THE USE OF MRI FOR STERNOCLEIDOMASTOID MUSCLE VOLUME MEASUREMENT AND ITS ASSOCIATION WITH INTERNAL CAROTID ARTERY VELOCITY IN A HEALTHY COHORT

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Abstract:

Impairments or changes in the neck musculature's functional capacity has been shown to lead to cervicogenic symptoms (Rubin et al., 1995; Ryan & Cope., 1955; Wrisley et al., 2000). This study measured the sternocleidomastoid muscle (SCMM) volume to explore sex-related differences and its relationship with internal carotid artery (ICA) blood flow velocity (BFV). Imaging of the SCMM was conducted using Phase-Contrast Magnetic Resonance Imaging (PC-MRI) and the ICA BFV was examined by Doppler Ultrasound (DU) of 34 healthy participants (19 females, 15 males). Significant sex differences were established in SCMM volumes ($p<0.05$). There was excellent absolute agreement amongst raters measuring SCMM volume (ICC=0.916). Body Mass Index (BMI) was significantly correlated with females' right SCMM volume and ICA BFV ($p<0.005$). Overall bilateral and particularly the right males' SCMM volume was significantly correlated with ICA BFV ($p< 0.05$). These results will be an essential comparison group of healthy SCMM volume values for patients suffering from concurrent concussion and whiplash-related injuries.

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Terminology

Abbreviation	Expanded word
MRI	Magnetic resonance imaging
PC MRI	Phase contrast magnetic resonance imaging
DU	Doppler ultrasound
ICA	Internal carotid artery
CCA	Common carotid artery
VA	Vertebral artery
MCA	Middle cerebral artery
ECA	External carotid artery
SCM	Sternocleidomastoid
mTBI	Mild traumatic brain injury
WAD	Whiplash associated disorder
PCS	Post concussion syndrome
CBF	Cerebral blood flow
BFV	Blood flow velocity
SCAT5	Sport Concussion Assessment Tool 5th Edition
ECG	Electrocardiogram
NPDI	Neck Pain Disability Index
BMI	Body Mass Index
ECG	Electrocardiogram

Chapter 1: Introduction

Concussion & Whiplash Injuries

Over the last decade there has been considerable research conducted on the etiology, pathophysiology and recovery time for mild traumatic brain injury (mTBI) and concussion. Despite the influx of research into this topic, there remains a lack of consensus on the definitions for both mTBI and concussion. In the past, mTBI has been defined as an acute brain injury resulting from a biomechanical force leading to disturbances in brain function (McCrory et al., 2013). The term concussion, broadly used to define a Sport Related Concussion, is best defined by the latest Consensus Statement on Concussion in Sport, as a traumatic injury caused by a biomechanical force due to either a direct or indirect blow to the head, face, neck or body resulting in a transient disturbance in neurological function (McCrory et al., 2017). Within both definitions, brain trauma is caused by a biomechanical force, yet they are both used separately and interchangeably in the literature. For the purposes of this paper the term concussion will be used. Regarding epidemiology, 7 million individuals within North America are affected by a traumatic brain injury yearly (McNair, 1999). From 2003 to 2012, there were 176,685 pediatric visits for concussions in Ontario emergency departments (Zemek et al., 2017). As of 1999, approximately 5.3 million individuals in the United States live with the consequences of traumatic brain injury which associates with a $48.3 billion cost per annum within the United States alone (Kiraly & Kiraly., 2007). Therefore, it is clear from the literature that there is a high prevalence of concussions. The vast majority of concussions, approximately 80-90% are resolved within 7-10 days, with 10-15% of patients continuing to have persistent symptoms 10 days post injury (McCroy et al., 2013).

Symptoms following a concussion can be divided into 3 categories; physiologic, vestibulo-ocular and cervicogenic (Ellis et al., 2015). This paper will focus on physiologic and cervicogenic symptoms. The pathophysiology of physiological concussion symptoms is consistent changes in “neuronal depolarization, cell membrane permeability, mitochondrial function, cellular metabolism and cerebral blood flow” (Ellis et al., 2015). To explore physiological symptoms, this paper will examine blood flow velocity (BFV) within the neck. The pathophysiology of cervicogenic concussion symptoms are “muscle trauma and inflammation along with dysfunction of cervical spine proprioception” (Ellis et al., 2015). It must be noted that these symptoms are not specific to concussions, therefore it is crucial to consider other conditions which may have resulted from the injury (McCroy et al., 2013). Cervicogenic symptoms are closely related to those experienced by whiplash-associated disorders (WAD).

Currently, the literature supports that a relationship exists between WAD/cervicogenic neck pain and concussion injuries (Marshall et al., 2015; Leddy et al., 2015; Schneider et al., 2014; Hynes & Dickey, 2006). The term whiplash injury was first introduced by Gay and Abbot (1953) in which the mechanics of this injury involved a sudden and forceful flexion of the neck, which in some cases is followed by several less violent oscillations of the neck as it is flexed or extended. Most whiplash injuries occur in rear-end car collisions (Linnman et al., 2009). The challenge with a whiplash injury is that there is high variability and low predictability of its prognosis, even the duration of the condition is inconsistent (Gay & Abbot, 1953). Patients with whiplash injuries present a wide variety of signs and symptoms ranging from simple neck pain to combinations of several musculoskeletal and neurological symptoms (Gay & Abbot, 1953). The most commonly reported symptoms for cervical whiplash injuries are neck pain, headache, dizziness and neck stiffness (Wallis et al., 1997; Treleaven et al., 2006; Treleaven & Jull., 2003). To date there are no specific diagnostic biomarkers for WAD, however, a relationship between the initial post-traumatic stress symptoms following the injury and a poor outcome has been established (Rodriquez et al., 2004; Buitenhuis et al., 2006; Sterling et al., 2006). The overall prognosis for recovery varies with each individual case, however active as opposed to passive treatment during the first 6 months post injury has proven to be effective in improving outcomes for patients (Rodriquez et al., 2004). Slower recovery has been associated with many socio-demographic factors such as gender being female, older age, being employed for less than full-time hours and having dependents (Harder et al., 1998). Approximately 25% of WAD patients will develop chronic pain-related disability along with potentially poor functional recovery (Sterling et al., 2010). Along with impacting patients physically, the injury takes an economic toll on patients as well with an estimated cost of $2500 per patient in indirect costs only in Canada (Suissa et al., 1995). On average in North America and Europe, approximately 300 per 100,000 individuals suffer from WAD (Holm et al., 2008). Therefore, the prevalence and impact of concussions and WAD both economically and physically on individuals is quite high.

Blood flow:

Presently there are two ways in which cerebral blood flow (CBF) can be measured. Total CBF can be estimated by either the flow in the capillaries within the head or by measuring the flow in the vessels supplying blood flow to the brain (Spilt et al., 2002). The first method provides an estimation of regional CBF whereas the latter provides a global CBF volume quantification of individual vessels. For the purpose of this study the latter method will be focused on, specifically measuring the variables of CBF velocity. BFV of a vessel is its blood supply over a period of time obtained by a sample volume that covers the entire diameter of the vessel (Kaps et al., 1992). Blood flow volume is the product of the mean flow velocities of the vessel over a given period time and its cross-sectional area (Kaps et al., 1992). The estimation of total cerebral blood flow volume is calculated by adding ICA and VA bilaterally together, for which the normalized values were collected by DU in 1994 by Schöning et al.

CBF supplied to the brain begins at the heart from the aortic arch, which branches off directly into the left CCA and the brachiocephalic artery which gives rise to the right CCA (Drake et al., 2015). The thoracic section of the left and right CCA differ due to their origin, but the path that both common carotid arteries follow in the cervical section is similar as they obliquely travel upwards to the level of the thyroid cartilage (Drake et al., 2015). At the level of the fourth cervical vertebra, the CCA bifurcates into the ICA and ECA (Drake et al., 2015). A study by Williams & Nicolaides (1987) identified a close relationship in the ICA and CCA vessel diameter as opposed to ECA. Therefore, ICA is a better predictor of CCA blood flow.

The bilateral vertebral arteries originate from the subclavian arteries and merge together to give rise to the basilar artery, which travels up the brainstem, pons and midbrain where it branches off to give rise to the Circle of Willis along with the bilateral ICA (Drake et al., 2015).

Within the Circle of Willis, the ICA bifurcates to give rise to the anterior cerebral artery and the MCA. The basilar artery bifurcates into the posterior cerebral artery which by the posterior communicating artery connects with the MCA (Drake et al., 2015). Within the literature, a direct relationship between ICA and MCA in terms of blood flow has been explored, however the input of the posterior circulation isn’t taken into account in this relationship (Weber et al., 1990). When comparing CCA and ICA as a predicator for cerebral blood flow, ICA was identified as more reliable than the CCA (Schöning et al., 1994). In a study with 250 adults, it was found that the right and left ICA contribute 41% and 40% respectively to cerebral blood flow calculated as a sum of ICA and the basilar artery (Buijs et al., 1998). The VA is estimated to contribute approximately 24.7% to the global cerebral blood flow volume through the posterior communicating artery, connecting with the MCA (Kashimada et al., 1995). From an anatomical perspective, it too should be considered when estimating MCA blood supply (Scheel et al., 2000).

In consideration of noninvasive imaging techniques, only ultrasonography and PC MRI allow the assessment of individual vessels (Scheel et al., 2000). DU is a noninvasive method of measuring blood flow volume and velocity of the vessels supplying blood to the brain (Albayrak et al., 2007). Benefits of DU include low operational costs, its efficient use for bedside monitoring, and the accessibility to repeated measurements (Albayrak et al., 2007). A common disadvantage of DU is its overestimation of blood flow volume (Albayrak et al., 2007). When performing measurements via DU time-averaged mean, BFV is more commonly used to calculate blood flow volume (Albayrak et al., 2007). During the systole portion of a cardiac cycle, the velocity profile is mostly flat; during diastole, the velocity profile becomes parabolic (Albayrak et al., 2007). Therefore, during systole the mean velocity and maximum velocity are very close whereas during diastole the mean velocity is approximately half of the maximum velocity (Albayrak et al., 2007). As a result, in this study taking the measurements of time averaged mean blood flow velocity will help to control for overestimating values.

Ultrasonography is the most common technique used to evaluate blood flow in vertebral and carotid arteries as it has shown to be reliable, inexpensive and produce real time images (Scheel et al., 2000; Quesnele et al., 2014). However, the measurements can be affected by the technician, angle of insonation and the unidirectional velocity encoding; as a result, MRI examinations have shown to be more reliable (Quesnele et al., 2014). PC MRI has greater sensitivity than standard techniques such as DU and is considered the criterion standard for blood-flow volume quantification of carotid and vertebral arteries (Quesnele et al., 2014). When blood flow volume measurements were compared with three noninvasive imaging techniques including color velocity imaging, spectral Doppler imaging and PC MRI quantifications, it was determined that PC MRI and color velocity imaging are preferable (Ho et al., 2002). Despite MRI being the most accurate quantification of blood flow in the literature, it has numerous limitations. There are physiologic and anatomical limitations resulting from breathing wherein vessel movements cause inconsistency in vessel diameter measurements (Ho et al., 2002). In vitro and in vivo studies have demonstrated a close correlation between PC MRI and Doppler ultrasound in measuring blood flow volume of internal carotid and vertebral arteries (Oktar et al., 2006). Despite DU overestimating blood flow volume and velocity, it is a practical and widely available technique compared to others (Oktar et al., 2006). In a majority of published studies using DU for examination to measure cerebral blood flow, CCA has been investigated the most as it is easily accessible when compared to ICA and VA (Donis et al., 1988; Keller et al., 1976; Uematsu et al., 1983; Muller et al., 1987). ICA and VA require a high angle of correction, and there are many variations in vessel diameter during a cardiac cycle (Schöning et al., 1994). Furthermore, obtaining ultrasonographic measurements of ICA CBF may be difficult due to the high carotid bifurcation, plaques, bends, tortuosity or stenosis (Yazici et al., 2005). Despite these challenges, DU remains a common and acceptable means of measuring ICA and VA blood flow, therefore it will be used in this study (Scheel et al., 2000; Quesnele et al, 2014; Schöning et al., 1994; Dörfler et al., 2000; Soustiel et al., 2003). In further investigation, it was seen that CCA vessel diameter was resulting in unreliable CCA blood flow volume measurements as it had ample changes throughout the cardiac cycle resulting in a higher blood flow volume than the sum of ICA and ECA blood flow volumes (Donis et al., 1988; Schöning et al., 1994).

BFV changes can occur after whiplash injuries; one study demonstrated statistically significant disturbances in the vertebrobasilar circulation within the first month following a whiplash injury, and in a 6-month follow up only 50% of the patients' blood flow velocities returned to normal (Šerić et al., 2000). Furthermore, altered regional cerebral blood flow in WAD patients has been significantly correlated to the patients' neck disability ratings, especially in areas of the brain responsible for the perception of pain such as, within the middle frontal gyrus, right temporo-occipital transition zone and right precentral gyrus (Linnman et al., 2009). Blood flow disturbances have also been reported after concussion injuries. Studies have shown increased blood velocities in the MCA and reduced blood flow velocities in the ICA following a concussion (McQuire et al., 1998; Weber et al., 1990). Alterations in cerebral blood flow volume have been observed in not only acute concussions but also post-concussion syndrome (PCS) (Leddy & Willer, 2013). It is important to consider sex differences when examining cerebral blood flow, as structural sex differences exist in males' and females' brains (Ritchie et al., 2018; Ruigrok et al., 2014). Sex differences also exist in brain weight as males on average have a 10% higher brain weight compared to females of the same age group (Ho et al., 1980; Dekaban et al., 1978). Likewise, when compared to male brains, female brains are approximately 8-13% smaller in size (Ruigrok et al., 2014). Some studies have demonstrated that females have a higher cerebral blood flow when compared to males (Mathew et al., 1986; Rodriguez et al., 1988; Gur et al., 1987; Rootwelt et al., 1986). However, many DU studies did not find sex differences in ICA, BFV, or CBF (Schebesch et al., 2004; Scheel et al., 2000; Buijs et al., 1998).

<u>Sternocleidomastoid Muscle:</u>

The SCMM is a cervical flexor muscle of the neck, which passes obliquely across the side of the neck bilaterally, with an origin of two heads; the first from the sternum and the second from the clavicle (Drake et al., 2015; Conley et al., 1980). The SCMM is a cervical flexor when acting bilaterally, however it is capable of ipsilateral rotation and side flexion when acting unilaterally (Drake et al., 2015; Conley et al., 1980). Both heads of the SCMM merge into a thick, rounded muscle below the middle of the neck, which then has an insertion at the lateral half of the superior nuchal line of the occipital bone and the mastoid process (Drake et al., 2015; Conley et al., 1980). This muscle has several functions, one of which is to serve as protection for the carotid arteries, with its placement anterior to the carotid sheath (Drake et al., 2015; Conley et al., 1980). Other functions of the muscle include being transposed to other body parts to improve function, assisting in rehabilitation of facial paralysis and assisting in shoulder elevation (Conley et al., 1980). The SCMM has been used for reconstructing regional defects within the body, rehabilitating oral cavity defects, reanimating the face and aiding shoulder elevation (Conley et al., 1980).

Cross sectional area measurements of the SCMM have been explored to some extent however at different cervical vertebrae levels. There is a gap within the literature examining the total volume of the SCMM as it has only been explored in one study with a very small sample size (Zheng et al., 2013). A study measuring total neck volume has demonstrated significant sex differences in SCMM volume (Zheng et al., 2013). SCMM volume has been calculated as the sum of the cross-sectional area over the cervical vertebrae levels to provide a cumulative cross-sectional area, which is then multiplied by the MRI slice thickness size in order to provide total SCMM volume (Zheng et al., 2013). Similar muscle volume calculation by PC MRI has been explored in thigh muscles by Yamauchi et al. (2017). Computerized axial tomography (CT) and MRI have widely been used to measure skeletal muscle volume however the disadvantage of using these methods is the costs and time (Miyatani et al., 2002). Similar to measuring blood flow, DU is a cost-effective alternative to measuring skeletal muscle volume and it has shown a significant correlation in measuring muscle thickness to muscle volume (Miyatani et al., 2004). Despite the cost-effective advantage of DU, CT and MRI are considered gold standards in measuring skeletal muscle volume as DU requires the observer to accurately select the site of measurement which can be especially difficult in muscles such as the SCMM which passes obliquely across the body (Miyatani et al., 2004).

A study exploring the relationship between the deep cervical muscle flexor dysfunction and WAD demonstrated increased muscle activation in the superficial neck muscle flexors which may be in response to compensating for the decreased activation of the SCMM (Jull, 2000). A comparison of the SCMM between healthy individuals and those with a whiplash injury demonstrated that individuals with a whiplash injury had significantly greater muscle fatty infiltrates of the SCMM (Elliott et al., 2010). It is interesting to note that whiplash injuries result in similar symptoms to concussion cervicogenic symptoms. In fact, within concussion injuries, inflammation of the neck muscles has been noted, however the physiologic and/or biomechanical connection between the overlapping symptoms has not been investigated. In addition, the SCMM volume relationship with whiplash and head injuries has not been extensively explored within the literature. Therefore, there may a relationship between concussion and WAD symptoms with SCMM volume changes.

Changes in Neck Anatomy with Neck-Related Disorders

Impairment or changes in the functional capacity of the neck musculature has shown to lead to cervicogenic symptoms such as recurrent or chronic headaches (Oksanen et al., 2008). In addition, sex differences have been explored in terms of muscle fibres and total muscle cross sectional area of the neck, which demonstrated that females typically have 60 to 85% of males' muscle fibres and total muscle cross sectional areas (Drinkwater., 1984). One study demonstrated increased SCMM activity in females with whiplash-related chronic neck pain compared to healthy controls (Juul-Kristensen et al., 2013). Several other studies have also demonstrated increased whiplash injuries (Harder et al., 1998; O'Neill et al., 1972) and neck pain (Mäkela et al., 1991) incidences in females. The biomechanical rationale behind this finding is being explored however not yet verified. A systematic review reported that in patients with chronic neck pain, there is a tendency of an increased cross-sectional area of the cervical flexors (De Pauw et al., 2016). A study by Vasavada et al. (2008) demonstrated significantly weaker female necks by 32% in flexion and 20% in extension when compared to male necks. It is possible that females are more susceptible to neck injuries because they have weaker supporting muscles in the cervical spine and less body weight for back support (Otremski et al., 1989). In a group of non-chronic neck pain individuals, females had a considerable weakness in neck flexor and extensor muscles (Ylinen et al., 2004). Females' total neck volume has been shown to be 59% lower than males' total neck volume (Zheng et al., 2013). Therefore, it is essential to consider sex differences when examining neck-related disorders. Symptomology of whiplash injuries has been explored in relation to the cross-sectional area of the SCMM (Juul-Kristensen et al., 2013; De Pauw et al., 2016) however the measurement of the volume will help further this interaction. This study will explore the relationship between SCMM volume and the neck's blood flow velocity to help further our understanding of how the pathophysiology of the cervicogenic symptoms may be related to the pathophysiology of the physiologic symptoms in concussion injuries. This study will be essential in providing healthy control values for the SCMM, which may serve as a comparison group in the future.

Chapter 2: Manuscript

Introduction:

A concussion is best defined by the latest Consensus Statement on Concussion in Sport, as a traumatic injury caused by a biomechanical force due to either a direct or indirect blow to the head, face, neck or body resulting in a transient disturbance in neurological function (McCrory et al., 2017). There are three categories for concussion symptoms; physiologic, vestibulo-ocular and cervicogenic (Ellis et al., 2015). A relationship has been established between WAD/cervicogenic neck pain and concussion injuries in terms of concurrent injuries (Marshall et al., 2015; Leddy et al., 2015; Schneider et al., 2014; Hynes & Dickey, 2006). Whiplash is defined as a sudden and forceful flexion of the neck, which is sometimes followed by several less violent oscillations of a flexed or extended neck (Gay & Abbot., 1953). After a WAD injury, approximately 25% of patients develop chronic pain-related disability and the potential for poor functional recovery (Sterling et al., 2010). WAD injuries also take an economic toll on patients with an estimated cost of $2500 in indirect costs, in Canada (Suissa et al., 1995). Approximately 300 per 100,000 individuals suffer from WAD in North America and Europe (Holm et al., 2008).

Anatomically total CBF can be measured at the neck from the cumulative bilateral ICA and VA blood flow velocities and volume. At the level of the fourth cervical vertebra, the CCA bifurcates into the ICA and ECA (Drake et al., 2015). A study identified a close relationship in the ICA and CCA vessel diameter as opposed to ECA (Williams & Nicolaides., 1987). Therefore, ICA is considered a better predictor of CCA blood flow.

The SCMM is a cervical flexor muscle of the neck, which passes obliquely across the side of the neck bilaterally (Drake et al., 2015; Conley et al., 1980). The SCMM is capable of ipsilateral rotation and side flexion when acting unilaterally and forward flexion when acting bilaterally (Drake et al., 2015; Conley et al., 1980). In particular, SCMM cross-sectional area is greater in individuals suffering from WAD compared to healthy controls at the C2-3 and C5-6 vertebral levels (Elliot et al., 2010).

PC MRI allows the assessment of individual vessels and is considered a “gold standard” and DU is a noninvasive method of measuring blood flow volume and velocity (Scheel et al., 2000; Albayrak et al., 2007). Benefits of DU include low operational costs, its use for bedside monitoring, and the accessibility to repeated measurements (Albayrak et al., 2007). However, a common disadvantage of DU is its overestimation of blood flow volume (Albayrak et al., 2007). Despite these disadvantages, DU remains a common and acceptable means of measuring ICA blood flow (Scheel et al., 2000; Quesnele et al, 2014; Schöning et al., 1994; Dörfler et al., 2000; Soustiel et al., 2003). SCMM volume has been calculated as the sum of the cross-sectional area over the cervical vertebrae levels, which is multiplied by the slice thickness size in order to provide total SCMM volume (Zheng et al., 2013). This method of measurement is similar to total body skeletal muscle volume calculations seen in previous studies (Janssen et al., 2000; Lee et al., 2000).

Currently the literature demonstrates some blood flow changes following a concussion and/or whiplash injury. At a 6-month follow up only 50% of the patients’ vertebrobasilar blood flow velocities returned to normal compared to the first month post whiplash injury (Šerić et al., 2000). An increase in MCA blood velocities and reduction in ICA blood flow velocities were seen following a concussion (McQuire et al., 1998; Weber et al., 1990). Therefore, it can be inferred that both injuries result in altered blood flow within the neck and head. Structural sex differences exist in males' and females' brains; hence sex differences should be considered when examining cerebral blood flow in order to further complete the picture about blood flow changes following a concussion and/or whiplash injury (Ritchie et al., 2018; Ruigrok et al., 2014). Males have a 10% higher brain weight compared to females of the same age group (Ho et al., 1980; Dekaban et al., 1978). Females brains are approximately 8-13% smaller compared to male brains (Ruigrok et al., 2014). Since CBF can be measured from the neck, sex differences in neck sizes should also be considered. Female's total neck volume is 59% lower than male's total neck volume (Zheng et al., 2013). Female necks are significantly weaker than males by 32% in flexion and 20% in extension (Vasavada et al., 2008). However, these anatomical sex differences in brain weight and neck sizes do not always align with the sex differences in CBF within the literature. Some studies have demonstrated sex differences in CBF identifying females as having a higher cerebral blood flow compared to males (Mathew et al., 1986; Rodriguez et al., 1988; Gur et al., 1987; Rootwelt et al., 1986). Conversely, many DU studies did not find sex differences in ICA BFV or CBF (Schebesch et al., 2004; Scheel et al., 2000; Buijs et al., 1998). As a result, within the literature there are CBF changes following a concussion or whiplash, which may be a result of the anatomical influences of brain weight and/or neck size. Sex differences exist in brain weight and neck size however the literature is inconsistent in terms of sex differences in blood flow therefore it is unclear if the blood flow changes seen following concussion and whiplash injuries is inherently due to anatomical sex differences in blood flow.

The use of DU to measure blood flow velocity is not uncommon but the desire to evaluate the ICA blood flow velocity is due to the vessel's location in the anterior of neck (Ekroll et al., 2014; Schöning et al., 1994). The SCMM is situated anterolateral to the carotid sheath surrounding the ICA and is commonly injured in those who have suffered from Whiplash Associated Disorders (WAD) or experiencing non-WAD cervicogenic neck pain (Vasavada et al., 2007). The rationale proposed in this study is novel in nature for evaluating the relationship between the muscle and its surrounding vasculature as it takes into account the proximity of the ICA to the SCMM along with the potential spasm and swelling of the injured SCMM which may apply undue pressure on the carotid sheath, thereby creating a potential distortion of the ICA vessel wall. This in turn could result in changes in arterial blood flow and pressure output which could be a contributing factor to headaches, a common symptom associated with cervicogenic neck pain and post-concussion syndrome. The purpose of this study is to establish healthy SCMM volume values to serve as a control cohort for an injured population and explore if a relationship exists between SCMM volumes as measured by Magnetic Resonance Imaging (MRI) proton density weighted imaging, and blood flow velocity in the internal carotid artery (ICA) as measured with Doppler Ultrasound (DU). We hypothesize, PC MRI can reliably and accurately measure SCMM volume in healthy males and females and there will be sex differences in the SCMM volume. In addition, we hypothesize, there will be a negative linear relationship between SCMM volume and ICA BFV for males and females.

<u>Methods:</u>

Participants

Thirty-four healthy, non-sedentary (15 males and 19 females), university aged (average age for males is 23 years old; average age for females is 24 years old) participants with no concussion history for the past 2 years were recruited from the York University, Keele St. campus. The weight and height of participants was recorded during the MRI scans and the BMIs were calculated, with the average BMI of male participants being 24.51 kg/m^2 and for females 23.34 kg/m^2. To ensure participants had no current neck pain the NPDI questionnaire was administered prior to any evaluation. To ensure no concussion symptoms were present a SCAT5 was also administered. In all the measures used to collect blood flow data, electrocardiogram (ECG) data was simultaneously recorded (gated ECG) for the purpose of recording a minimum of 5 cardiac cycles to calculate an average measurement of blood flow velocity. The order of DU and MRI evaluations were scheduled according to the availability of the MRI scanner, on the same day consecutive to one another. All measurements were taken in a supine position while the participants rested to allow for stabilization in heart rate. Participants were also instructed to refrain from caffeine for at least 12 hours before the testing. All participants signed an informed consent form and the study protocols were approved by the Office of Research Ethics at York University.

MRI data collection and analysis

MRI evaluations were conducted using a 3.0 T Siemens TIM MAGNETOM MRI Scanner. All MRI evaluations were conducted by the same MRI technologist. Firstly, 3D Time of flight (TOF) MR angiography scans were taken of the neck to identify the structures of interest and their anatomical landmarks. A turbo spin echo sequence with proton density (PD) weighting was used to visualize the SCM musculature of the neck. The parameters for the PD imaging were: TR = 3520 ms, TE = 38 ms, voxel size = 0.7 x 0.5 x 3.0 mm, FOV = 200 x 200 mm, 35 slices, FA = 120º, total acquisition time 5:18. In order to analyze and extract the data collected from the MRI, Segment by Medvisio, a MRI analysis software was used. As seen in

Figure 1, regions of interest (ROIs) were drawn on MRI images in the Segment software. To keep consistent with and obtain accurate measures comparable to the literature, 3 manual ROIs were drawn by 3 different individuals, who were trained on how to use the software, to obtain an average and decrease variability in the measures. ROIs were drawn around the cross-sectional area of the SCMM for a total of 35 slices per participant. Each slice's cross-sectional area was multiplied by the slice thickness of 0.33 cm to obtain the volume per slice. The volumes of all 35 slices were summed together to obtain a cumulative SCMM volume. These steps were performed bilaterally. BMI was calculated by the weight and height collected during the MRI scans from which the BMI adjusted SCMM volume was calculated by dividing the SCMM volume by the BMI.

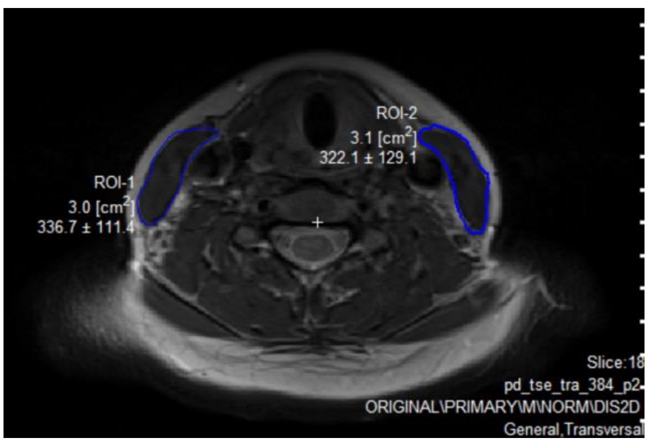

Figure 1: PC-MRI slice image of ROI analysis in Medvisio Segment program of SCMM to measure cross-sectional area to produce SCMM volume measurements.

Doppler Ultrasound Data Collection and Analysis

Ultrasound images were collected on a Logiq e Ultrasound System (GE Healthcare). ICA velocities were collected using a 4-12MHz linear array transducer. The ICA was measured 1 cm above the bifurcation point of the CCA, estimated to be between the levels of the C2-C4 vertebrae to account for individual variability (Drake et al., 2015). Upon identifying the vessel of interest, a video was recorded for 15 seconds, of which measurements were taken over five cardiac cycles. To ensure accuracy and consistency for the measurements, video recordings of the ICA were taken at a straight segment of at least 0.5 cm. As seen in Figure 2, in the recordings, the parallel walls of the vessels were visible with the largest longitudinal section. The angle of insonation was aimed to be kept at an approximate 60° angle for all measurements. Time average mean velocity were measured over 5 cardiac cycles per participant and an average value was obtained for average ICA BFV. All vessel scans were taken bilaterally. Measurements for 17 participants were completed by rater A and measurements for the other 17 participants were completed by rater B.

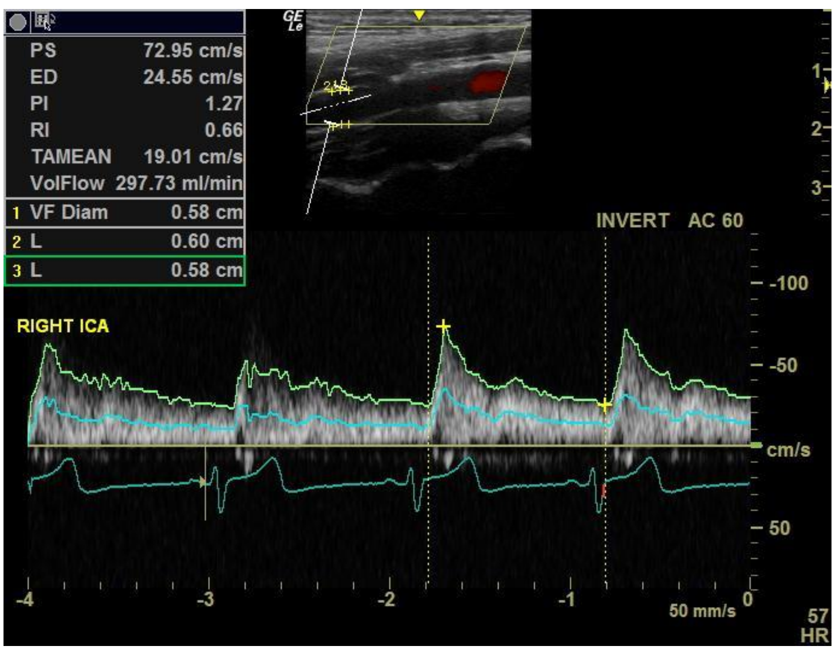

Figure 2: Measurement of blood flow velocity in Colour Duplex Doppler Ultrasound over the cardiac cycles. High- resolution B-mode imaging of the ICA, with an ECG trace for beat-by-beat recordings of blood flow.

Statistical Analysis

All statistical analyses were performed within SPSS version 24 (IBM Inc, Armonk, New York). For SCMM volume statistical analysis, the intra-class correlation coefficient test was used to determine absolute agreement between the 3 raters drawing the ROIs for the muscle. An independent t-test was used to determine if sex differences exist bilaterally in the SCMM volume and BMI-adjusted SCMM volume. A Pearson correlation was used to explore if a relationship exists between the SCMM/BMI-adjusted SCMM volume and the blood flow velocity of the ICA.

Results

Table 1: Demographic Characteristics and BMI of Participants and Independent T-Test for Sex Differences

	Male (n=15)	**Female** (n=19)	**Overall** (n=34)	**P Value**
Age (years)	23.13	23.84	23.53	0.61
BMI (kg/m^2)	24.51	23.24	23.86	0.34

Male and female participants had similar ages and BMI, the respective characteristics are shown in Table 1. No significant difference in age and BMI were seen between both sexes of participants and the distribution of participants for each sex was similar.

Bilateral SCMM volume and BMI-Adjusted SCMM volume averaged over values measured by 3 raters is summarized in Table 2. The inter-rater reliability of the 3 raters' measurements was in excellent absolute agreement with an intra-class correlation coefficient of 0.916. Bilaterally both sexes had similar absolute and relative SCMM volumes, with males absolute and relative SCMM volume being higher than the females. Average bilateral ICA BFV for both sexes are summarized in Table 2. Males and females had similar ICA BFV both between sexes and bilaterally.

Table 2: Overall Average Values for SCMM Volume, BMI-Adjusted SCMM Volumes and Internal Carotid Artery Blood Flow Velocity with 95% Confidence Intervals

	Left Side				**Right Side**			
	Male	**95% CI**	**Female**	**95% CI**	**Male**	**95% CI**	**Female**	**95% CI**
Mean SCMM volume (cm^2)	46.16	41.60 – 50.72	29.57	27.65 – 31.49	47.04	41.82 – 52.27	29.34	27.25 – 31.44
Mean BMI Adjusted SCMM volume (cm^2)	1.90	1.72 – 2.08	1.27	1.19 – 1.35	1.93	1.74 – 2.12	1.26	1.18 – 1.34
ICA BFV (cm/s)	20.94	17.03 – 24.86	23.75	19.81 – 27.69	23.28	18.44 – 28.11	24.58	20.67 – 28.48

As summarized in Table 3 and 4 there was no significant correlation between age, BMI and males' bilateral SCMM volume, BMI-Adjusted SCMM volume and ICA BFV. There was no significant correlation between age and females' bilateral SCMM volume, BMI-Adjusted SCMM volume and ICA BFV. However, there were significant correlations between females' bilateral SCMM volume and BMI-adjusted SCMM volume and BMI. There were significant correlations between females' right ICA BFV and BMI, however no significant correlation between females left ICA BFV and BMI. There were moderate positive linear relationships between bilateral females' absolute SCMM volume, right ICA BFV and BMI. There were moderate negative linear relationships between bilateral females' relative SCMM volume and BMI.

Table 3: Pearson Correlation Coefficients of Left SCMM volume, BMI-Adjusted SCMM volume and ICA BFV with Age and BMI for Both Sexes

	Male Age		**Male BMI**		**Female Age**		**Female BMI**	
	R	**P Value**	**R**	**P Value**	**R**	**P Value**	**R**	**P Value**
Mean SCMM volume (cm^2)	-0.07	0.81	0.45	0.092	0.25	0.29	0.61	0.01
Mean BMI Adjusted SCMM volume (cm^2)	-0.24	0.38	-0.39	0.152	0.04	0.89	-0.56	0.02
ICA BFV (cm/s)	0.45	0.09	-0.03	0.930	-0.19	0.43	0.34	0.15

Table 4: Pearson Correlation Coefficients of Right SCMM volume, BMI-Adjusted SCMM volume and ICA BFV with Age and BMI for Both Sexes

	Male Age		**Male BMI**		**Female Age**		**Female BMI**	
	R	**P Value**	**R**	**P Value**	**R**	**P Value**	**R**	**P Value**
Mean SCMM volume (cm^2)	-0.02	0.96	0.49	0.06	0.18	0.47	0.62	0.004
Mean BMI Adjusted SCMM volume (cm^2)	-0.18	0.53	-0.27	0.33	-0.03	0.91	-0.47	0.04
ICA BFV (cm/s)	-0.04	0.87	0.43	0.11	-0.02	0.96	0.46	0.05

Anatomical differences between sexes, bilaterally are summarized in Table 5. Independent t-tests demonstrated significant sex differences between males and females for both left and right SCMM volume and BMI-Adjusted SCMM volume. For bilateral SCMM volume and BMI-adjusted SCMM volume, males had larger volumes when compared to females. ICA BFV did not significantly differ between males and females on both the left and right sides.

Table 5: Independent T-Test for Sex Differences in Bilateral SCMM volume and ICA BFV

	Left Side			**Right Side**		
	Mean	**SD**	**P Value**	**Mean**	**SD**	**P Value**
Male Mean SCMM volume (cm^2)	46.158	8.23	$8.18\ e^{-9}$	47.04	9.44	$2.89\ e^{-8}$
Female Mean SCMM volume (cm^2)	29.575	3.98		29.34	4.35	
Male Mean BMI Adjusted SCMM volume (cm^2)	1.90	0.33	$3.49\ e^{-8}$	1.93	0.35	$1.37\ e^{-8}$
Female Mean BMI Adjusted SCMM volume (cm^2)	1.27	0.16		1.26	1.56	
Male ICA BFV (cm/s)	20.94	7.06	0.30	23.27	8.73	0.66
Female ICA BFV (cm/s)	23.75	8.18		24.58	8.11	

Anatomical differences bilaterally for each sex are summarized in Table 6. In both sexes, there were no significant differences between the left and right side of SCMM volume, BMI-adjusted SCMM volume and ICA BFV.

Table 6: Independent T-Test P values for Bilateral Differences in SCMM Volume in Males and Females

	Male			**Female**		
	Mean	**SD**	**P Value**	**Mean**	**SD**	**P Value**
Left Mean SCMM Volume (cm^2)	46.16	8.23	0.79	29.57	3.99	0.86
Right Mean SCMM Volume (cm^2)	47.04	9.44		29.34	4.35	
Left Mean BMI Adjusted SCMM Volume (cm^2)	1.90	0.33	0.81	1.27	0.16	0.76
Right Mean BMI Adjusted SCMM Volume (cm^2)	1.93	0.35		1.26	0.16	
Left ICA BFV (cm/s)	20.94	7.06	0.43	23.75	8.18	0.75
Right ICA BFV (cm/s)	23.27	8.73		24.58	8.11	

Correlations identified through Pearson correlation coefficients for females between bilateral and overall ICA blood flow velocity and absolute and relative SCMM volume are summarized in Table 7. There were no significant correlations between bilateral and overall ICA blood flow velocity and SCMM volume and BMI-adjusted SCMM volume in the female participants.

Table 7: Pearson Correlation Coefficients Between Bilateral SCMM/BMI-Adjusted Volume and ICA Blood Flow Velocity in Females

	Left ICA Blood Flow Velocity (cm/s)		**Right ICA Blood Flow Velocity (cm/s)**		**Bilateral ICA Blood Flow Velocity** (cm/s)	
	R Correlation Coefficient	**P Value**	**R Correlation Coefficient**	**P Value**	**R Correlation Coefficient**	**P Value**
Mean SCMM Volume (cm^2)	0.06	0.79	0.36	0.13	0.22	0.19
Mean BMI Adjusted SCMM Volume (cm^2)	-0.36	0.13	-0.18	0.46	-0.27	0.09

Correlations identified through Pearson correlation coefficients for males between bilateral and overall ICA blood flow velocity and absolute and relative SCMM volume are summarized in Table 8. There were no significant correlations between bilateral and overall ICA blood flow velocity and absolute SCMM volume in the male participants. There was no significant correlation between the left ICA BFV and relative SCMM volume however there was a significant correlation between the right and overall ICA blood flow velocity and relative SCMM volume. There was a negative moderate relationship for both right and bilateral ICA BFV and relative SCMM volume.

Table 8: Pearson Correlation Coefficients Between Bilateral SCMM/BMI-Adjusted Volume and ICA Blood Flow Velocity in Males

	Left ICA Blood Flow Velocity (cm/s)		**Right ICA Blood Flow Velocity (cm/s)**		**Bilateral ICA Blood Flow Velocity** (cm/s)	
	R Correlation Coefficient	**P Value**	**R Correlation Coefficient**	**P Value**	**R Correlation Coefficient**	**P Value**
Mean SCMM Volume (cm^2)	-0.36	0.14	-0.19	0.49	-0.26	0.16
Mean BMI Adjusted SCMM Volume (cm^2)	-0.40	0.14	-0.56	0.03	-0.48	0.008

Discussion

The present study calculated SCMM volume reliably and accurately with an excellent absolute agreement amongst the three raters. SCMM volumes were explored within this study, to add to the literature's normative values. There were significant sex differences in absolute and relative SCMM volume. However, there were not significant sex differences in ICA BFV. Age was not significantly correlated with SCMM volume and ICA BFV. BMI was also not significantly correlated with SCMM volume and ICA BFV in males, however there was a significant correlation between females' right SCMM volume and right ICA BFV. The second objective of this study was to explore the relationship between the neck's musculature and cerebral blood flow. This study, in a healthy cohort demonstrated a significant correlation in right and bilateral relative SCMM volume and ICA BFV.

The method of measurement for SCMM volume is in accordance to total body skeletal muscle volume calculations done in previous studies (Janssen et al., 2000; Lee et al., 2000). Within the literature it has been seen that males have significantly greater SCMM volumes than females (Zheng et al., 2013). Cross-sectional area of a muscle is also linked to the capacity to generate force, hence since males had significantly greater SCMM volumes in the present study, they may have a greater capacity to generate force when compared to females (Zheng et al., 2013). It has been seen in previous studies that females have weaker necks, which can be inferred in this study as well through the sex differences in SCMM volume as females had significantly smaller SCMM volumes (Vasavada et al., 2008). Overall the current study's sex difference in SCMM volume is consistent with the literature in terms of whole body skeletal muscle volume. Multiple studies have demonstrated that males usually have larger skeletal muscles than females overall in an absolute and relative reference to body mass (Janssen et al., 2000; Lee et al., 2000; Gallagher et al., 1998; Gallagher et al., 1997). Therefore, the SCMM is a sex-specific muscle which supports the second hypothesis and establishes a larger participant pool for healthy control values that may be used in future research in comparison to an injured population.

A significant correlation was not identified for males and females SCMM volume in relation to age. Similarly, another study identified a non-significant relationship between posterior neck muscle cross-sectional areas and age (Rankin et al., 2005). A decrease in overall body skeletal muscle volume is seen in both sexes beginning at the age of 40 years old, however a significant decrease is seen at approximately 67-69 years (Janssen et al., 2000). Since the age range for the participants within the current study was 18 to 34, a significant difference in muscle volume with an increase age was not expected to be seen as the age group was not broad enough. Therefore, consideration should be given to expanding the age range, to explore the relationship between age and SCMM volume.

In the current study, a non-significant relationship was seen between BMI and males bilateral absolute SCMM volume. Whereas, a significant correlation for females in bilateral absolute SCMM volume and BMI was noted. However, it is important to note that BMI has many flaws as it does not take into account age, sex, bone structure, fat distribution and muscle mass (Rothman, 2008). As age increases it has been seen that muscle mass decreases with sometimes no change in BMI (Rothman, 2008). Therefore, the inter-relationship between BMI, age and SCMM volume should be explored in future studies.

Blood flow volume is a measure which takes into consideration blood flow velocity therefore BFV and blood flow volume are proportional, however most studies focus on blood flow volume which will be used as references for blood flow velocity (Schöning et al., 1994). The average values of unilateral ICA BFV for males and females within the current study range from 20.94 cm/s to 24.58 cm/s as seen in Table 2; these values are quite similar to the average ICA BFV seen in the study by Schöning and colleagues (1994) of 24.9 cm/s. Within this study no significant side to side differences were found in ICA BFV in males and females. This is consistent with a study by Schebesch and colleagues (2004) in which no significant side to side differences in ICA blood flow volume were seen. Despite neck size differences between sexes, this study did not find significant sex differences within ICA BFV (Martin et al., 1997; Rowley et al., 2000). This is in accordance with multiple DU studies that also demonstrated non-significant sex differences in ICA BFV or CBF (Schebesch et al., 2004; Scheel et al., 2000; Buijs et al., 1998). However, several studies measuring CBF taking brain weight and volume into account demonstrated sex differences in which females had a higher CBF in comparison to males (Mathew et al., 1986; Rodriguez et al., 1988; Gur et al., 1987; Rootwelt et al., 1986). These differences in CBF calculated by DU and in reference to brain weight may be due to sex differences in brain weight as on average males have a 10% higher brain weight compared to females within the same age group (Ho et al., 1980; Dekaban et al., 1978). However, in terms of sex differences in ICA BFV, many studies have demonstrated non-significant sex differences (Schebesch et al., 2004; Scheel et al., 2000; Buijs et al., 1998).

In the present study, a significant relationship between age and BFV did not exist for males and females, however this may be due to the small age range. Multiple studies have identified that with an increase in age, ICA blood flow volume and velocity decrease significantly (Schebesch et al., 2004; Scheel et al., 2000). Since ICA BFV is proportional to CBF, some studies found no correlation between CBF and age similar to the current study (Schöning et al., 1994; Waldemar et al., 1991; Yamaguchi et al., 1986; Mathew et al., 1986). However, other studies have found an inverse relationship between CBF and age (Bujis et al., 1998; Leenders et al., 1990). These differences between studies may be due to the demographic differences of the participants, in terms of age, sex and race, or another contributing factor could be the method of measurement.

A significant correlation was only seen in females' BMI and the right ICA BFV. To the best of our knowledge the relationship between BMI and ICA BFV has not been explored. Regardless of age and sex, an increase in BMI was associated with a reduction in MCA BFV in healthy, type-2 diabetes mellitus, hypertension and stroke patients (Selim et al., 2008). A higher BMI is associated with decreased regional CBF in Broadmann areas 8,9,10,11, 32 and 44; which are responsible for attention, reasoning and executive function (Willeumier et al., 2011). Within the same study a decrease of regional CBF was seen to the prefrontal cortex (Willeumier et al., 2011). A similar significant correlation may not have been seen in the present study because participant sample sizes or since ICA only accounts for approximately 40-41% of CBF so it may not be the artery leading to the decrease CBF (Buijs et al., 1998).

A significant negative correlation was only seen in males' right and bilateral relative SCMM volume and ICA BFV, therefore an increase in SCMM volume resulted in a decrease in ICA BFV. An increase in an artery's diameter will inversely result in a decrease in the vessel's BFV, as seen in a study by Valdueza et al. (1997) in which an increase of 10% in the MCA diameter correlated to a 23% decrease in the MCA BFV. Therefore, the present study's negative correlation is supported by the literature and proposes a potential relationship between vasculature and the surrounding muscle volume. The exploration of this relationship between SCMM volume and ICA BFV is novel as it takes into account the SCMM's proximity to the ICA and potential spasms or swelling of the injured SCM tissue which may apply undue pressure on the carotid sheath, thereby creating a potential distortion of the ICA vessel wall. A significant hypertrophy in cervical muscles has been seen in WAD patients within multiple studies when compared to healthy individuals (Elliott et al., 2010; Elliot et al., 2008). The SCMM cross-sectional area is greater in individuals suffering from WAD compared to healthy controls at the

C5-6 vertebrae which is quite close to the bifurcation of the CCA into the ICA and at the C2-3 vertebrae (Elliot et al., 2010). Hence, in future studies the hypertrophied SCMM in WAD patients should be explored in relation to changes in ICA BFV.

The treatment for concussions has historically been rest as opposed to whiplash injuries in which active recovery is recommended (McCroy et al., 2013; Rodriquez et al., 2004). However, within the literature it can be seen that these injuries often occur concurrently therefore the treatment being on two different ends of a spectrum is concerning. In multiple studies, it was seen that the activation of the SCM and upper trapezius muscles is vital for decreasing the severity of head injuries (Bauer et al., 2001; Choi et al., 2017; Ito et al., 1995; Ito et al., 1997; Schmidt et al., 2014; Simoneau et al., 2008; Tierney et al., 2004). This demonstrates the close relationship between the neck and head when concussion injuries occur concurrently with whiplash injuries. Brain injuries are not limited to a young athletic group; in fact, a study demonstrated that 37% of falls for 227 seniors result in traumatic brain injuries (Robinovitch et al., 2013). Furthermore, it has been seen in the literature that low neck strength is a potential modifiable risk factor for concussion injuries (Eckner et al., 2014). Within the current study it was seen in a healthy cohort the bilateral relative SCMM volume was significantly correlated with ICA BFV, which is a great portion of cerebral blood flow within the brain. Therefore, if a potential relationship between the neck and brain exists within healthy male individuals in terms of neck musculature and brain blood flow then this relationship should be further explored in future studies with an injured population.

Limitations

The present study is without potential limitations. Firstly, DU has numerous factors that may influence measurements including the following; it is greatly dependent on ultrasound beam geometry, sample depth adjustment and insonation angle (Gill 1985; Kohler et al., 1985). Within the current study, two different users performed DU measurements although both users had equal distribution of participants. Studies have shown that DU is highly dependent on the user's skills and experience hence having a possible impact on ICA BFV measurements; however, with experience reproducible measurements can be made by a hand-held probe (Saeed et al., 2012). ICA BFV from the current study are similar to those measured in other studies which demonstrates reliability in these reproducible values (Schöning et al., 1994; Farhoudi et al., 2011). The ICA BFV from the present study was lower than one study by Saeed et al., 2013 which may be due to BFV measurements being taken on an angle-independent dual beam DU instead of an angled fixed device as done in the reference study. Within the current study to control for variability, both raters maintained an angle of insonation at approximately 60° to the best of their ability and all measurements were taken from an average of 5 cardiac cycles from B-mode imaging videos, which were 15 seconds in length. Furthermore, DU has been established as a reliable method of measurement in comparison to the 133-xenon technique, (Soustiel et al., 2003).

In addition, due to the lack of neck circumference collection the relationship between the neck circumference and BFV and SCMM volume could not be explored. Since males on average have a larger neck circumference than females, it would be important to control for this factor when examining the differences in SCMM volume in order to explore the possible reasons behind the sex differences in SCMM volume. Hence, the measurement of neck circumference would be ideal to help control for it in future multiple regression analyses. Despite this shortcoming, the present study did account for BMI. Previous studies, that matched individuals for BMI found neck circumference was always larger in males compared to females, hence it is difficult to find participants where both sexes can be matched for neck circumference (Martin et al., 1997; Rowley et al., 2000). Since it has been difficult to match both sexes by their neck circumference, it is possible that this shortcoming may be persistent in future studies due to the significant anatomical sex differences in muscle volume.

Furthermore, in the future when altering the study design the age group of the participants should be given great consideration. Within the literature, it is apparent that a decrease in overall body skeletal muscle volume is seen in both sexes beginning at the age of 40 years old. (Janssen et al., 2000). With an increase in age, a decrease in muscle mass has been seen with sometimes no change in BMI (Rothman, 2008). The age group of this study was 18 to 34 years old; consequently future studies should include a larger participant group with a wider range of age and BMI to further support the literature. In addition, in future studies the injured SCMM should be explored in terms of it its potential impact ICA BFV due to its close proximity to the vessel.

Conclusion

In conclusion, the present study successfully measured SCMM volume expanding on the normative values previously collected by Zheng et al. (2013), hence reproducible estimates with excellent absolute agreement can be made in a healthy population. Significant sex differences were seen between absolute and relative SCMM volume as previously seen in SCMM volume and skeletal muscle studies (Zheng et al., 2013; Janssen et al., 2000; Lee et al., 2000; Gallagher et al., 1998; Gallagher et al., 1997). No significant sex differences in ICA BFV were seen in the present study, however right and bilateral ICA BFV was significantly correlated with males' SCMM volume. There were no significant correlations between age and SCMM volume nor ICA BFV, however this may be due to a small age range. BMI was significantly correlated with right females' SCMM volume and right ICA BFV. Significance may not have been found in the male population due to a smaller sample size compared to females. This study shows promise in serving as a control cohort for an injured group to explore if a relationship exists between SCMM volume and ICA BFV, in order to explore the relationship between the neck's musculature and cerebral blood flow. The importance of the SCMM in terms of muscle activation has been seen as a modifiable risk factor in concussion injuries, hence this muscle of interest should be further explored to see how other factors of the muscle such as volume are related to these injuries. This could possibly further establish a diagnostic rationale behind cervicogenic symptoms experienced by concussed and WAD injury patients.

Chapter 3: Discussion & Conclusions

SEX DIFFERENCES IN ABSOLUTE AND RELATIVE SCMM VOLUME

This study was able to use a method to calculate SCMM volume reliably and accurately with an excellent absolute agreement amongst three raters, hence supporting the first hypothesis of this study. The method of measurement for SCMM volume is similar to how total body skeletal muscle volume has been calculated in previous studies (Janssen et al., 2000; Lee et al., 2000). Specifically, for neck muscles, this method has previously been used to measure the volume of several neck muscles albeit being with a small sample size (Zheng et al., 2013). Similar to this study, Zheng et al. (2013) found sex differences in the SCMM volumes where males had significantly greater SCMM volumes than females. Since cross-sectional area is directly proportional to muscle volume, several studies measuring cross-sectional area alone have also found sex differences in the neck muscles (Vasavada et al., 2008; Rankin et al., 2005; Drinkwater.,1984; Ulbrich et al., 2011). SCMM volume sex differences may be due to significant differences between neck sizes for males and females, as on average males have larger necks than females (Martin et al., 1997; Rowley et al., 2000). Cross-sectional area of a muscle is also linked to the capacity to generate force, hence implying that since males had significantly greater SCMM volumes they have a greater capacity to generate force when compared to females (Zheng et al., 2013). When compared to male necks, females have significantly weaker necks by 32% in flexion and 20% in extension (Vasavada et al., 2008). Overall females have weaker supporting muscles in the cervical spine and less body weight for back support hence females may be more susceptible to WAD (Otremski et al., 1989). Overall this sex difference in SCMM volume is consistent with the literature in terms of whole-body musculature. It has been seen within studies that males usually have larger skeletal muscles than females overall in absolute and relative reference to body mass (Janssen et al., 2000; Lee et al., 2000; Gallagher et al., 1998; Gallagher et al., 1997). Therefore, within the neck the SCMM is a sex-specific muscle, which supports the second hypothesis of this study, and the larger participant pool within this study helps the literature expand on establishing control values for the SCMM volume.

THE RELATIONSHIP OF AGE WITH ABSOLUTE AND RELATIVE SCMM VOLUME

When exploring the relationship between demographic characteristics with absolute and relative SCMM volumes, a significant relationship was not identified for males and females. Likewise, Rankin and coworkers (2005) identified that a significant relationship did not exist between posterior neck muscle cross-sectional areas and age. In terms of overall skeletal muscle distribution, an increase in age has been correlated with a decrease in skeletal muscle volume in males and females from the age of 40 years, however a significant decrease is seen at approximately 67-69 years old (Janssen et al., 2000). Since the age range for the participants within the current study was 18 to 34 a significant difference in muscle volume with an increase age would not have been seen. In future studies, consideration should be given to expanding the age range in order to explore the relationship between age and SCMM volume.

THE RELATIONSHIP OF BMI WITH ABSOLUTE AND RELATIVE SCMM VOLUME

In the current study, BMI did not demonstrate a significant relationship with males bilateral absolute SCMM volume. However, there was a significant correlation for females between BMI and bilateral absolute SCMM volume. BMI was first introduced in the 1800s as a measure of body fat however this assumption has since been shown to be very flawed (Rothman, 2008). BMI does not take into account age, sex, bone structure, fat distribution and muscle mass; as a result, it should not be held as a comparator to SCMM mass (Rothman, 2008). As age increases it has been seen that muscle mass decreases with sometimes no change in BMI, therefore it would be interesting to see in future studies the relationship with age and SCMM volume (Rothman, 2008). In the current study, SCMM volume was adjusted for BMI for males and females however participants were not matched for neck circumference as in previous studies. In two different studies the neck circumference was found to be larger in males than females, despite females and males having the same or larger BMI (Martin et al., 1997; Rowley et al., 2000). The present study did not control for possible differences in neck circumference and its relationship to SCMM volume, however it is possible that it may be a difficult variable to control given the significant sex differences in neck size.

SEX DIFFERENCES IN BILATERAL ICA BLOOD FLOW VELOCITY

Most studies focus on blood flow volume, which is a calculation which takes into consideration blood flow velocity, therefore these two measures are proportional (Schöning et al., 1994). Schöning and colleagues (1994) were the first to establish normative values for ICA blood flow velocity and volume. The average values of ICA blood flow velocity for males and females within the current study range from 20.94 cm/s to 24.58 cm/s as seen in Table 2; these values are quite similar to the average ICA blood flow velocity seen in the study by Schöning and colleagues (1994) of 24.9 cm/s. However, within the study by Schöning and colleagues (1994) there were more participants than the current study with an average age of 12 years higher than this study, which could account for the slight differences between the results. Within this study no significant side to side differences were seen in blood flow velocity in males and females. This finding is consistent with the literature as Schebesch and colleagues (2004) also did not find significant side to side differences in blood flow volume within the ICA. It is interesting to note that despite sex differences reported within the literature for neck circumference, this study did not find significant sex differences within ICA BFV (Martin et al., 1997; Rowley et al., 2000). This is in accordance with DU studies that also did not find sex differences in ICA BFV or CBF which is also an indication of ICA BFV (Schebesch et al., 2004; Scheel et al., 2000; Buijs et al., 1998). Hence in future studies it would be interesting to explore the relationship between neck circumference and ICA BFV to determine the possible influence. However, within the literature there are also studies measuring cerebral blood flow in relation to brain weight or volume which have shown sex differences with females having a higher cerebral blood flow when compared to males (Mathew et al., 1986; Rodriguez et al., 1988; Gur et al., 1987; Rootwelt et al., 1986). These differences in cerebral blood flow calculated by DU and in relation to brain weight may be explained by the sex differences in brain weight as males on average have a 10% higher brain weight compared to females of the same age group (Ho et al., 1980; Dekaban et al., 1978).

THE RELATIONSHIP OF AGE WITH BILATERAL ICA BLOOD FLOW VELOCITY

Within this study a significant relationship between age and blood flow velocity did not exist for males and females, however this may be due the age range being quite small. Within the literature it has been seen that both ICA blood flow volume and velocity decreases significantly with age (Schebesch et al., 2004; Scheel et al., 2000). When exploring cerebral blood flow in relation to age, some studies have found no correlation similar to what the current study would suggest as ICA BFV is proportional to CBF (Schöning et al., 1994; Waldemar et al., 1991; Yamaguchi et al., 1986; Mathew et al., 1986). However, other studies have found a decline in cerebral blood flow with an increase in age (Bujis et al., 1998; Leenders et al., 1990). The reason for the differences between these studies could be the demographics of the participants in the studies in terms of age, sex and race, however another contributing factor could be the method of measurement. For example, within the study by Schöning and colleagues (1994) DU was used as opposed to the study by Bujis and colleagues (1998) in which MRI was used to measure cerebral blood flow. MRI is considered the “gold standard” in measuring blood flow and this may be the reason for the difference (Quesnele et al., 2014). Studies by Mathew et al. (1986) and Waldemar et al. (1991) both found no correlation between age and CBF however they both used the same Xenon Inhalation technique for measurement. Lastly, Yamaguchi et al. (1986) and Leenders et al. (1990) both used the positron-emission tomography method and found a decrease in the cerebral metabolic rate of oxygen with age. Only Leenders et al. (1990) found a negative correlation between CBF and age, this may be due to participant sample size differences. This inconclusive relationship between age and ICA BFV should be further explored as Mathew et al. (1986) found a marked decrease in CBF with age especially within the frontal region where the ICA anatomically provides blood flow to. Furthermore, there were sex differences found within the frontal region where females had a higher CBF compared to males (Mathew et al., 1986). The current study’s findings of higher ICA BFV supports higher frontal region blood flow as females had a higher average ICA BFV bilaterally when compared to males as reported in Table 2. Therefore, in future studies cerebral blood flow specifically in the frontal region along with ICA blood flow should be explored along with age.

THE RELATIONSHIP OF BMI WITH BILATERAL ICA BLOOD FLOW VELOCITY

A significant correlation was only seen in females' BMI and the right ICA BFV. To the best of our knowledge a study directly exploring BMI and ICA BFV has not been done, however there are studies exploring the relation between BMI and cerebral blood flow. A study by Selim et al. (2008) exploring the relationship between BMI and cerebral blood flow in healthy, type-2 diabetes mellitus, hypertension and stroke patients demonstrated that regardless of age and sex an increase in BMI was associated with reduced cerebral blood flow measured via the MCA. A healthy brain study explored the relationship between regional cerebral blood flow and BMI in healthy adults over the span of 3 years and found that a higher BMI is associated with decreased regional cerebral blood flow in Broadmann areas 8,9,10,11, 32 and 44; these brain regions are responsible for attention, reasoning and executive function (Willeumier et al., 2011). Within the same study a decrease of regional cerebral blood flow was seen to the prefrontal cortex (Willeumier et al., 2011). Despite the link between BMI and cerebral blood flow, it was not seen within this study bilaterally for both sexes, possibly due to sample sizes or that ICA only accounts for approximately 40-41% of cerebral blood flow so it may not be the artery associated with the decreased cerebral blood flow (Buijs et al., 1998). A study exploring the relationship between aerobic exercise with BMI and MCA BFV in healthy sedentary and endurance-trained men demonstrated a higher MCA BFV in men that did regular aerobic exercise (Ainslie et al., 2008). Therefore, in future studies it would be interesting to explore the relationship between BMI and ICA BFV with a difference of training in the two groups.

THE RELATIONSHIP BETWEEN ABSOLUTE AND RELATIVE SCMM VOLUME AND ICA BLOOD FLOW VELOCITY

A significant negative correlation was only seen in males' right and bilateral relative SCMM volume and ICA blood flow velocity. Hence with an increase in SCMM volume a decrease in ICA blood flow velocity can be seen. The measurement approach proposed in this study is novel in nature for evaluating the relationship between SCMM volume and ICA BFV as it takes into account the fact that due to the SCMM's proximity to the ICA, potential spasm and swelling of the injured SCM tissue may apply undue pressure on the carotid sheath, thereby creating a potential distortion of the ICA vessel wall. It has been noted within the literature that an increase in an artery's diameter will inversely result in a decrease in the vessel's blood flow velocity. This was seen in a study by Valdueza et al. (1997) where an increase of 10% in the MCA diameter correlated to a 23% decrease in the MCA BFV. As a result, this negative correlation is supported by the literature and proposes a potential relationship between vasculature and the surrounding muscle volume particularly in the neck. Despite having a small sample size in this current study, a significant relationship was noted, hence this relationship should be further explored within a larger sample size. Of particular interest would be the impact of altering SCMM volume as seen in WAD on ICA BFV and cerebral blood flow, since the SCMM is situated anterolateral to the carotid sheath surrounding the ICA and is commonly injured in those who have suffered from WAD or experiencing non-WAD cervicogenic neck pain, which can often accompany concussion injuries (Vasavada 2007, 2002; Marshall et al., 2015; Leddy et al., 2015; Schneider et al., 2014; Hynes & Dickey, 2006). Overall, it has been seen that cervical muscles hypertrophied significantly in patients with chronic WAD compared to healthy individuals (Elliott et al., 2010; Elliot et al., 2008). In particular, SCMM cross-sectional area has been shown to be greater in individuals suffering from WAD compared to healthy controls at the C2-3 and C5-6 vertebral levels (Elliot et al., 2010). The C5-6 vertebrae are very close to the location at which the CCA bifurcates into the ICA. The greater SCMM volume at this level could in turn could result in changes in arterial blood flow and pressure output which could be a contributing factor to headaches, a common symptom associated with cervicogenic neck pain and concussions. Interestingly in many regions of the brain increased regional cerebral blood flow was seen at resting state with WAD which could be correlated to the increased SCMM cross-sectional area seen in another study which may be impacting BFV of the ICA by applying undue pressure to the vessel (Linnman et al., 2009; Elliot et al., 2010). Furthermore, a study has demonstrated a correlation between altered regional cerebral blood flow in WAD patients and patients' neck disability ratings (Linnman et al., 2009). This is the rationale behind why the current study should be used as control normative values to compare to a participant pool suffering from WAD in the future.

RELATIONSHIP BETWEEN CONCUSSIONS, WAD AND NECK VOLUME AND BLOOD FLOW VELOCITY

To date concussion injuries and whiplash injuries have been treated very differently; traditionally rest was prescribed for concussion injuries whereas active recovery was most commonly prescribed for whiplash injuries (McCroy et al., 2013; Rodriquez et al., 2004). Recently, however, the treatment prescriptions have moved away from complete rest. These differences are an essential reason behind the exploration of the neck's role in concussions with concurrent whiplash injuries. Low neck strength is a potential modifiable risk factor for concussion injuries as lower neck strength is associated with an increased risk for concussions

(Eckner et al., 2014). Multiple studies have demonstrated the importance of activation of the SCM and upper trapezius muscles for head stabilization and decreasing the severity of head impact in young adults in head injuries. This again demonstrates the relationship between the neck and head in concussion injuries and concurrent occurrence of WAD and concussions (Bauer et al., 2001; Choi et al., 2017; Ito et al., 1995; Ito et al., 1997; Schmidt et al., 2014; Simoneau et al., 2008; Tierney et al., 2004). The role of the SCMM in decreasing the severity of head injuries through activation has been explored within the literature. In turn, the SCMM is the muscle of interest in this study which helps explores the potential relationship between the muscle and concussion and WAD symptomatology. Not only is it important to explore the relationship between the neck and head when considering concussion and WAD for athletes; it is also vital for seniors as with age neck muscle strength and muscle activation decline resulting in the inability to control for these injuries in falls (Wood et al., 2019). In a study examining 227 falls of seniors, it was seen that 37% of falls were accompanied with traumatic brain injuries (Robinovitch et al., 2013). Brain injuries impact a wide age range and the exploration of a modifiable risk factor such as neck strength would be essential in preventing injuries across the lifespan. Within this study, the relationship between the neck muscle volume and brain blood flow was explored. It was seen that in males the bilateral relative SCMM volume was significantly correlated with the neck blood flow which accounts for a significant portion of cerebral blood flow. This demonstrates rationale for more studies using a larger participant pool to further explore this relationship. The results of this study suggest that for males, a concussion with a concurrent whiplash injury that injures the SCMM could be significantly correlated with changes in ICA BFV which in turn could be responsible for the cervicogenic and concussion symptoms experienced by patients. Exploring this further could help distinguish the best treatment for concussion and whiplash injuries.

Limitations

The present study has some limitations. Firstly, DU is not always considered the first choice when selecting a tool for to measure BFV as there are various factors that may influence the results. DU measurements are greatly dependent on ultrasound beam geometry, sample depth adjustment and insonation angle (Gill 1985; Kohler et al., 1985). In this study, DU measurements were performed by two different evaluators, although both users had equal distribution of participants. Studies have shown that DU is highly dependent on the user's skills and experience; however, with experience reproducible measurements can be made by a hand-held probe (Saeed et al., 2012). Despite these limitations to DU the values obtained for ICA BFV are similar to those obtained in other studies (Schöning et al., 1994; Farhoudi et al., 2011). The values found in this study were lower than Saeed et al., 2013 but may be due to blood flow velocity measurements being taken on an angle-independent dual beam DU in the current study versus an angled fixed device as done in the reference study. In order to control for variability, both raters maintained the angle of insonation as close to 60° as possible and all measurements were taken from an average of 5 cardiac cycles across 15-second B-mode imaging videos. In previous studies, DU measurements of ICA BFV have been closely correlated to the measurements performed by the 133-xenon technique, supporting the use of DU as a reliable method of measuring ICA BFV (Soustiel et al., 2003).

SCMM volume measurements are highly dependent on the raters' ability to identify the SCMM and precisely outline the muscle. Despite room for variability, the current study had an excellent absolute agreement amongst the 3 raters.

Finally, neck circumference was not collected as a component of this study design, limiting the ability to explore the relationship between the neck circumference and BFV and SCMM volume. On average, males have a larger neck circumferences than females, hence it is important to try to control for this factor in future studies especially with multiple regression analyses in order to clearly identify the possible reasons behind the sex differences in SCMM volume. The present study did account for BMI; despite using BMI matched individuals in previous studies, neck circumference was always larger in males than females, which makes the possibility of matching neck circumference between the two sexes difficult (Martin et al., 1997; Rowley et al., 2000).

Conclusion

In conclusion, the present study successfully applied the method of skeletal muscle volume analysis via PC-MRI to the SCMM volume, expanding on the normative values previously collected by Zheng et al. (2013) and that reproducible estimates with excellent absolute agreement can be made in a healthy population. Significant sex differences were established between SCMM volumes in absolute and relative terms as previously seen in SCMM volume and skeletal muscle studies (Zheng et al., 2013; Janssen et al., 2000; Lee et al., 2000; Gallagher et al., 1998; Gallagher et al., 1997). There were no significant sex differences in ICA BFV, however right and bilateral ICA BFV were significantly correlated with males' SCMM volume. No significant correlations were seen between age and SCMM volume and ICA BFV, however this may be due to a small age range within the participants of the present study. BMI was significantly correlated with females' right SCMM volume and right ICA BFV; it is possible that significance was not found in the male population due to a smaller sample size compared to females. These significant correlations give support to future studies that should focus on a

WAD population in which the SCMM is injured and could potentially impact ICA BFV due to its close proximity to the vessel. Furthermore, a larger age group should be explored in order to further explore correlations with age and BMI. Since WAD symptoms remain persistent at rest, the present study establishes reliable, healthy control group values for future WAD studies (Linnman et al., 2009). To date concussion injuries and whiplash injuries have been treated differently, however presently active recovery is prescribed for both injuries (McCroy et al., 2013; Rodriquez et al., 2004). In fact, a relationship between the SCMM activation and decreasing the severity of traumatic brain injuries has been explored in multiple studies (Bauer et al., 2001; Choi et al., 2017; Ito et al., 1995; Ito et al., 1997; Schmidt et al., 2014; Simoneau et al., 2008; Tierney et al., 2004). Therefore, it is essential to explore the relationship between the neck's musculature and cerebral blood flow in an effort to determine the cause behind the symptoms within both injuries. This study has promising implications in serving as a control cohort for an injured group and explore if a relationship exists between SCMM volume and ICA BFV in order to possibly establish diagnostic rationale behind cervicogenic symptoms experienced by concussed and WAD injury patients.

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