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INVITED REVIEW

Evaluating the methods used for measuring cerebral blood flow at rest and during exercise in humans

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Abstract

The first accounts of measuring cerebral blood flow (CBF) in humans were made by Angelo Mosso in ~1880, who recorded brain pulsations in patients with skull defects. In 1890, Charles Roy and Charles Sherrington determined in animals that brain pulsations—assessed via a similar method used by Mosso—were altered during a variety of stimuli including sensory nerve stimulation, asphyxia, and pharmacological interventions. Between 1880 and 1944, measurements for CBF were typically relied on skull abnormalities in humans. Thereafter, Kety and Schmidt introduced a new methodological approach in 1945 that involved nitrous oxide dilution combined with serial arterial and jugular venous blood sampling. Less than a decade later (1950's), several research groups employed the Kety-Schmidt technique to assess the effects of exercise on global CBF and metabolism; these studies demonstrated an uncoupling of CBF and metabolism during exercise, which was contrary to early hypotheses. However, there were several limitations to this technique related to low temporal resolution and the inability to measure regional CBF. These limitations were overcome in the 1960's when transcranial Doppler ultrasound (TCD) was developed as a method to measure beat-by-beat cerebral blood velocity. Between 1990 and 2010, TCD further progressed our understanding of CBF regulation and allowed for insight into other mechanistic factors, independent of local metabolism, involved in regulating CBF during exercise. Recently, it was discovered that TCD may not be accurate under several physiological conditions. Other measures of indexing CBF such as Duplex ultrasound and magnetic resonance imaging, although not without some limitations, may be more applicable for future investigations.

Keywords Kety-Schmidt technique · Transcranial Doppler ultrasound · Duplex ultrasound · Cerebral blood flow · Exercise

Abbreviations

ACAv	Anterior cerebral artery
ACAv	Anterior cerebral artery blood velocity
BOLD	Blood oxygenation level dependent
CBF	Cerebral blood flow
CBV	Cerebral blood velocity
CCA	Common carotid artery
CMR	Cerebral metabolic rate
ECA	External carotid artery
gCBF	Global cerebral blood flow
ICA	Internal carotid artery
ICAv	Internal carotid artery blood velocity
MCA	Middle cerebral artery
MCAv	Middle cerebral artery blood velocity
MRI	Magnetic resonance imaging
$PaCO_2$	Partial pressure of arterial carbon dioxide
PCA	Posterior cerebral artery
PCAv	Posterior cerebral artery blood velocity
$P_{ET}CO_2$	Partial pressure of end-tidal carbon dioxide
PET	Positron emission tomography
TCD	Transcranial Doppler ultrasound.
VA	Vertebral artery

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Introduction

The brain is a formidable organ, accounting for ~2% of body weight, while receiving ~15–20% of the total cardiac output, which relates to approximately 750–1000 ml/min of blood

flow. This magnitude of cerebral perfusion is appropriate to maintain a constant supply of oxygen and nutrients due to the brains poor ability to store glycogen (< 10 μmol/g). The brain is kept in an enclosed cavity (i.e. the skull), but the extension of the cerebral vasculature originates proximally beyond the skull and into the neck with the vertebral and common carotid arteries (VA and CCA, respectively). The common carotid arteries emerge directly from the aortic arch (left CCA) and the brachiocephalic artery (right CCA). Traveling distally from the heart, both CCA's bifurcate into an internal carotid artery (ICA) and an external carotid artery (ECA), the latter being responsible for supplying blood to facial tissue. The internal carotid arteries are responsible for supplying blood flow to the anterior portion of the brain, and when considering both ICA's, they account for ~ 70% of global cerebral blood flow (gCBF). Distally, the ICA's trifurcate into the middle cerebral arteries (MCA), anterior cerebral arteries (ACA), and posterior communicating arteries. The posterior cerebral circulation is comprised of the left and right VA's, which branch off from the left and right subclavian arteries, respectively. The VA's supply blood flow distally through the cervical vertebrae and eventually combine forming the basilar artery—responsible for ~ 30% of gCBF. The basilar artery, although has many smaller arteries branching perpendicularly, eventually bifurcates into the left and right posterior cerebral artery (PCA) segments. The combined anterior and posterior cerebral vascular structure that is responsible for cerebral perfusion is known as the circle of Willis. Although the majority of circle of Willis show vascular anomalies (Iqbal 2013), the circle of Willis, is an effective vascular structure capable of supplying blood flow to regions of the brain in the presence of small obstructions in blood flow (e.g. reductions in ICA or VA flow). Distal from the large intracranial conduit arteries (e.g. MCA, ACA, and PCA), is a dense network of arterioles (i.e. pial vessels), that are present along the surface of the brain. These vessels are highly reactive to a variety of stimuli such as changes in blood gases (Willie et al. 2012), and cerebral perfusion pressure (Lassen 1959; Paulson et al. 1990). The pial arterioles then penetrate beneath the surface of the cortex, into the pia mater, through what is known as the Virchow–Robin space. Here, the pial arterioles progress into small microvessels, and eventually become encapsulated by pericytes, astrocytes, microglia and neurons, together known as the "neurovascular unit", and although these vessels are vasoactive, their contribution to cerebral blood flow regulation (CBF) is controversial.

The first known published documents of dynamic cerebral perfusion were made by Angelo Mosso around 1880, where he measured changes in cerebral blood volume using plethysmothgraphy in participants with skull defects (Mosso 1880; Zago et al. 2009). This device recorded brain pulsatility, and could detect changes in cerebral blood volume (see Fig. 1). Notably, Angelo Mosso observed that the brain pulsations of participants who engaged in tasks such as mathematical calculations increased in magnitude (see Fig. 1d). This was a primitive methodology at the time, but likely the very first demonstration of neurovascular coupling (i.e.

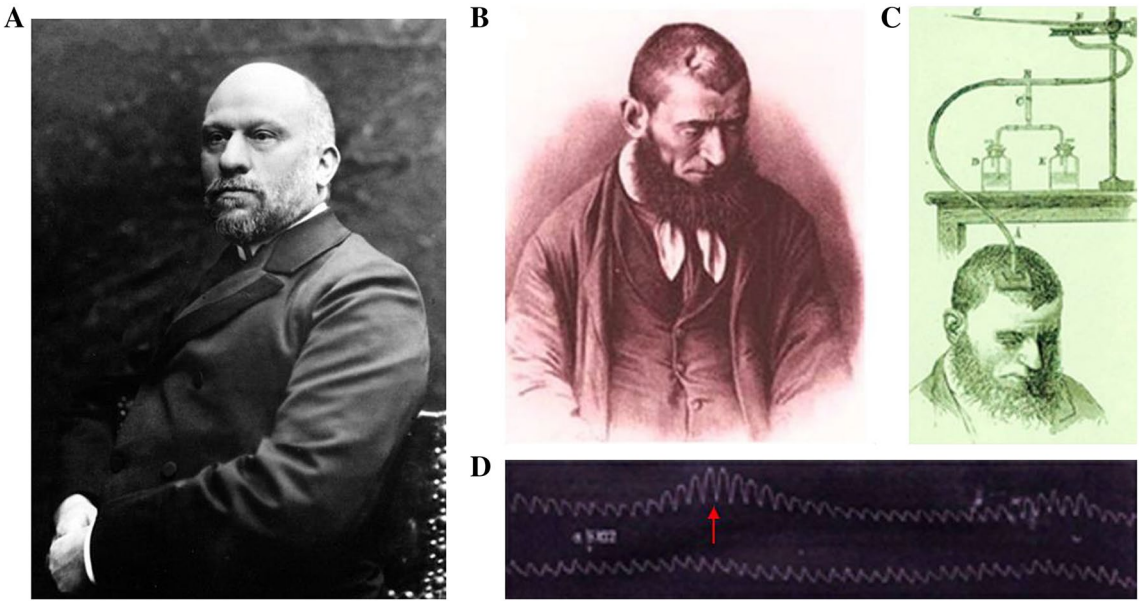

Fig. 1 Panel **A** represents a picture of Angelo Mosso. In panel **B**, Michele Bertino, one of Mosso's patients is fitted with Mosso's device to record brain pulsations (panel **C**). During this experiment, Mosso asked Bertino to multiply 8 × 12, and the resulting change in brain blood volume was observed, indicated by the red arrow (panel **D**). Modified with permission from Zago et al. (2009). (Color figure online)

changes in CBF in response to changes in neural activity). Although Mosso's findings were ground breaking, his measures did not index CBF per se, and using this approach he could only conduct experiments on individuals with specific skull defects. In addition, another major limitation to Mosso's technique was later outlined by Roy and Sherrington (1890):

> *"We cannot, however, in man measure the pressure either in the systemic arteries or in the veins, so that we cannot tell whether any of the changes in the volume of the brain are or are not due to active changes in the calibre of the cerebral blood vessels".*

Mosso went on to develop what was known as the 'human circulation balance' in an effort to further explore CBF in humans. Soon after Mosso's pioneering studies, Roy and Sherrington conducted a series of experiments investigating CBF in an unspecified number of dogs, cats, and rabbits (see Fig. 2). Using robust methodological approaches they recorded variations in the "vertical thickness" of the cerebral hemispheres responding to a number of stimuli such as: sensory nerve stimulation, asphyxia, perturbations to cerebral blood inflow and outflow, and during the administration of a wide variety of pharmacological interventions (Roy and Sherrington 1890). Here, they provided the first description of the complex integration of local and systemic mechanisms that interact to regulate CBF.

Due to methodological challenges, the primary being that non-invasive measurements of CBF were not obtainable in humans, most of the early work was done either in the animal model, or in humans with skull defects (e.g. the "Mosso Method"). Approximately, 50 years after the seminal work of Mosso, and Roy and Sherrington, the first quantitative measurement of gCBF in humans was achieved by Kety and Schmidt in 1945. Herein, we describe the initial work of Kety and Schmidt, and the more recent techniques (e.g. ultrasound) and landmark experimental approaches that have been used to quantify CBF during exercise.

History of cerebral blood flow during exercise

Kety-Schmidt technique

The first experiments that accurately quantified CBF were done by Kety and Schmidt in 1945. In these landmark experiments, the authors used an inert tracer (nitrous oxide), and the calculated differences between the rate of appearance and clearance of the tracer from concurrent arterial and internal jugular venous blood samples (i.e., Fick principle) to quantify the volume of blood flowing through the brain (see Fig. 3). One of the advantages of the technique that Kety and Schmidt developed is that since the arterial-venous sampling sites are identical (e.g. radial artery and jugular vein), and gCBF is measured (the measured arterio-venous difference of the inert tracer), therefore, cerebral metabolic rate (CMR) can be calculated. Since the development of this experimental approach, it has been discovered that any freely diffusible tracer (e.g. Xenon, Hydrogen) can be used to quantify changes in gCBF (Edvinsson and Krause 2002). Although at the time this was the gold-standard method of

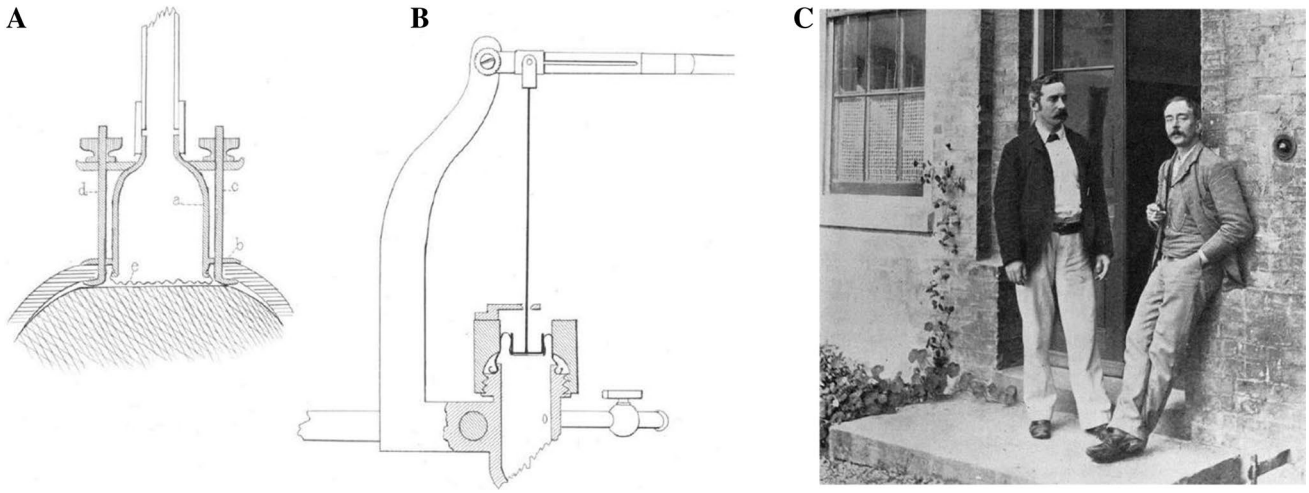

Fig. 2 A picture of the device employed by Roy and Sherrington (Panel **A**). Here, a bell shaped capsule is enclosed by an animal membrane (*e*), which moves in accordance to changes in brain blood volume. The apparatus is fixed into position by two metal pins that hook around the cranial opening (*c, d*). Changes in brain volume results in changes in bell capsule (*a*) position, and these are detected with the recording apparatus as depicted in panel **B**. Panel **C** Is a picture of Charles Roy (left) and Charles Sherrington (right). Reprinted with permission from Roy and Sherrington (1890)

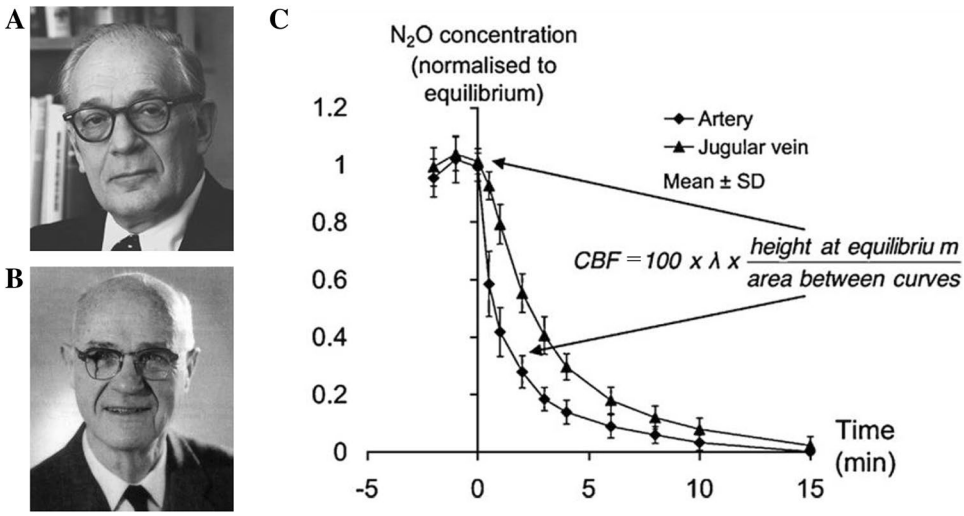

Fig. 3 Panels **A** and **B** are pictures of Seymour Kety and Carl Schmidt, respectively. Panel **C** represents an example of the Kety-Schmidt technique using N_2O. Arterio-venous samples are drawn, and gCBF can be calculated using the Kety-Schmidt equation. Using arterio-venous samples and calculated gCBF, global CMR can be quantified. λ is the brain-blood partition coefficient (ml/g). Reprinted with permission from Willie et al. (2011)

quantifying gCBF, there are several limitations that reduce the impact of these original findings: (1) the Kety-Schmidt technique lacked temporal resolution as measurements were averaged over ~ 10-min, and (2) gCBF, not regional CBF, was measured, and (3) this technique assumed that venous outflow was symmetrical between both jugular veins, an assumption that may not be true even at rest (Lichtenstein et al. 2001), let alone during various interventions (e.g. changes in body position, and exercise).

Shortly after these landmark studies by Kety and Schmidt, the first studies investigating the effects of exercise on gCBF occurred using the nitrous oxide dilution technique (Kleinerman and Sokoloff 1953; Lambertsen et al. 1953; Scheinberg et al. 1953, 1954). Out of these studies, only one reported that gCBF was significantly elevated during exercise (Kleinerman and Sokoloff 1953). Here, the authors observed an ~ 18% increase in gCBF during low-intensity exercise in the supine position (i.e. ~15–20% of VO_2 max; Kleinerman and Sokoloff 1953). In contrast, other investigations reported insignificant increases in gCBF of ~ 5% (Scheinberg et al. 1954), and ~ 10% (Scheinberg et al. 1953), during low-intensity supine exercise, respectively. The second study by Scheinberg et al. (1954), measured resting gCBF in the supine position, and then compared it to gCBF measured in upright position, which could potentially lead to an underestimation of gCBF during exercise. Similarly, Lambertsen et al. (1959) observed no change in mean gCBF during supine exercise; however, they reported that exercise resulted in a wide range of reduced relative partial pressure of arterial CO_2 ($PaCO_2$; − 0.9 to − 11.1 mmHg), indicating that participants were ventilating at different rates, thus, potentially exercising at a wide range of relative workloads. Therefore, the observed gCBF response to exercise in the study by Lambertsen et al. (1959) should be cautiously interpreted due to various levels of hypocapnia-related cerebral vasoconstriction. During this similar time period, another study investigated the effects of continuous low intensity supine exercise (20-min at ~10% of their maximum workload), and reported a reduction in both $PaCO_2$ (~ 2 mmHg) and gCBF (~ 13%; Kleinerman and Sancetta 1955). The potential factors responsible for these contrasting reports from these early studies are three-fold: (1) the gCBF response to exercise is intensity related, requiring a minimum of ~ 15% of exercise workload to elevate gCBF (Kleinerman and Sokoloff 1953; Kleinerman and Sancetta 1955); (2) methodological issues associated with posture (i.e., baseline gCBF in the supine position compared to gCBF measured during exercise in the upright position); and (3) variability in the relative individual exercise capacities and fluctuations in $PaCO_2$ could be responsible for the observed unchanged (Lambertsen et al. 1953), or reduced (Kleinerman and Sancetta 1955), gCBF response to exercise. Finally, considering that a major limitation of the Kety-Schmidt technique was that it required ~ 10-min of steady-state data, ensuing studies that aimed to accurately quantify the gCBF response to exercise demanded techniques with improved temporal resolution to limit the impact of confounding factors during exercise (e.g., thermal stress, fatigue and metabolism). These factors even when using contemporary techniques can prove difficult to control during a bout of exhaustive exercise, and are even more challenging when using the Kety-Schmidt technique, which requires a longer steady-state assessment period which can increase the chance that changes in these factors may influence gCBF assessment. The importance of scrutinizing data in studies that utilize traditional and contemporary methodology will continue to improve our understanding of the mechanism(s) involved in maintaining adequate cerebral perfusion during exercise.

The utilization of radioactive tracers (e.g., Xe clearance and radio labeled erythrocytes) and gamma imaging improved the resolution of gCBF measurements over the next 30 years after Kety and Schmidt's initial experiments.

Three studies were published during this time using the Xe clearance technique. One study reported an unchanged gCBF (Globus et al. 1983), while two others observed an increase in gCBF ranging from ~ 13% (Herholz et al. 1987), to ~ 26% (Thomas et al. 1989), above baseline values during low-intensity semi-recumbent cycling exercise (~ 20% of maximum workload). The increases in gCBF were similar to those observed by researchers utilizing the nitrous oxide washout technique (~ 15%; Zobl et al. 1965) as well as those utilizing radio labeled erythrocytes, injected directly into the ICA during low-intensity exercise (~ 18%; Hedlund 1965). Further increases in exercise intensity (i.e., low-to-moderate) augmented the elevated gCBF response to exercise (~ 30%; Herholz et al. 1987; Thomas et al. 1989), whilst maximal intensity exercise (> 70% maximum workload) failed to induce further elevations in gCBF from baseline values (Thomas et al. 1989). The potential factors contributing to the variability observed in the magnitude of the gCBF response to exercise when assessed using radioactive tracer contrasts (i.e., Xe and erythrocytes) are two-fold: (1) in order for the gamma camera to detect the radioactive tracers study participants are required to remain motionless, a feat which may be difficult near maximal exercise intensities; and (2) because the entire body is subjected to venous injections of a radiolabelled isotope, quantification of gCBF using gamma detection may be influenced by skin blood flow through the scalp. However, observations made by Thomas et al. (1989), utilizing a special cooling cap to avoid thermally induced increases in scalp blood flow during exercise, indicated that the gCBF responses at maximal exercise were similar whether blood flow to the scalp was controlled or not. In summary, the collective consensus using multiple techniques indicates that gCBF is elevated from baseline values during mild-to-moderate intensity exercise, however, it does not increase further at maximal exercise intensities. Regardless, the requirement of invasive and radioactive approaches to dynamically assess gCBF during exercise reduced the applicability and increased the ethical considerations needed to utilize these techniques in broader academic and worldly settings. Thus, the need for novel non-invasive approaches that did not require radioactive tracers led to the employment of Doppler ultrasound techniques to explore the effects of exercise on CBF.

Transcranial doppler and duplex ultrasound

Although it was several years after that Doppler ultrasound was used to assess CBF during exercise, it was first described in the early 1960's for assessing blood velocity in extracranial cerebral vessels (Miyazaki and Kato 1965). Due to the thickness of the skull, it is difficult for ultrasound waves to penetrate the cerebral vasculature. However, Aaslid et al. (1982) demonstrated that insonation of the cerebral vessels can be achieved in the low frequency range of 1–2 MHz. The thinning of the temporal bone on the side of the skull provides an "acoustic" window to non-invasively measure the velocity of red blood cells within an insonated cerebral vessel using Doppler ultrasound (see Fig. 4). This breakthrough allowed for beat-by-beat measurements of cerebral blood velocity (CBV), which was reflective of CBF under the condition that the conduit cerebral vessel diameter (e.g. MCA and PCA) did not change. Despite the limitations of TCD (outlined below), which were not known at the time, the measurement of CBF using TCD is still considered a robust and precise method depending upon the experimental question. This technique was the champion at measuring cerebrovascular function in the early 1990s, and has enabled researchers to quantify the temporal pattern of CBF during exercise, and identify the factors that regulate CBF during exercise such as blood pressure, neurogenic activity, arterial blood gases, and cerebral metabolism (reviewed in Smith and Ainslie 2017).

One of the first investigations of CBF during exercise using Doppler ultrasound was done by Huang et al. (1991), where the authors measured ICA blood velocity (ICAv) and observed a somewhat similar relative increase in CBF (~ 16%) in response to incremental exercise, compared to the gCBF response (~ 26%) measured using the Xe approach (Thomas et al. 1989). Shortly afterwards, Jorgensen et al. (1992b) compared the CBF response between the MCA (measured via TCD), and gCBF (measured via Xe clearance technique) during low (30 and 60 W), and moderate (149 W), exercise intensities. The findings from this study indicated that gCBF and MCAv followed a similar trend during exercise when gCBF was indexed using the initial slope index (Jorgensen et al. 1992b), and the authors observed a similar plateau in both gCBF and MCAv at or above moderate workloads [i.e., > 60% maximum achieved workload (%WMax)]. The study by Jorgensen et al. (1992b) was the first to establish the use of MCAv (measured via TCD) as a non-invasive index of gCBF during exercise.

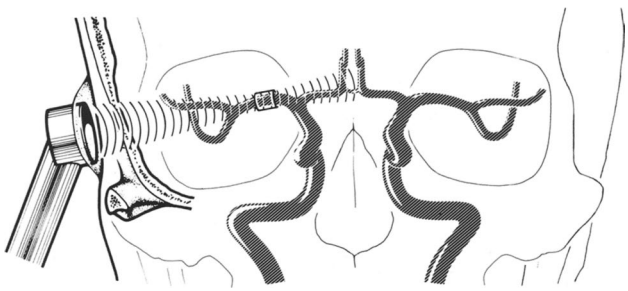

Fig. 4 A picture of a TCD ultrasound probe directed towards the MCA, measured through the temporal window. Reprinted with permission from Aaslid et al. (1982)

One of the primary advantages for the use of TCD is its temporal resolution, where a fixed ultrasound probe can obtain beat-by-beat measurements of CBV, allowing for the first time, high resolution CBV data across a wide range of exercise intensities. Just over two decades ago, two landmark studies were conducted using TCD during incremental exercise (Hellstrom et al. 1996; Moraine et al. 1993). Moraine et al. (1993) measured MCAv during an incremental exercise protocol to exhaustion (total duration ~ 16–26 min), and Hellstrom et al. (1996) measured MCAv while increasing the cycling exercise intensity every 2-min until 225 W was achieved, and then participants were asked to continue cycling at this workload until complete exhaustion (total duration 10–15 min). Since then, there have been a total of 19 studies that have measured anterior CBF during exhaustive exercise in otherwise healthy individuals (Brugniaux et al. 2014; Fan and Kayser 2013; Fisher et al. 2013; Gonzalez-Alonso et al. 2004; Hellstrom and Wahlgren 1993; Hellstrom et al. 1996; Imray et al. 2005; Larsen et al. 2008; Marsden et al. 2012; Moraine et al. 1993; Olin et al. 2011; Sato et al. 2011; Smirl et al. 2012; Smith et al. 2012, 2014, 2016; Subudhi et al. 2008, 2011; Trangmar et al. 2014), with the vast majority of these studies (17 out of 19) observing significantly elevated MCAv from rest during submaximal exercise intensities (20–80% VO_2 max), followed by a relative reduction in MCAv when approaching maximal exercise intensities (see Fig. 5). However, this bi-phasic MCAv response to incremental exercise was not observed by all investigations (Gonzalez-Alonso et al. 2004; Larsen et al. 2008). For example, during an incremental bout of semi-recumbent cycling to exhaustion (239 ± 42 W), Larsen et al. (2008) observed that the mean relative change in MCAv remained ~ 27 ± 17% elevated above baseline values at 100% WMax, despite significant hypocapnia (-8 ± 4.2 mmHg of $PaCO_2$ from baseline). Similarly, Gonzalez-Alonso et al. 2004 found that MCAv (averaged between left and right MCAv) was elevated by ~ 7.8 and ~ 14.0% compared to baseline at 40% workload max and at maximal exercise, respectively, despite substantial reductions in $PaCO_2$. The latter mentioned study employed a slightly different study design, which could be responsible for the observed findings. Gonzalez-Alonso et al. (2004) instructed participants to exercise at 40% of their work load max, and then undergo a brief (~ 7-min) maximal exercise bout to exhaustion, with a short (i.e. 5-min) recovery time in-between the moderate and maximal exercise bouts. Interestingly, during the maximal exercise bout, MCAv was elevated to ~ 20% above baseline, and then slightly decreased from that time-point to ~ 14% above baseline values when participants reached exercise exhaustion. Although a clear biphasic relationship between MCAv and exercise intensity was not observed in this study between 40% workload max and maximal exercise time-points, the maximal exercise trial indicates that MCAv was reduced at the point of participant fatigue compared to the beginning of exercise at this workload. These findings have been extensively reviewed previously with

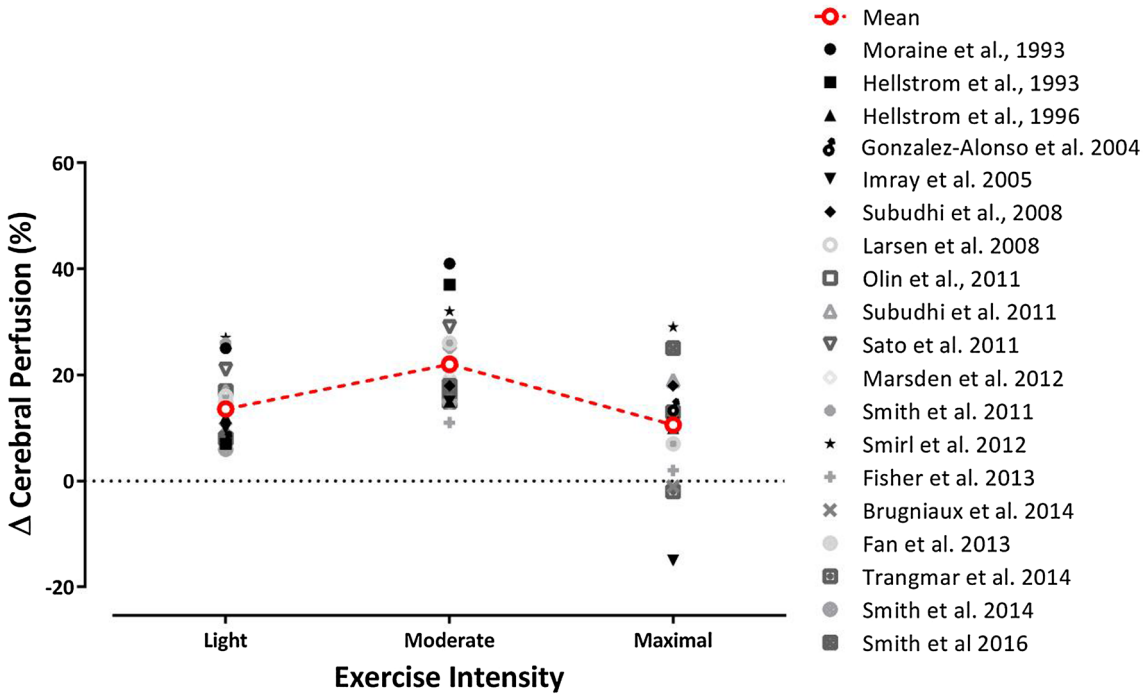

Fig. 5 Relative change in cerebral perfusion during light, moderate, and maximal intensity cycling exercise in the 19 studies investigating cerebral hyperemia utilizing TCD and Duplex ultrasound during incremental exercise

regards to the mechanistic factors (e.g. arterial blood pressure) contributing to the subtle differences observed between these studies (Smith and Ainslie 2017). The biphasic CBF response during incremental exercise is likely in part due to the hypercapnic vasodilatory and hypocapnic vasoconstrictive stimuli during submaximal and maximal exercise intensities, respectively. To date, there are three studies that have demonstrated that controlling $PaCO_2$ during incremental exercise alters the CBF response (Olin et al. 2011; Subudhi et al. 2011; Siebenmann et al. 2013). The studies by Olin et al. (2011), Subudhi et al. (2011) and Siebenmann et al. (2013) clamped the partial pressure of end-tidal carbon dioxide ($P_{ET}CO_2$) slightly above resting levels, and maintained relative hypercapnia throughout incremental exercise. This approach resulted in an elevated MCAv response during exercise, and abolished the typical observed reduction in MCAv upon approaching maximal exercise. In contrast, the study by Smith et al. (2016) did not show differences in gCBF between isocapnia and poikilocapnia exercise trials, but this was likely because gCBF was not measured > 80% of workload max; therefore, ventilatory related reductions in $PaCO_2$ were yet to be observed.

As the use of TCD cerebrovascular assessment progressed, there was a great deal of interest in whether regional differences in CBF existed. For example, Herholz et al. (1987) observed similar increases in CBF throughout the various regions of the anterior and posterior circulation (i.e., frontal, parietal, temporal, central and occipital cortices) at 25 and 100 W (10–20% and 15–30% of relative workload max) of cycling exercise, using the 133Xe method. Whereas, Jorgensen et al. (1992a) compared ACAv and MCAv during incremental cycling exercise and reported that the increases in MCAv were greater than ACAv. Rationale for the elevated MCAv vs ACAv exercise response was thought to be related toa greater contribution of the MCA blood flow to specific cortices involved in controlling muscular effort during cycling (e.g. frontal, central, parietal and temporal), whilst the ACA supplies blood flow to cortical areas involved in activities that are less influential to sustain motor control (Jorgensen et al. 1992a).

Early work attempted to validate the TCD technique by demonstrating that MCA diameter does not change during physiological stimuli (Serrador et al. 2000), however, more recent studies suggest that exercise and changes in $PaCO_2$ may in fact elicit changes in MCA diameter (Ainslie and Hoiland 2014; Coverdale et al. 2014, 2015, 2017; Verbree et al. 2014, 2017). Further discussion on the validity of TCD to measure CBF is described in the "Magnetic resonance imaging" section below. A series of studies were conducted over the past decade that used Duplex ultrasound, which makes simultaneous measurements of blood vessel diameter and blood velocity, therefore, making it possible to calculate the volumetric blood flow through a vessel instead of merely indexing it via a velocity dependent measure. Sato et al. (2011) was one of the first to utilize both TCD and Duplex ultrasound to demonstrate that relative changes in blood flow through the ICA correlates with relative changes in MCAv during incremental recumbent cycling (80% of workload max; see Fig. 6). The authors also reported that relative VA blood flow was significantly greater compared to both relative ICA blood flow and MCAv at 40, 60, and 80% of work load max. Similarly, Willie et al. (2011b) observed a greater relative increase in posterior cerebral circulation (i.e. PCAv) compared to the anterior cerebral circulation (i.e. MCAv) during constant load exercise (i.e. 40-min at 60% of maximum workload). However, these findings are in contrast to a series of studies conducted by Smith et al. (2012, 2014), which did not observe any specific regional differences in anterior and posterior CBF during normoxic exercise. Interestingly, a more recent study (Smith et al. 2016), which measured regional CBF using both TCD (i.e. MCAv and PCAv) and Duplex ultrasound (i.e. ICA and VA CBF), found that anterior CBF was elevated during exercise compared to posterior CBF (Smith et al. 2016). This study, for the first time, employed a dynamic end-tidal forcing system to maintain $P_{ET}CO_2$ during exercise; therefore, it is possible that the regional differences reported by previous studies were due to differences in $PaCO_2$ sensitivity during exercise. Nevertheless, more research is needed using both TCD, Duplex ultrasound and possibly MRI or positron emission tomography (PET) to characterize the CBF response to exercise.

Magnetic resonance imaging

In concurrence with the technological development of TCD and Duplex ultrasound, neuroimaging techniques such as magnetic resonance imaging (MRI) have been utilized to measure the effects of exercise on cerebral blood volume, cerebral structures, and CMR. Although these techniques have been employed for the previous 50+ years, high resolution neuroimaging techniques have only been available in recent decades. Although the robustness of MRI is excellent and its non-invasive nature makes it an attractive measurement technique, it has several caveats such as poor temporal resolution, and it requires a motionless patient while the MRI scan is taking place, therefore, imaging cannot occur during exercise.

Moreover, it is important to acknowledge that the majority of MRI techniques are akin to the other aforementioned methods (Kety-Schmidt technique, TCD, and Duplex ultrasound), intravascular blood flow cannot be measured directly. All measures are surrogate and the various techniques measure something that relates to blood flow. A common method in MRI is using the blood oxygenation level dependent (BOLD) signal as a surrogate for blood flow.

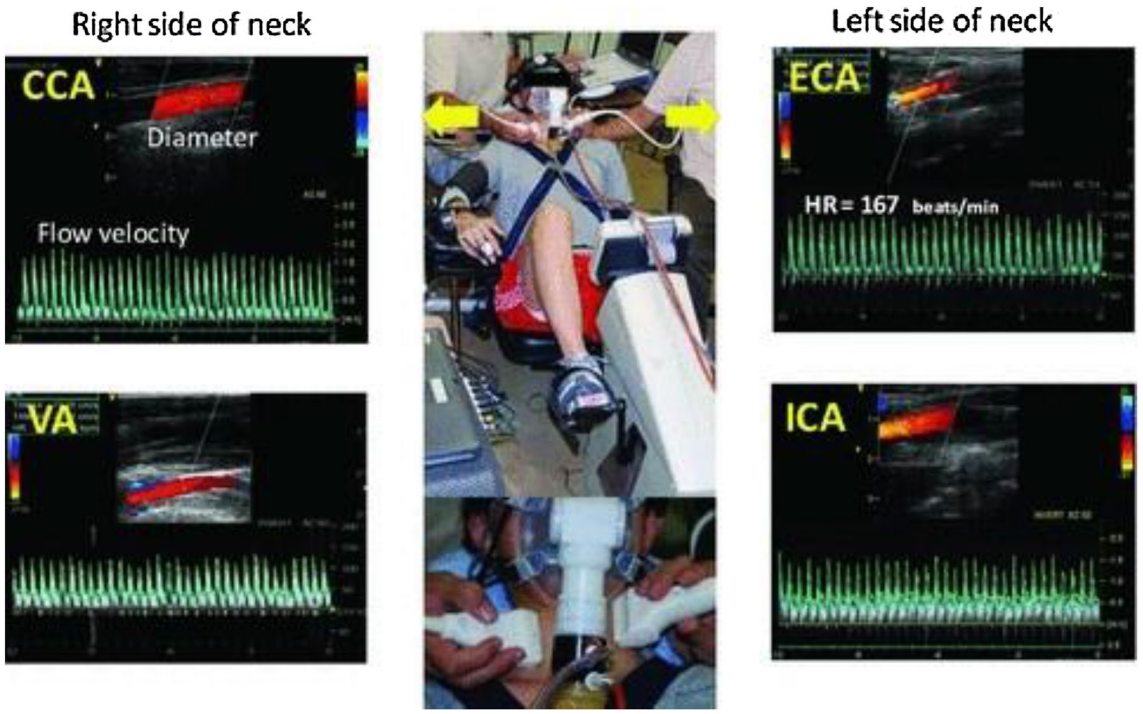

Fig. 6 A picture of the experimental set-up employed by Sato et al. (2011). Recordings of CCA, VA, ICA, and ECA using Duplex ultrasound were completed in participant's during submaximal exercise. Reprinted with permission by Sato et al. (2011)

In the magnetic field, deoxygenated hemoglobin is paramagnetic, which weakens the BOLD signal. Since O_2 consumption can be assumed to be constant (at least rest), the greater the blood flow, the less there is of the deoxygenated hemoglobin, and the stronger the signal—stronger signal means more blood flow. Despite not measuring actual CBF, however, BOLD can measure the change in blood flow following an exercise intervention. The validity of BOLD MRI following exercise, if the assumption of stable O_2 consumption is not met, is unclear. Although there are many different types of MRI approaches, each with advantages and disadvantages, however, further discussion of this topic is beyond the scope of this review.

In addition to providing valuable information into cerebral structures and CBF, MRI has proven useful in measuring cerebral vessel diameters. Briefly mentioned in a previous section (see "Transcranial doppler and duplex ultrasound"), a highly cited MRI study demonstrated that the MCA does not change in diameter during changes in $PaCO_2$, measured via a 1.5 Tesla MRI in healthy humans (Serrador et al. 2000). Likely, due to the small sample size ($n = 6$) and insufficient imaging resolution, these findings have since been challenged in that the MCA has been demonstrated to change in diameter in response to alterations on $PaCO_2/P_{ET}CO_2$ (Coverdale et al. 2014, 2015, 2017; Verbree et al. 2014; see Fig. 7a), and importantly, in the context of the current review, during handgrip exercise, but only a ~ 1% reduction in MCA diameter was reported (Verbree et al. 2017; see Fig. 7b), which probably won't challenge the interpretation of most handgrip exercise studies. However, cerebral vessel diameter changes in response to different modalities of exercise should be explored in future work. Importantly, these experiments were all conducted using higher resolution MRI (i.e. 3 and 7 T), which in future studies, could be used in concert with TCD and duplex ultrasound to dynamically model and map blood flow throughout the entirety of the brain under a variety of conditions.

Perspectives and future directions

A wide range of methodological techniques are currently employed to measure CBF at rest and during exercise—each of these having its advantages and disadvantages (see Table 1). The use of the direct Fick principle is still a powerful method to measure CBF, oxygen delivery, and CMR, especially when paired with ultrasound (Smith et al. 2014; Trangmar et al. 2014, 2015). However, the requirement of invasive measurements and steady-state assessments demands an advanced level of training and support staff to ensure participant safety, and reliable data. Over a wide range of studies, TCD has proven to have excellent utility in indexing dynamic CBF, especially during exercise, and has the distinct advantage to allow for the continuous

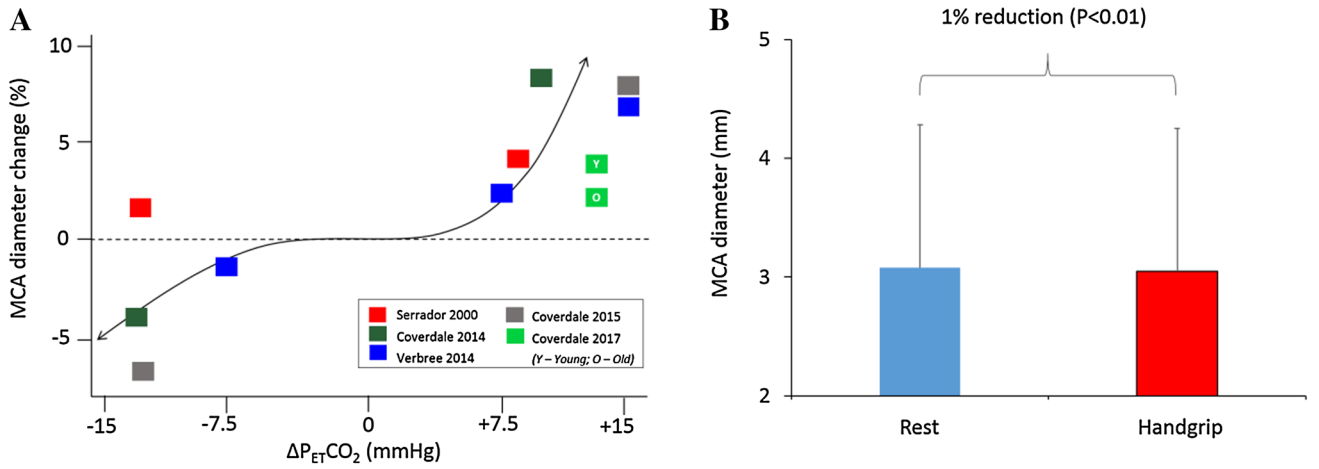

Fig. 7 Panel **A** represents a summary of the studies that investigated the effects of changes in $P_{ET}CO_2$ on MCA diameter measured using MRI. Panel **B** illustrates the findings highlighted by Verbree et al. (2017), which demonstrated that MCA diameter is reduced by a ~ 1% during rhythmic handgrip exercise. The study by Verbree et al. (2017) reports cross sectional area of the MCA, based on these data reported in their manuscript MCA diameter was calculated

Table 1 Comparison of methodologies to measure the effects of exercise on CBF

Technique	Reliability	Temporal resolution	Strengths	Weaknesses
Kety-Schmidt	Good	Poor	Robust measurement technique	Only measures gCBF, and requires highly trained personnel
TCD ultrasound	Fair	Excellent	Easy to use, and the probe can be fixed into position	Assumes that blood vessel diameter does not change
Duplex ultrasound	Fair	Excellent	Measures both CBV and blood vessel diameter	Reliability is largely based on sonography experience
MRI or PET	Excellent	Poor	High-quality images	Unable to take images during exercise due to movement

measurement of beat-by-beat CBV with the ultrasound probe locked in a stable position. However, recent data indicates that TCD as a standalone index of CBF during exercise may not be a valid tool due to the potential for changes in the diameter of cerebral arteries (Coverdale et al. 2014, 2015, 2017; Verbree et al. 2014, 2017), and its inability to quantify shear stress (due to no measurement of vessel diameter), which has been recently linked to cerebrovascular regulation during exercise (Smith and Ainslie 2017). Due to these reasons, Duplex ultrasound may be a more useful methodology in the context of exercise because it measures blood vessel diameter and velocity, therefore, it provides a reliable continuous measurement of CBF and shear stress. However, there are several limitations when employing Duplex ultrasound. For example, to obtain reliable, accurate, and reproducible dynamic cerebrovascular images, a considerable amount of high quality training is required (> 100 hrs of practice), as well as a state of the art edge-detection software to capture and quantify beat-to-beat diameter, velocity, blood flow and shear stress in cerebral arteries (Woodman et al. 2001). An important consideration with Duplex ultrasound is that there are anatomical differences between individuals that can hinder ultrasound image quality (e.g. an ICA located underneath the jaw line). In contrast to Duplex ultrasound, MRI provides high temporal resolution at rest; however, high quality MRI images are unobtainable during exercise due to participant movement. Additionally, MRI is expensive to employ and most research groups often have limited access. These two factors greatly reduce the accessibility of MRI as a tool to assess the effects of exercise on CBF and brain structure. Collectively, future studies should aim to incorporate multiple methodologies that support excellent image quality in combination with techniques that enable dynamic cerebrovascular function assessment (i.e. MRI with TCD and/or Duplex ultrasound). It is necessary to implement such studies, to gain a better understanding of the factors that regulate CBF during exercise, and to determine the extent to which these factors contribute or trigger the structural and cognitive adaptations associated with exercise training and life-long physical activity (Colcombe et al. 2006). A summary of the key seminal work on CBF and exercise is provided in Table 2.

Table 2 Summary of seminal work

Publication	Rest/exercise	Technique	Significance
Mosso (1880)	Rest	"Mosso Method"	First known recordings of neurovascular coupling in man
Kety and Schmidt (1945)	Rest	Fick principle	First accurate measurement of gCBF in man
Kleinermen and Sokoloff (1953)	Rest and Exercise	Fick principle	One of the first documents of the effects of exercise on gCBF
Aaslid et al. (1982)	Rest	TCD	Established TCD as a method to measure CBV as a surrogate for CBF
Jorgensen et al. (1992a, b)	Rest and Exercise	TCD	One of the first documents of using TCD to measure CBV during dynamic exercise in humans
Sato et al. (2011)	Rest and Exercise	TCD and Duplex ultrasound	Used both TCD and Duplex ultrasound to measure CBF during exercise
Verbree et al. (2017)	Rest and Exercise	MRI	Determined that MCA diameter is altered with handgrip exercise indicating that TCD is unreliable during exercise

Summary

Measurements of CBF has significantly advanced since the nineteenth century, which has expanded our understanding of cerebrovascular function in humans—from the early work of rudimentary human and comparative physiology, and through a period of invasive, and non-invasive experimentation aimed towards the quantification of gCBF and regional CBF in exercising humans. Due to the development of these techniques, researchers have identified multiple factors that are involved in cerebrovascular regulation at rest, and during exercise. Additionally, these studies have provided evidence that exercise induces structural and functional changes in the brain, which have been linked to cognitive function and cerebrovascular disease (Glodzik et al. 2013; Gupta et al. 2012). Future studies combining multiple imaging techniques (i.e. MRI, PET, Duplex ultrasound, and TCD) will help identify to what extent that these individual factors influence cerebrovascular adaptation and dysfunction in health and disease.

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Compliance with ethical standards

Conflict of interest The author(s) declare that they have no conflict of interest.

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