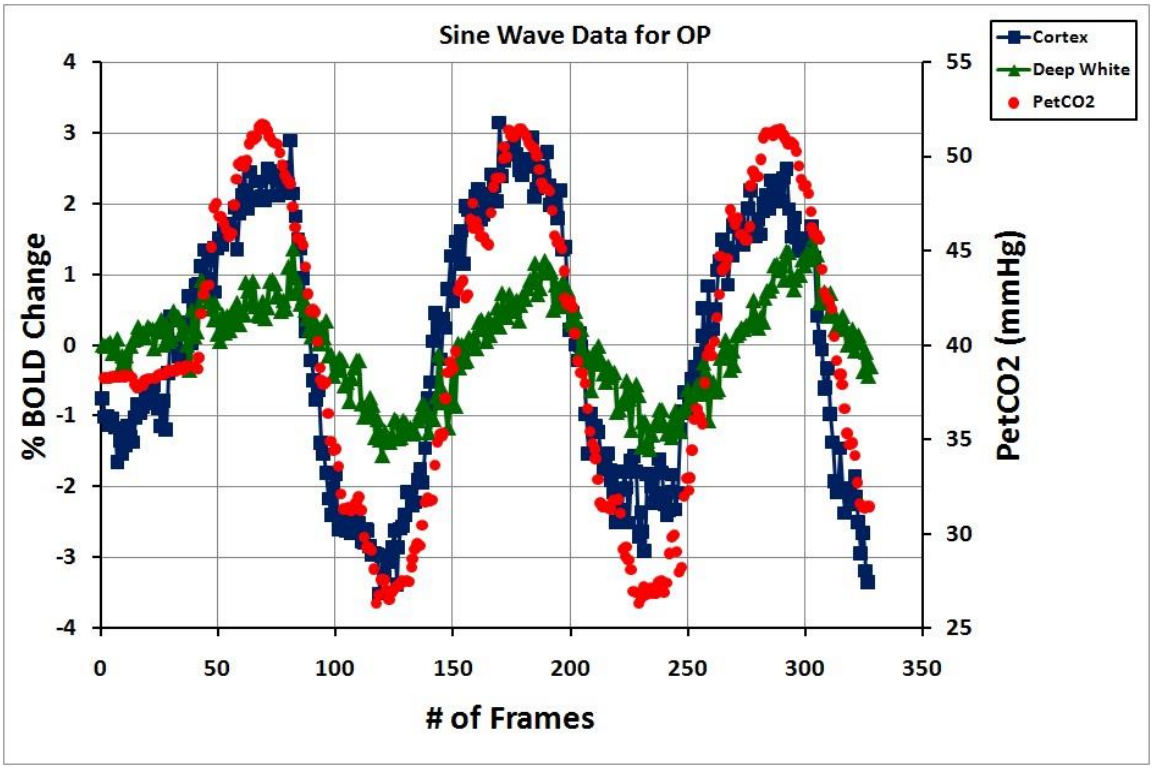


Figure 1.4: Illustration of the close tracking of BOLD signals in cortex (blue squares) and deep white matter (green triangles) with end-tidal PCO_2 (red dots, axis on right), over a wide range of PCO_2.

Methods

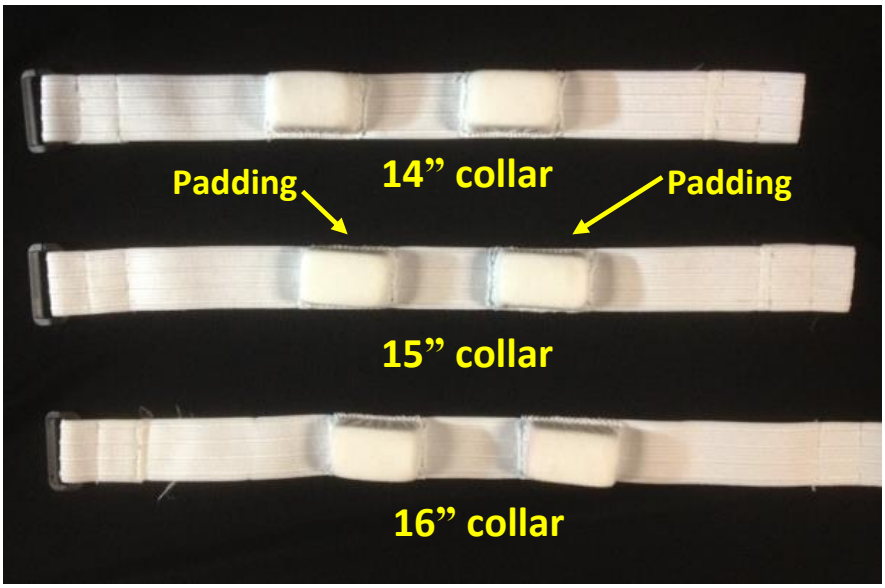
Subjects and Ethical Approval

This study conformed to the standards set by the latest revision of the Declaration of Helsinki. After approval from the Research Ethics Board of the University Health Network and written informed consent, 11 (7 m) healthy non-smoking subjects of mean (SD) age 30.6 (8.06) years were recruited for the study. All subjects were asked to refrain from caffeine, over-the-counter drugs or engage in heavy exercise for at least 12h before each test day.

Collar

The entire experimental protocol was repeated twice on each subject. During the first run, a collar was placed around the subject's neck prior to being placed in the MRI. The neck collars incorporated two bulges localized over the sites of the internal jugular veins bilaterally and had Velcro clasps to facilitate tightening (Figure 1.1). To account for different neck sizes, three collars of different sizes were available (14 inches, 15 inches and 16 inches). Collars were determined to be of correct size for each subject, such that compression pressure was felt around the neck but was comfortable. Those subjects that had smaller neck sizes (specifically female subjects) where the 14inch collar was still loose, gauze was added under the collar at the bulge regions to increase pressure on the internal jugular vein. A second sequence of tests was performed with the same protocol without compression from a collar.

a)



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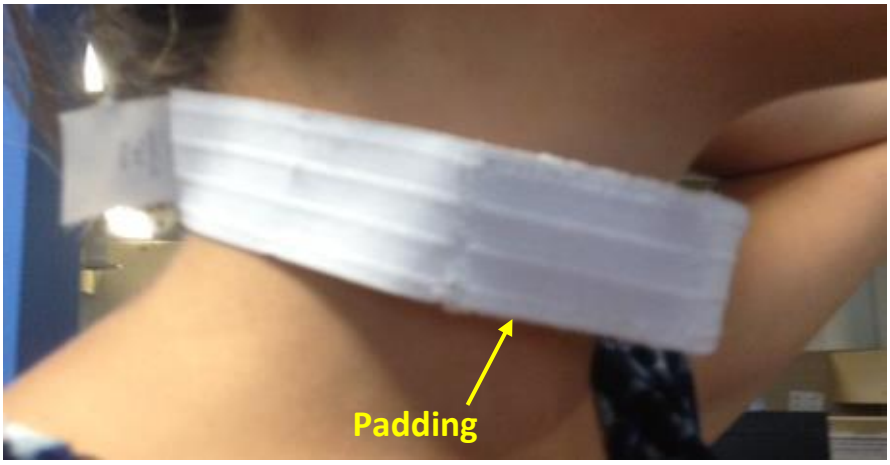

Figure 2.1: a) Neck collar at three different sizes supplied by Q30. Arrows indicate area where padding is located on the collar which, b) when worn applies localized compression to the internal jugular veins bilaterally.

Study 1: Effect of jugular compression on cerebral blood flow and cerebral blood volume

As the BOLD signal is proportional to blood flow and blood volume, the aim of this study was to compress the IJV intermittently and look for changes in the distribution of BOLD signal. In particular we focus on the change in signal in the great veins and sinuses in order to confirm that compressing IJV increased backpressure into the brain and distended the large venous sinuses with blood.

Methods

Protocol

This study was performed on 8 of the healthy subjects. The collar was initially placed loosely around the subject's neck. The subject was prompted to compress (test) and release (baseline) their own jugular veins alternately at 60 s intervals for 7 minutes. Subjects were instructed to apply considerable force on their neck but not to the point of discomfort.

Data acquisition and analysis

BOLD acquisition consisted of echo planar imaging (EPI) gradient echo (TR 2000, TE 30 ms, 3.75 x 3.75 x 5 mm voxels). The images were volume registered and slice-time corrected and co-registered to the axial 3-D T1-weighted images. The difference in signal is calculated as [mean BOLD signal during compression- mean BOLD signal at rest] so that increases in BOLD signal during compression are positive in the graphs and brain maps. The differences in signal were calculated for each voxel and color-coded to

generate slice by slice maps of changes in BOLD signal, with the color scale indicating magnitude and direction of signal difference.

Increases in BOLD signal indicate increases in blood flow (leading to an increase in O_2Hb, and/or a decrease in blood volume; reductions in signal, the opposite. Mean differences were compared for specific region of interests (ROIs): middle cerebral arteries, cerebellum, which were taken to indicate changes in blood flow in anterior and posterior circulations, and the superior sagittal sinus, sigmoidal sinus and straight sinus, to assess changes in intracranial venous volume or hemoglobin saturation.

Results

Figure 2.2 shows a typical BOLD signal response over time in specific ROIs, during the compression on/off task in a subject. In venous territories there was a progressive decrease in BOLD signal after the onset of compression. This observation was consistent with the accumulation of dOHb in the expanding great veins. After release of IJV compression there was a rapid recovery in BOLD signal, to the point of 'overshoot', best seen in the superior sagittal sinus, as the distended great veins drain rapidly and lose dOHb. We interpret the increase in BOLD signal in the arterial territories as a transient accumulation of oxygenated blood by distension or the recruitment (Norris, 2006) of arterioles and capillaries on the arterial side of the vascular tree. This phenomenon would occur largely in voxels without large venous capacitance, increasing the ratio of O_2Hb to dOHb in the voxels.

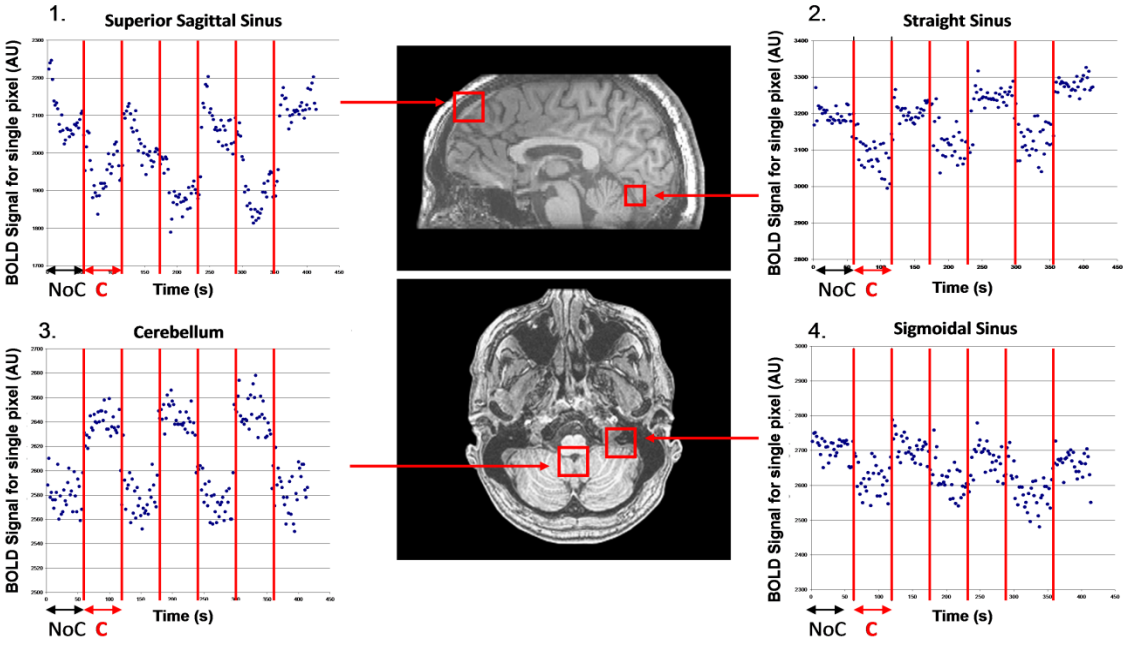

Figure 2.2: Compression on/off task related BOLD-signal changes over time for four ROIs: 1) Superior Sagital Sinus, 2) Straight Sinus, 3) Cerebellum and 4) Sigmoidal Sinus. NoC denotes the time elapse during no compression and C indicates time during compression.

Mean difference maps between the first compression and first baseline (no compression) BOLD response were calculated voxel-by-voxel for each subject as shown in Figure 2.3. These maps denote the areas of the brain where BOLD signal increased or decreased during compression from baseline.

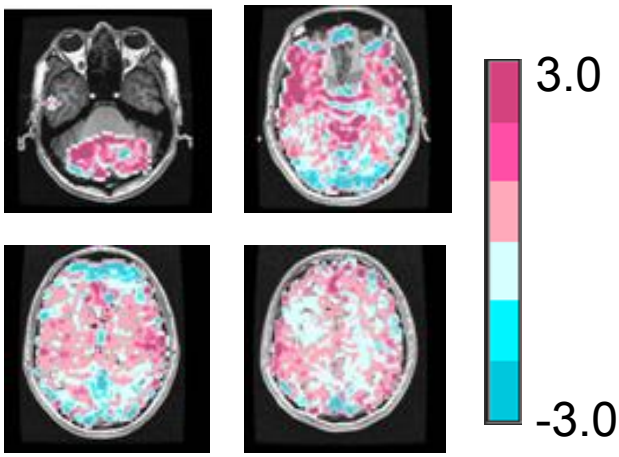

Figure 2.3: Example for a single subject of compression on/off-task difference maps. The colour scale denotes positive differences (representing an increase in BOLD signal response during compression from baseline) ranging in different shades of pink from 0 to 3. Negative differences (indicative of a decrease in BOLD signal response during compression from baseline) range in different shades of turquoise from 0 to -3.

Mean(SD) differences were compared for each ROI over all 8 subjects and plotted in Figure 2.4. The middle cerebral artery and cerebellum had an overall mean positive BOLD difference indicating an increase in blood flow or accumulation of oxygenated blood during compression. The superior sagittal sinus had a mean difference close to zero, with some individuals having a positive response and others having a negative response. Both straight sinuses and sigmoidal sinuses displayed a mean negative difference in BOLD signal response between compression and baseline, indicating increases in venous volume during compression.

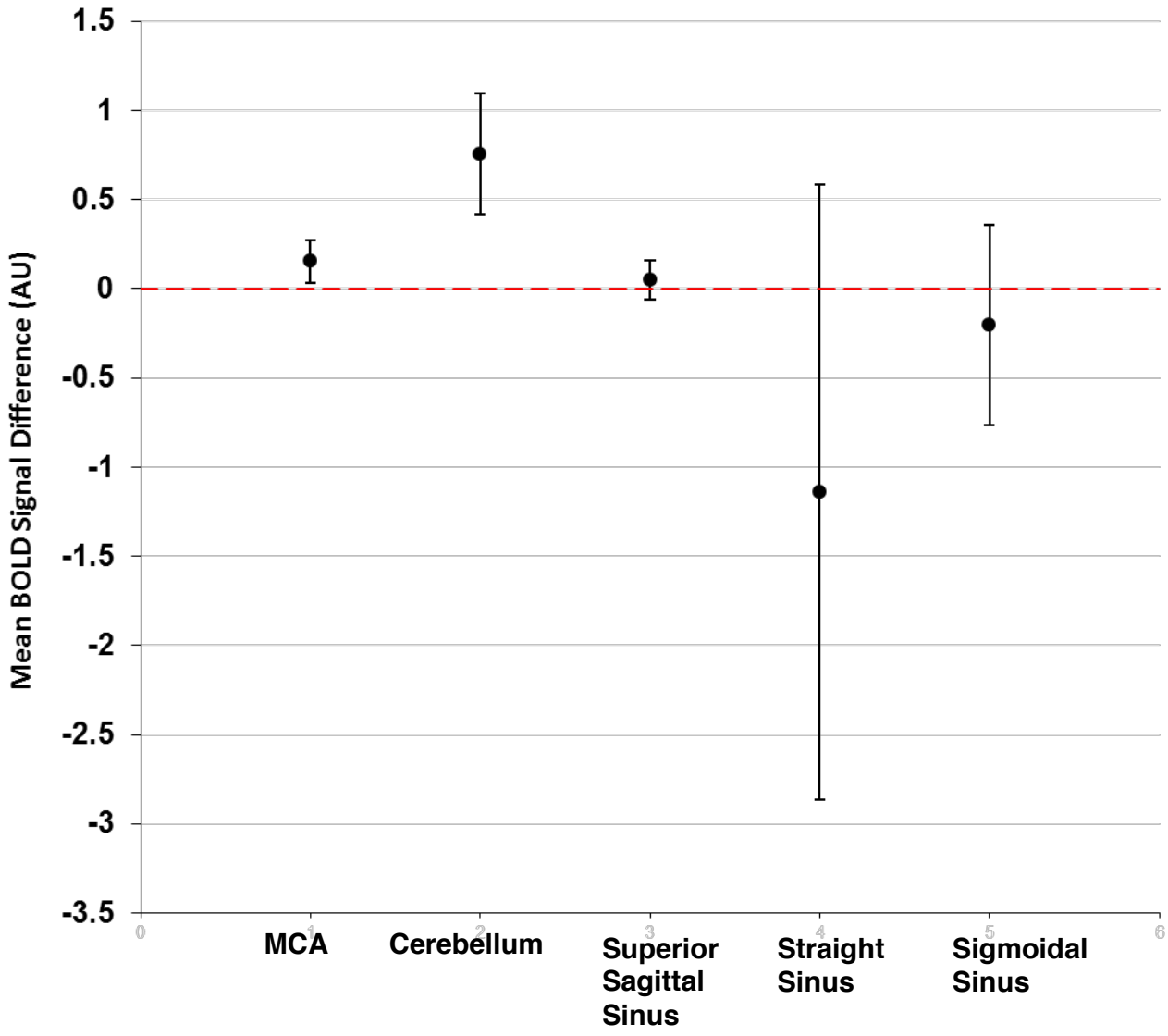

Figure 2.4: Mean +/-SD compression on/off task difference for each ROI across all 8 subjects.

Summary of findings and their significance

a) Compression of IJV increases the blood volume in venous sinuses, observable in particular in the straight and sigmoid sinuses. This confirms the effectiveness of the IJV compression.

b) Compression of IJV results in a small, but widespread reduction in BOLD signal over the brain. This observation is consistent with either a decrease in cerebral blood flow or increase in volume of deoxygenated blood. A reflexive

increase in cerebral blood flow in response to venous obstruction has previously been reported.(MOYER *et al*., 1954)

Our data also validates the use of BOLD to measure changes in intracranial blood volume as these findings are consistent with those from previous studies using other methods of measuring blood volume using methods such as radioactive labelling of albumin (KITANO *et al*., 1964).

Study 2: Experimental Protocol for assessing the role of venous backpressure on cerebral vascular reactivity.

It has previously been shown in humans that increasing venous backpressure as high as 150 mmHg does not reduce CBF (MOYER *et al*., 1954). However, it is not known if venous backpressure reduces cerebral vascular reactivity—i.e., the ability to increase CBF in response to an increase in demand. In this study, we used the BOLD signal as a surrogate for CBF and hypercapnia to simulate an increase CBF demand.

The aim of this study was to determine whether venous backpressure diminished the CBF response to a standardized hypercapnic stimulus.

Methods

Subjects were fitted with a face mask, and connected to a sequential gas delivery breathing circuit(Slessarev *et al*., 2007) (Prisman *et al*., 2007). The patterns of $P_{ET}CO_2$ and $P_{ET}O_2$ were programmed into the automated gas blender (RespirAct™, Thornhill Research Inc., Toronto, Canada), (Slessarev *et al*., 2007). Tidal gas was sampled and analyzed for $P_{ET}CO_2$ and $P_{ET}O_2$ and recorded at 20 Hz (RespirAct™).

Protocol

The $P_{ET}CO_2$ and $P_{ET}O_2$ in all subjects were adjusted to baseline values of 40 and 100mmHg, respectively. Then two iso-oxic square wave increases in $P_{ET}CO_2$ to 50 mmHg was implimented. The first increase was 45s in duration, followed by a return to baseline for 90s and then a second increase for 130s followed by a return to baseline. This is the standard CVR protocol we have used in, now, over 1000 subjects and patients (Spano *et al*., 2012).

MRI imaging consisted of 3D T1-weighted inversion-recovery prepared fast spoiled gradient-echo acquisition (voxel size 0.86x0.85x1.0mm) on a 3.0-Tesla HDx scanner (Signa; GE Healthcare, Milwaukee, Wisconsin). Cerebrovascular reactivity was evaluated using a BOLD acquisition as a surrogate for CBF. We used echo planar imaging (EPI) gradient echo (TR 2000, TE 30 ms, 3.75 x 3.75 x 5 mm voxels).

This protocol was repeated with and without the collar on each subject. Hypercapnic stimuli were kept within 1 mmHg for each run in each subject.

Data processing and analysis

The acquired MRI and $P_{ET}CO_2$ data were analyzed using AFNI software (National Institutes of Health, Bethesda, Maryland; http://afni.nimh.nih.gov/afni; (Cox, 1996). $P_{ET}CO_2$ data were time-shifted to the point of maximum correlation with the whole brain average BOLD signal. A linear, least-squares fit of the BOLD signal data series to the $P_{ET}CO_2$ data series was then performed voxel-by-voxel. The slope of the relation between the BOLD signal and the $P_{ET}CO_2$ was color-coded to a spectrum of colors corresponding to the direction (positive or negative) and the magnitude of the correlation. BOLD images were then volume registered and slice-time corrected and co-registered to the axial 3-D T1-weighted image that was acquired at the same time (Saad *et al*., 2009). This method has been described in greater detail elsewhere (Fierstra *et al*., 2010). CVR information was further analyzed by comparing the direction and magnitude of the change in BOLD signal of each voxel to that of the corresponding voxel in a previously studied normal cohort of 48 healthy individuals. The CVR maps from each individual in the healthy cohort were co-registered (Ashburner & Friston, 1999) using a MNI152 SPM

distributed template supplied by the Montreal Neurological Institute. The mean and SD of each corresponding voxel was calculated to form a "normal atlas". We next compared the CVR map of each subject to that of the atlas and re-scored the CVR of each voxel in terms of a z score for the corresponding atlas voxel. In other words, the value expressed in standard deviations (SD) of the CVR scores of the corresponding voxel in the atlas, ($z = \frac{r - \bar{r}}{\sigma_r}$)). Finally, attributing a color to each z-score; AFNI software (Cox, 1996)was used to indicate a magnitude and direction of the differences in z-scores compared to the atlas population.

Positive scores (where the CVR is greater than the mean of the atlas) were coloured with 15 different shades of green ranging between the values of 0 to 3.0. Negative scores (where CVR is less than the mean) were coloured purple with 15 shades of purple between 0 and -3. The calculated z-scores were superimposed on the anatomical scans to allow a perspective of the individuals CVRs (with and without the collar) compared to the atlas CVR.

To compare the CVR maps resulting from different ROIs (grey matter and white matter), and between subject conditions (collar on vs. collar off) a 2-way repeated measure analysis of variance (rmANOVA) was performed. If differences were found, post hoc Holm-Sidak all-pair-wise comparisons were used to determine which groups differed significantly from one another.

Results

All subjects completed both CVR runs (with and without collar), however one subject's data set was excluded due to motion artifacts. Figure 2.5 shows CVR data in a single subject as an example of CVR images with and without the collar. Statistical analysis in the form of z-maps shows that the CVR distribution in this subject is not different with and without the presence of jugular compression with the collar.

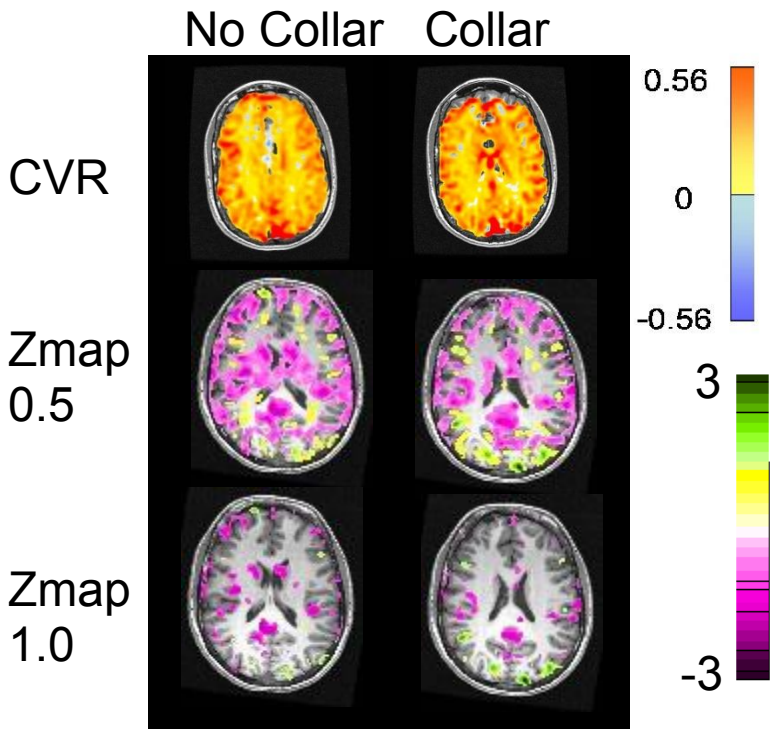

Figure 2.5: CVR and z-maps for an axial slice in one subject. The slice figures show the spatial distribution of CVR and z values with and without the collar, colored according to the scale shown. The CVR scale is in % BOLD change / mmHg $P_{ET}CO_2$ change. The z-maps are displayed at two different thresholds. Positive scores (where the CVR is greater than the mean of the atlas) are coloured green, ranging between the values of 0 to 3.0. Negative scores (where CVR is less than the mean) are coloured purple, ranging between the values of 0 and -3. For the purposes of interpretation, z-maps thresholded at 0.5 only display z-score values greater then 0.5, and z-maps thresholded at 1.0 only display z-scores greater then 1.0, and so on.

Comparisons of CVR in gray matter (GM) and white matter (WM) with, and without the collar are presented in Table 1. The results of this comparison showed that the only significant differences in CVR are those between GM and WM, which is normal, and not related to jugular compression. However, there were no significant differences found in CVR, in both GM and WM groups, when comparing collar on vs. collar off.

Table 1: 2-way rmANOVA of CVR population characteristics

Factor Comparison	p-value
Collar On vs. Off	0.23
GM vs. WM	0.002*
GM vs. WM Collar On	0.002*
GM vs. WM Collar Off	0.002*
Collar On vs. Off GM Only	0.277
Collar On vs. Off WM Only	0.196

*Less then significance level of 0.05. GM gray matter; WM white matter.

Mean gray matter CVRs for each subject (both collar on and collar off) were calculated and plotted in Figure 2.6. A paired t-test was performed for each voxel comparing no collar and collar CVR values; no significant differences were detected. The same analysis was performed for WM (Figure 2.7). CVRs for the majority of the subjects did not differ between collar and no collar, however 3 subjects decreased their CVR with the collar, and 2 subjects increased their CVR with the collar. In such a situation one can say that there is no systematic effect of the collar but one cannot rule out the possibility of individual variation in the response.

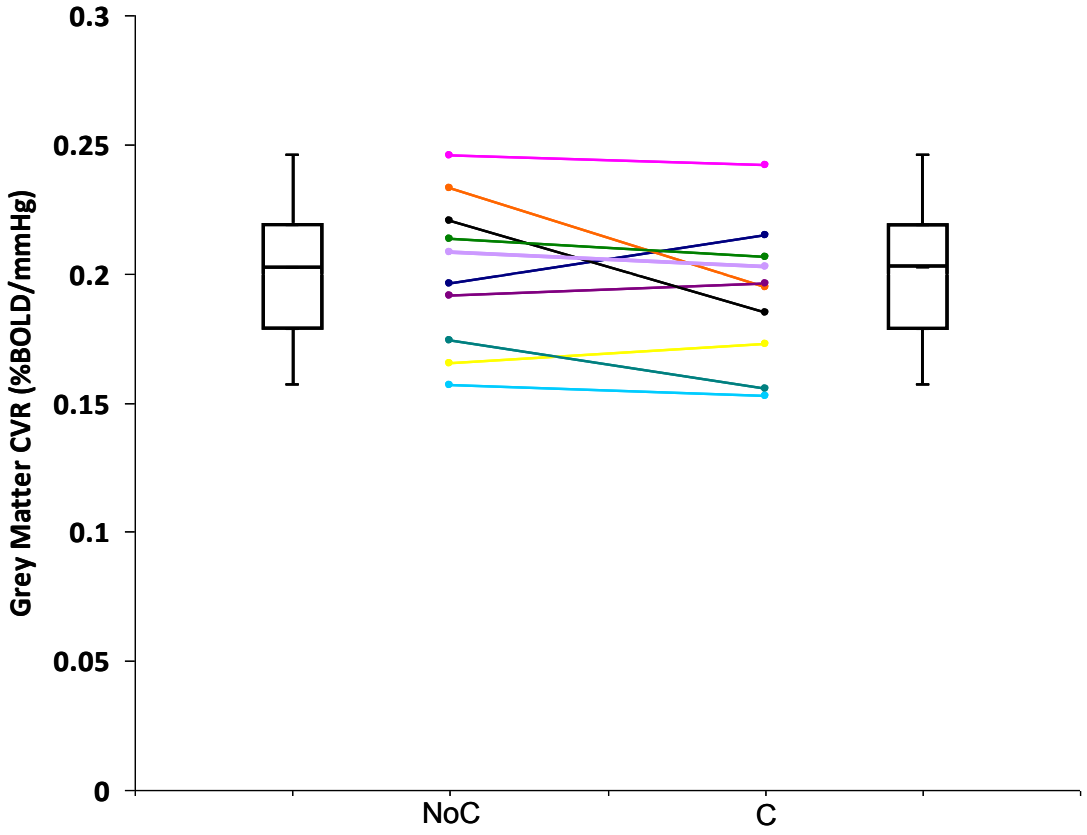

Figure 2.6: No collar (NoC) and collar (C) comparison of mean gray matter CVR values for each of the 10 subjects. Box plots on right and left of graph depicts mean, 25 percentile, 75 percentile and range determined from the maximum and minimum values in dataset. Colors represent individual subjects.

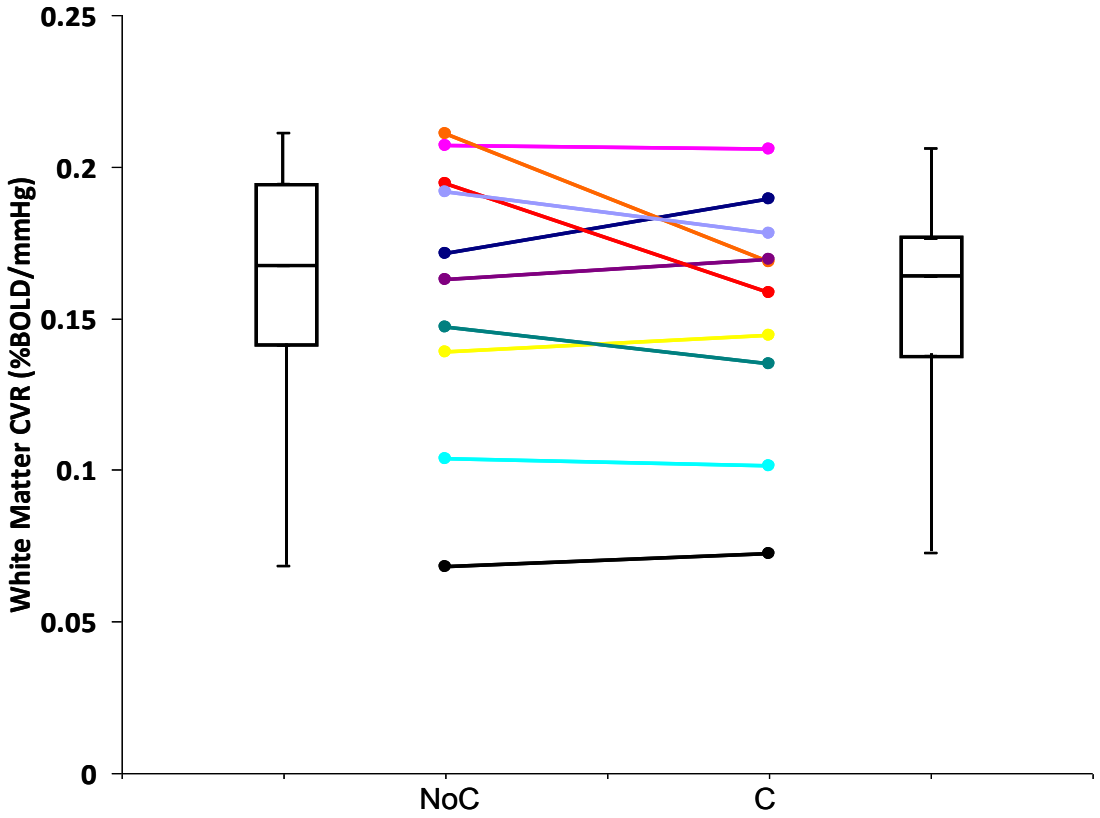

Figure 2.7: No collar (NoC) and collar (C) comparison of mean white matter CVR values for each of the 10 subjects. Box plots on right and left of graph depicts mean, 25 percentile, 75 percentile and range determined from the maximum and minimum values in dataset. Colors represent individual subjects as in Figure 2.6.

Summary of findings and their significance

Regional and general cerebral blood flow increases are required in response to neural activation and exercise. This study showed that the presence of the collar made no discernible difference in the magnitude or distribution of flow increase in response to a hypercapnic stimulus that simulated an increased demand.

Study 3: The effect of jugular compression on CBF at rest and during vasodilation

In Study 2 we found that the presence of the collar did not affect the ability of the brain vasculature to increase blood flow, as measured by the BOLD signal, in response to an increased blood flow demand, as simulated by hypercapnia, a vasodilatory stimulus. In this study we tested the effect of the collar on resting blood flow, and blood flow under hypercapnic stimulation. Arterial Spin Labeling (ASL) was used as a direct measure of CBF. ASL works on a different principle than BOLD, and is thus considered an independent measure. Furthermore, ASL, in contrast to BOLD, is a steady state measure which requires measurement at 2 steady states, normocapnia, and hypercapnia.

In brief, the ASL method entails labeling protons and using them as a contrast agent. The spins of protons synchronously and uniformly oriented by a radio-frequency pulse are known to deteriorate at a fixed rate in a magnetic field. Such protons are 'labeled' in the neck and their spins followed into the cerebral vessels over time. As the rate of decay of the spins are known, their residual spin states reveal the time since labeling and thereby the time required for the blood which contains them to reach a brain location.

Methods

Eight of the healthy subjects underwent direct measure of cerebral blood flow using ASL imaging at normocapnia (40mmHg) and hypercapnia (50mmHg). The scans were repeated with and without the application of the collar.

ASL was acquired using a 3D- GE arterial spin-labelling technique with phase–encoded fast spin echoes with spiral readout (TR: 4718ms; TE: 10.3ms; slice thickness: 4 mm). The images were then volume registered and slice-time corrected and coregistered with the axial 3-D T1-weighted images.

To compare CBF values resulting from different stimulus ranges (normocapnia and hypercapnia), and between subject conditions (collar on vs. collar off) a 2-way rmANOVA was performed in both grey matter and white matter ROIs. If differences were found, post hoc Holm-Sidak all-pair-wise comparisons were used to determine which groups differed significantly from one another.

Results

Figure 2.8 shows the images obtained from ASL measurement in a single subject, with and without the collar at both normocapnia and hypercapnia. Visually there is no difference between the ASL images between no collar and collar in both the normocapnia and hypercapnia stages. In addition, the normal increase in blood flow between normocapnia and hypercapnia is seen regardless of the presence or absence of the collar.

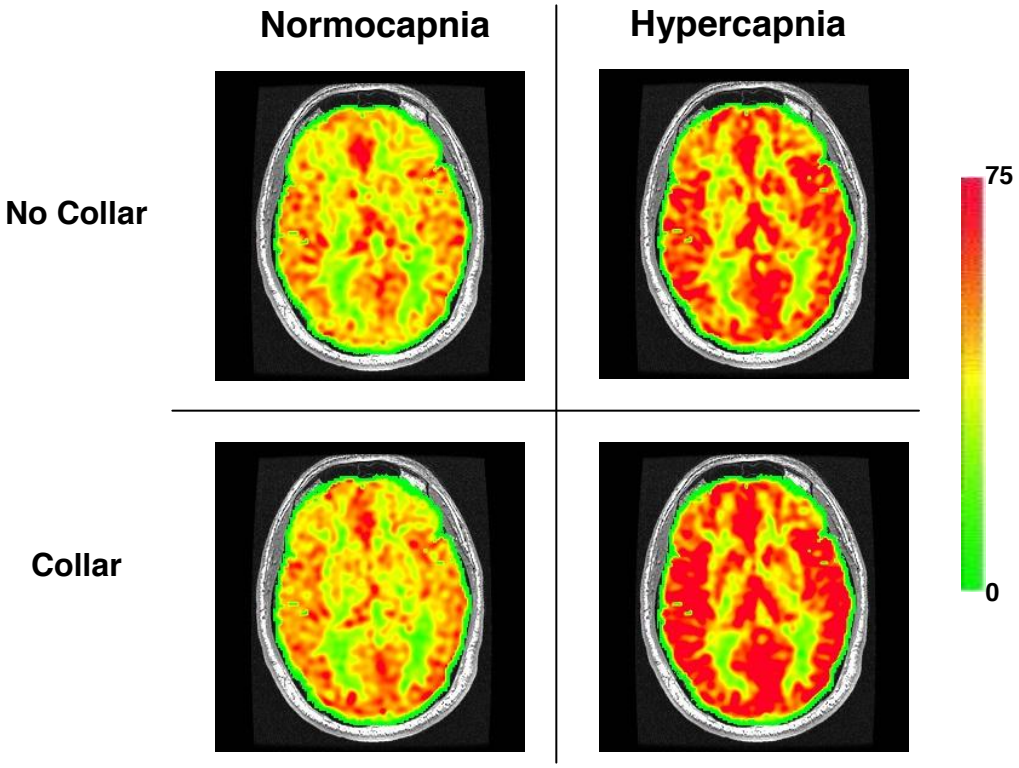

Figure 2.8: Mid-brain single slice ASL images for a single subject at both normocapnia and hypercapnia, with and without the collar. Color scale range from 0 to 75 in units of mL/100g/min.

The ASL results are listed in Table 2. The results of this comparison showed the known significant differences between normocapnia and hypercapnia but these changes were not affected by the collar on vs. collar off conditions.

Table 2: 2-way rmANOVA of CBF population characteristics

Factor Comparison	P-Value Gray Matter	P-Value White Matter
HC vs. NC	<0.001*	<0.001*
Collar On vs. Off	0.616	0.925
Collar On vs. Off during NC	0.494	0.763
Collar On vs. Off during HC	0.912	0.655
HC vs. NC Collar Off	<0.001*	<0.001*
HC Vs. NC Collar On	<0.001*	<0.001*

*Less than significance level of 0.05

Mean CBF values for gray matter with and without the collar were calculated for each of the 8 subjects. These values as well as population characteristics such as mean, maximum, minimum, 25 percentile and 75 percentile are displayed in figure 2.9. The same analysis was performed for white matter, results are found in figure 2.10. From these graphs we discerned that the mean CBF difference between normocapnia and hypercapnia was maintained, but there was no significant difference between CBF when comparing collar-on, and no-collar conditions.

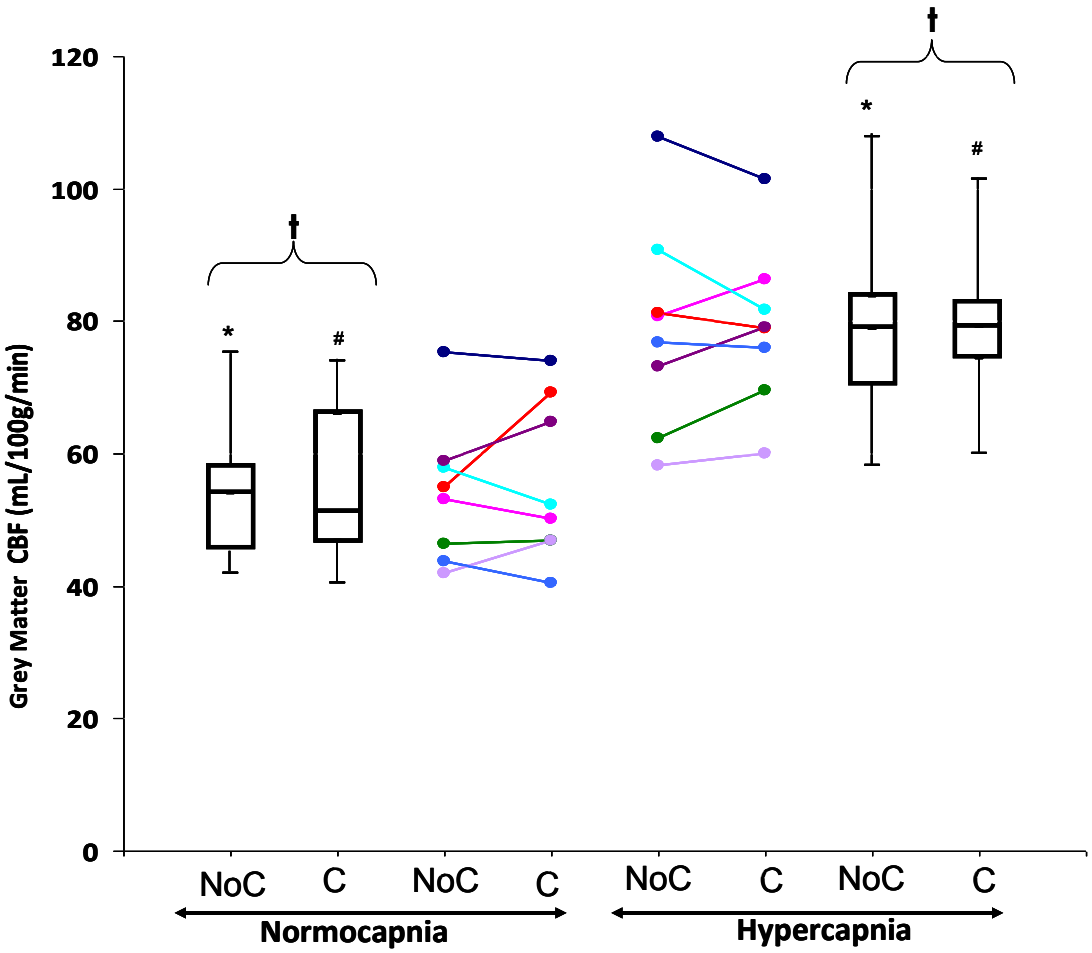

Figure 2.9: No collar (NoC) and collar (C) comparison of mean gray matter CBF values for each of the 8 subjects at both normocapnia and hypercapnia. *, # and † depicts groups comparisons which were found to be significantly different ($p<0.05$). Colors represent individual subjects.

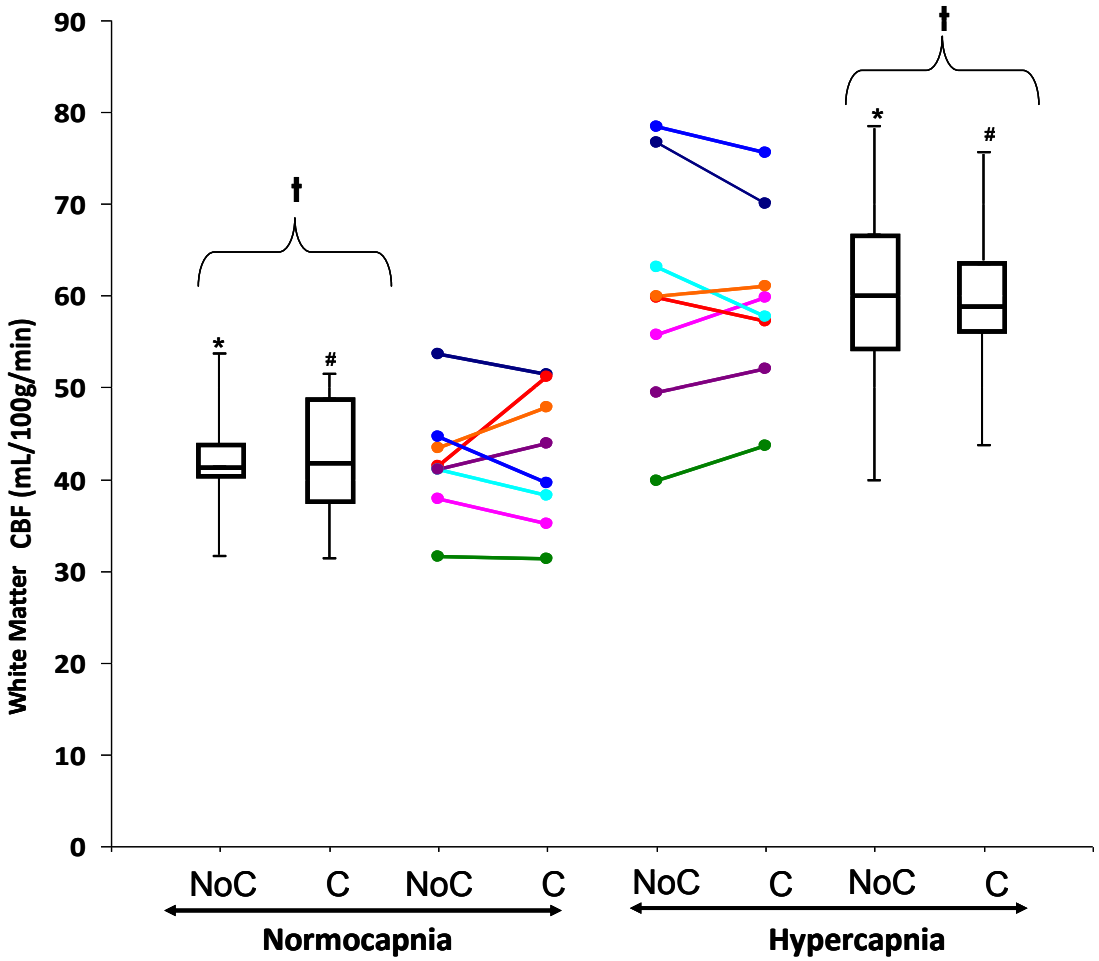

Figure 2.10: No collar (NoC) and collar (C) comparison of mean white matter CBF values for each of the 8 subjects at both normocapnia and hypercapnia. *, # and † depicts groups comparisons which were found to be significantly different ($p<0.05$). Colors represent individual subjects as in Figure 9.

Conclusions and significance

We concluded that CBF was unchanged in the presence of the collar at rest. CBF was measured with 2 independent measures. When CBF is increased, the presence of the collar does not change the CBF. This finding is consistent with the finding that the presence of the collar did not affect the CVR as demonstrated in Study 2. Together with Study 2, these findings support the notion that the presence of the collar does not reduce CBF, or affect the ability of the brain to recruit, or maintain increased blood flow.

Study 4: The effect of the collar on blood flow in individual vessels measured with NOVA

Study 4 is complementary to Study 1 where BOLD was used to determine the change and distribution of cerebral blood volume. In Study 1 subjects were at rest during the application of the collar, and we found localized changes in BOLD signal over the great veins indicating changes in blood volume, since there was little in the way of changes over the whole brain to indicate that the changes in BOLD signals were due to changes in blood flow.

The aims of this study are to (a) use NOVA as an independent confirmation of lack of changes in CBF with the application of the collar, and (b) to confirm that application of pressure on the IJV reduces its blood flow.

Methods

NOVA (NOVA-qMRA) (Zhao *et al*., 2007)is a non-invasive optimal vessel analysis quantitative magnetic resonance imaging software that works in tandem with MRI to construct a 3D model of the vasculature and quantify volumetric blood flow (ml/min) in the individual major vessels in the brain (Zhao *et al*., 2000). It does this by using time-of-flight (TOF MRA) and phase contrast magnetic resonance imaging to visualize the anatomy and quantify blood flow. NOVA technology is already being used in the clinical setting in the United States [www.vassolinc.com. Accessed September 10th, 2013], and is a powerful proprietary tool allowing for accurate determination of blood flow rates in the MRI environment.

Volumetric flow was measured in the IJV for 11 subjects. In 4 of these subjects, NOVA measurements were made on the major inflow arteries (ICAs-internal carotid arteries,

VAs- vertebral arteries) as well, to compare blood inflow and outflow, with and without the collar.

For the CVR data, a two-way rmANOVA with factors ROI (grey matter vs. white matter) and subject condition (collar on vs. collar off) was used to compare the effect of collar on CVR. If differences were found, post hoc Holm-Sidak all-pair-wise comparisons were used to determine which groups differed significantly from one another. A one-way ANOVA was performed on the NOVA data collected for the internal jugular veins with a factor of subject condition (collar on vs. collar on) to compare the effect the collar has on jugular flow.

Results

There was no significant difference in the outflow between collar on and collar off conditions (p value=0.407) at a significance level of 0.05. Values of outflow with collar off and on conditions for each subject are plotted in figure 2.11. A large variability was observed within each subject between collar on and off conditions due to errors in the measurement technique, which is highly dependent on operator and measurement placement. In addition, IJV flows are difficult for NOVA to measure. This difficulty is due to IJV shape variability and complex vein vasculatures seen in MR venograms at the neck level.

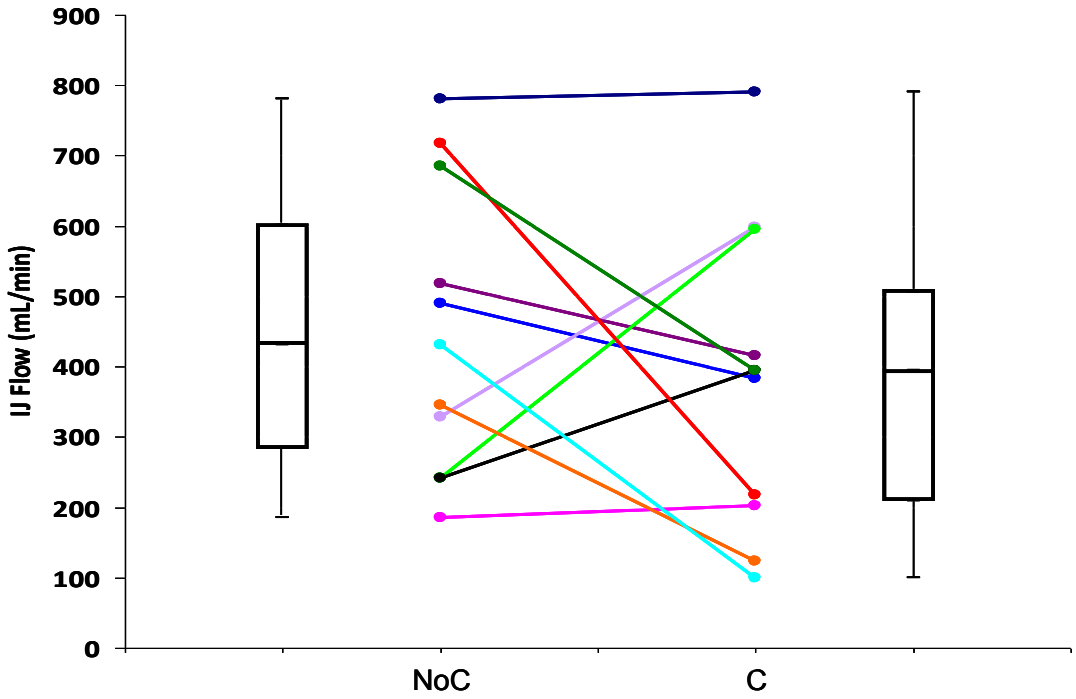

Figure 2.11: No collar (NoC) and collar (C) comparison of internal jugular (IJ) volumetric blood flow for each of the 11 subjects. Box plots on right and left of graph depicts mean, 25 percentile, 75 percentile and range determined from the maximum and minimum values in datasets. Colors represent individual subjects.

Volumetric flow in and out of the skull was measured in 4 subjects (Table 3). From these measurements a total percent flow in/out was calculated. In subjects 1 and 2 we observed that the percent total flow did not change between collar on vs. collar off conditions. Subject 3 decreased the percent total flow with the collar compared to without the collar; however, subject 4 increases the percent flow with vs. without the collar. Once again the results were highly variable due to an increased technique measurement error and complex vein vasculature in the neck, which makes obtaining IJ measurements difficult. Nevertheless there was no indication of a large flow effect due to the presence of the collar.

Table 3: Volumetric flow measurements of CBF in and out, with and without the collar present.

Subject	Collar	RVA (mL/min)	LVA (mL/min)	RICA (mL/min)	LICA (mL/min)	RIJ (mL/min)	LIJ (mL/min)	% Total Flow In/Out
1	Off	193	100	340	300	672	108	**83.60128617**
1	On	206	84	338	323	761	30	**83.17560463**
2	Off	128	125	288	185	110	92	**27.82369146**
2	On	119	123	248	179	108	78	**27.80269058**
3	Off	97	123	324	239	346	144	**62.5798212**
3	On	111	139	342	262	239	144	**44.84777518**
4	Off	96	218	311	362	120	209	**33.33333333**
4	On	94	205	328	358	189	409	**60.7106599**

Discussion

Limitations

All of these studies were done on healthy subjects, and thus represent normal physiology. Also, subjects were supine to enable MRI studies. In the supine attitude, most venous drainage is via the internal jugular veins. Therefore, the supine posture would be most sensitive in detecting physiologic derangements of CBF. In the upright posture, the jugular veins are reduced in size and much more drainage takes place via the vertebral venous plexus (Gisolf *et al.*, 2004), thereby reducing the influence of internal jugular compression on CBF. The posterior fossa dural venous sinuses connect with vertebral venous system via lateral, posterior, and anterior condylar veins, and mastoid and occipital emisary veins providing abundant alternate routes of venous drainage.

Sample Literature Review

1. Lavoie (Lavoie *et al*., 2008): Venous outflow was obstructed and intracranial pressure increased in 3 swine and 2 baboons. At 6 months, all animals were alive with no neurological deficits.
2. Rafferty (Rafferty *et al*., 2010): 40 healthy males had CVR performed with "breath hold index" method with and without a pneumatic constriction on the neck. Constriction was found to reduce CVR slightly, but still within normal range.
3. Greenberg (Greenberg *et al*., 1978): Cerebral blood volume is about 5 ml/100g and increases about 0.05 ml / 100 g / mmHg change in PCO_2, or about 10% when PCO_2 is changed 10 mmHg.
4. Gonzalez-Fajardo (Gonzalez-Fajardo *et al*., 1994): Complete clamping of superior vena cava in 6 dogs resulting in marked increase in central venous and intra-cranial pressure. Three animals had hemorrhagic infarction.
5. Masuda (Masuda *et al*., 1989): Clamping of superior vena cava for 6 hours in 6 cynomolgus monkeys with circulation similar to that in humans. Cerebral perfusion pressure remained in normal range. Brain histology showed slight edema in one monkey, the rest were normal.
6. Urayama (Urayama *et al*., 1997): Clamped superior vena cava and all other main outflow from brain of 6 dogs for 2 hours. Regional cerebral blood flow fell markedly during clamp. There were no changes in EEG, neurological defect, or brain histology with a 3 week follow-up.
7. Moyer (MOYER *et al*., 1954): CBF was measured in 7 patients with heart failure, before and after neck compression to over 150 mmHg. CBF did not decrease.

Conclusions:

Compression of the internal jugular veins in supine healthy volunteers…

5. …increases blood volume in the skull with venous blood distributed particularly to the large venous sinuses. A small increase in the BOLD signal seen in the MCA and cerebellum indicated the possibility of a slightly increased cerebral blood flow or the accumulation of arterial blood diffusely as well.
6. …does not change the magnitude or distribution of the resting blood flow in the brain.

7. …does not affect the response of brain blood flow to hypercapnia. This finding implies that it would not affect the brain blood flow in response to an increase in demand for flow.
8. …does not reduce the CBF if applied during increase in flow demanded, as simulated in this study by hypercapnia. This finding provides confidence that the brain would be able to maintain CBF if the collar is applied under other high flow states such as exercise.

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