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## **Systemic and cerebral hemodynamics in response to cardiovascular challenges : the heart-brain connection**

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# CHAPTER

# 6

## **Middle cerebral artery diameter changes during rhythmic handgrip exercise in humans**

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## ABSTRACT

Transcranial Doppler sonography (TCD) is a frequently employed technique for quantifying cerebral blood flow by assuming a constant arterial diameter. Given that exercise increases arterial pressure by sympathetic activation, we hypothesized that exercise might induce a change in the diameter of large cerebral arteries. Middle cerebral artery (MCA) cross-sectional area was assessed in response to handgrip exercise by direct MRI observations. 20 healthy subjects (11 female) performed three 5 minutes bouts of rhythmic handgrip exercise at 60% maximum voluntary contraction, alternated with 5 minutes of rest. High-resolution 7-Tesla MRI scans were acquired perpendicular to the MCA. Two blinded observers manually determined the MCA cross-sectional area. Sufficient image quality was obtained in 101 MCA-scans of 19 subjects (age-range 20–59 years). Mixed Effects modeling showed that the MCA cross-sectional area decreased by  $2.1 \pm 0.8\%$  ( $p = 0.01$ ) during handgrip, while the heart rate increased by  $11 \pm 2\%$  ( $p < 0.001$ ) at constant end-tidal CO<sub>2</sub> ( $p = 0.10$ ). In conclusion, the present study showed a 2% decrease in MCA cross-sectional area during rhythmic handgrip exercise. This further strengthens the current concept of sympathetic control of large cerebral arteries, showing in-vivo vasoconstriction during exercise-induced sympathetic activation. Moreover, care must be taken when interpreting TCD exercise studies as diameter constancy cannot be assumed.

### Keywords

Transcranial Doppler; Exercise; Cerebral Blood Flow; MRI; MR Angiography; Cerebral Blood Flow Measurement.

## INTRODUCTION

The regulation of cerebral blood flow (CBF) is critical for the maintenance of oxygen and nutrient supply to the metabolically active brain. CBF control is multifactorial, influenced largely by the partial pressure of arterial carbon dioxide, mean arterial pressure and cerebral metabolism [1]. Despite the abundance of sympathetic nerve fibers emanating from the cervical and stellate ganglia that innervate the cerebral arteries, the role of the autonomic nervous system in the control of cerebral blood flow (CBF) in humans has remained contentious for decades [2-10].

In peripheral circulations arterioles rather than large arteries are the main site of vascular resistance. However, for the brain, large vessels contribute ~50% to vascular resistance [11]. This noticeable contribution of large arteries in vascular resistance is most likely of importance in defending the cerebral microcirculation against surges in blood pressure by limiting fluctuations in microcirculatory pressure and cerebral perfusion [12-14]. Several observations have been put forward as an argument for sympathetic influence on the human cerebral vasculature [6,15]. At first, blocking sympathetic outflow to the human cerebral vasculature increases CBF, suggesting a sympathetically mediated restraint of cerebrovascular perfusion [15]. Second, systemic agents that impair sympathetic signaling (alpha-adrenergic) reduce measures of cerebral pressure autoregulation [15,16]. Finally, in healthy subjects, spillover of sympathetic neurotransmitters in the jugular vein is increased during sympathetic activation independently from neurotransmitters released in the brain [17]. These observations suggest that the cerebral vasculature is under sympathetic control, leaving the challenge to identify changes in human brain artery diameter during sympathetic activation, especially when these would impact interpretation of physiological measurements, such as Transcranial Doppler (TCD).

In humans, changes in CBF are commonly evaluated non-invasively by measuring the CBF-velocity (CBFv) by transcranial Doppler sonography (TCD) in the large cerebral arteries with real-time resolution [18]. A limitation of the majority of studies is that a constant diameter of the insonated brain artery is assumed to directly link changes in CBFv to changes in regional CBF. This assumption of constancy of brain artery diameter has been evidenced to be violated by high levels of PaCO<sub>2</sub> [19,20] and pressure pulsatility [21,22], but it is as yet unknown whether the assumed constancy of brain artery diameter holds true during exercise. Given that functional activation increases CBF and that sympathetic activation and arterial pressure increase with exercise, we hypothesized that exercise affects the diameter of large cerebral arteries. To that purpose we set out to quantify the middle cerebral artery (MCA) cross-sectional area in rest and in response to rhythmic handgrip exercise using high resolution 7 Tesla MRI.

## MATERIALS AND METHODS

### Subjects

Twenty healthy, non-smoking, non-diabetic, right handed subjects (9 males, 11 females) without history of cardiovascular disease underwent MRI during rhythmic handgrip exercise. No information about the active or chronic use of vasoactive medication or painkillers was obtained. One subject used a beta-adrenergic blocking agent for mild hypertension which was considered not to have an effect on the cardiovascular response to handgrip exercise [23]. Written informed consent was obtained from all participants prior to examination. The protocol was performed in accordance with the Helsinki protocol, as approved by the institutional Medical Ethics Committee of the Leiden University Medical Center.

### Measurements

MRI measurements were performed on a 7 Tesla Philips MRI system. Image acquisition parameters have been described previously [24] and are listed in Table 6.1. The MCA contralateral to the exercise hand was located on an orthogonally reconstructed axial Time-Of-Flight angiogram. Additional sagittal reconstruction was created along the course of the MCA to aid the planning procedure. Subsequently, the high resolution 2D scan based on the black-blood approach and with a voxel-size of  $0.2 \times 0.2 \times 5 \text{ mm}^3$  was positioned perpendicular to the MCA. This scan will be referred to as “MCA-scan” for the remainder of this article. Care was taken to select a straight part of the proximal MCA and exclude small branches whenever possible. Image blurring due to variations in vessel lumen across the cardiac cycle was minimized by prospectively triggering the acquisition to the subject’s heart beat using the finger pulse-oximetry unit of the scanner [22]. Acquisition duration was 2.5 to 3.5 minutes, depending on the heart rate (HR) of the subject. End-tidal  $\text{CO}_2$  (Pet $\text{CO}_2$ ) was continuously monitored via a nasal cannula connected to a capnograph (Capnomac Ultima, Datex), located in the control room adjacent to the scanner. Heart rate (HR) was obtained from the finger pulse-oximetry unit of the MRI scanner. After 10–15 minutes of preparatory scans, a brachial blood pressure measurement was performed on the right arm (Invivo Magnitude, Orlando, FL).

### Protocol

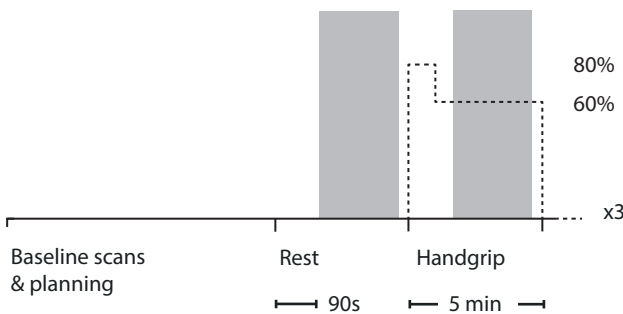
A graphical representation of the protocol is illustrated in Figure 6.1. Exercise consisted of 5 minutes of dynamic handgripping, i.e. repeated 2 sec hand contractions alternated with 2 sec of relaxation. Prior to scanning the subjects were familiarized with the protocol and handgrip-device (Gripforce 500N, Curdes, Philadelphia PA, USA). Maximum voluntary contraction (MVC) of the dominant hand was assessed by taking the maximum of 3 attempts. To achieve and maintain a steady state, rhythmic handgrip started the first minute at 80% MVC to be directly followed by 60% MVC for 4 minutes. Pilot-studies proofed that this approach results in a robust and steady response for the complete duration of the exercise bout. Graphical representations of the imposed exercise rhythm as well as the force applied by the volunteer were displayed onto a screen in the scanner to provide visual feedback to the subject. The exercise protocol started with 5 minutes of

rest followed by 5 minutes of handgrip. This was repeated three times to facilitate the detection of small differences in cross-sectional area. Acquisition of the MCA-scan was started 90 seconds after start of the rest or exercise bout. A total of 6 MCA scans (3 at rest and 3 during exercise) were obtained per subject.

**Table 6.1** | MRI scan parameters.

	Time-of-Flight Angiogram	Black blood T2-weighted (MCA-scan)
Planning orientation	3D acquisition covering the circle of Willis	Single slice, perpendicular to the MCA
Scan Technique	Fast Field Echo	Spin Echo
Acceleration type	none	Turbo spin echo; factor 12 + 4 dummy
Repetition time/echo time	12.6 / 3.7 ms	2 heart beats / 86 ms
Flip/ Refocusing angle	20° / –	110° / 105°
Field of view	180 x 170 x 40 mm <sup>3</sup>	240 x 180 x 5 mm <sup>3</sup>
Acquisition matrix	544 x 540	1200 x 900
Scan duration	4 min	2.5–3.5 min, depending on heart rate
Additional parameters	–	Trigger delay 50ms

The time-of-Flight angiogram was used to locate the MCA and additional reconstructions ensured perpendicular planning of the high-resolution black blood T2 MCA-scan. The MCA-scan was used to determine the cross-sectional area of the MCA.



**Figure 6.1** | Graphical representation of the exercise protocol. Relative timing of the MCA-scan is indicated by the gray area and the relative force level by a dashed line.

## Data processing

The MCA-scans were anonymized and viewed in proprietary Philips viewing software. Two observers blinded to subject-number and condition (JV, AGTB) manually drew elliptical regions of interests to determine the MCA cross-sectional area twice. Quality of the MCA-scans was visually assessed, and scans with visible motion artefacts or a badly discernable vessel wall were

excluded from analysis. All PetCO<sub>2</sub> recordings and HR data were visually inspected and artifacts were manually removed. Values during rest and exercise were averaged per subject. Relative (percentage) change was calculated by normalizing with respect to the resting condition: relative = 100% · (handgrip–rest) / rest.

## Statistics

The Intraclass Correlation Coefficient assessed the consistency within- and between observers (ICC C-k, SPSS v23). The effect of handgrip was assessed with a Mixed-effect model (Statistics Toolbox, Matlab v2015b) taking the percentage change in vessel cross-sectional area, HR and PetCO<sub>2</sub> as dependent variable. A fixed intercept with subjects as random intercept was used to model the change in cross-sectional area. The possible effects of time, age, and gender and change in PetCO<sub>2</sub> on the cross-sectional area change were investigated in separate models, using a fixed effect analysis. Normality of the residuals was assessed using Lilliefors test. Based on a sample-variance of 8.19±1.32 mm<sup>2</sup> as measured during normocapnia in a previous study [19], the a-priori power was set to 0.9 to detect a difference of 5% in cross-sectional area resulting in a sample size of n = 20. Significance threshold was set at 0.05. Values are mean±SE except when stated otherwise.

## RESULTS

Sufficient image quality was achieved in 101 of 120 scans, such that 19 subjects entered final analysis. The median (range) age was 30 (20–59) years and resting systolic- and diastolic arterial pressures were 112±9 and 69±10 mmHg (mean±SD) respectively.

During exercise HR increased 11.2±1.7% (p<0.001), without change in PetCO<sub>2</sub> (-0.9±0.5%, p<0.10, see Table 6.2).

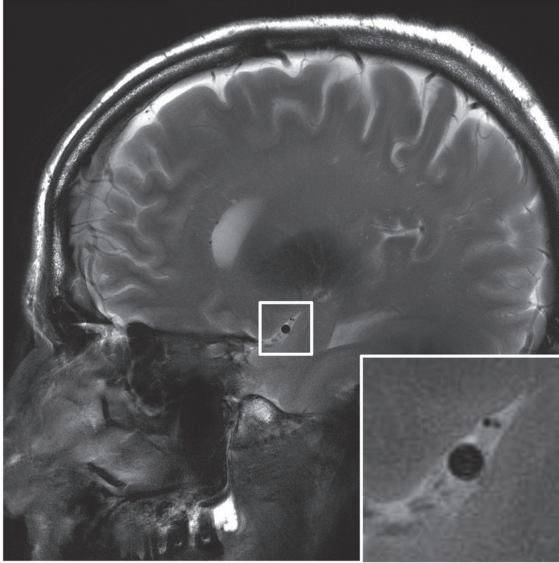
**Table 6.2** | Average response to rhythmic handgrip exercise.

	Rest (mean ± SD)	Handgrip (mean ± SD)	Effect Estimate (mean ± SE)	
Area (mm <sup>2</sup> )	7.47±1.18	7.31±1.15	-2.1±0.81%	p = 0.01
HR (bpm)	62.0±12.5	68.4±12.5	11.2±1.7%	p<0.001
PetCO <sub>2</sub> (kPa)	5.06±0.36	5.02±0.36	-0.9±0.5%	p = 0.10

Fixed effect estimates determined by corresponding Mixed Effects model. Area: cross-sectional area of the middle cerebral artery; HR: heart rate; PetCO<sub>2</sub>: end-tidal carbon dioxide.



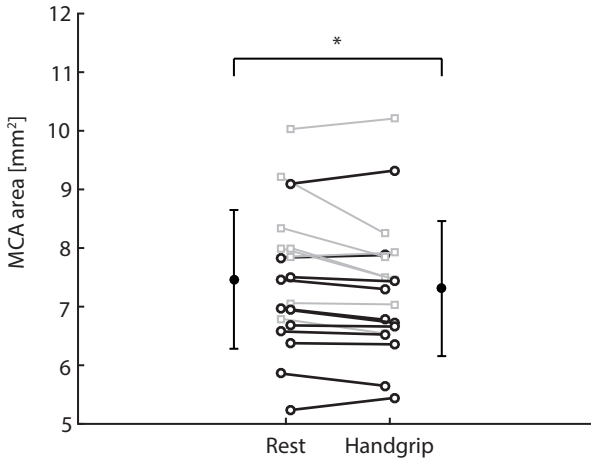
Both the consistency of MCA area measurements within- (ICC A-k = 0.978 and 0.977 for JV and AGTB respectively) and between observers (ICC C-k = 0.981) were considered sufficient to accept the reliability of the average MCA cross-sectional area measurement. A representative example of the image quality of the MCA-scan is depicted in Figure 6.2.



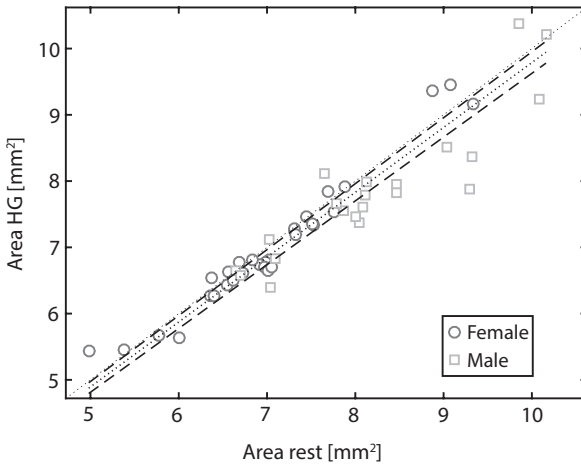
**Figure 6.2** | Example of a high resolution cardiac triggered MRI scan of the MCA; based on the black blood principle. The white box indicates the zoomed area showing the lumen of the MCA.

The mean cross-sectional area during rest was  $7.47 \pm 1.18 \text{ mm}^2$  (diameter of 3.1 mm), which decreased during handgrip exercise by  $0.16 \text{ mm}^2$  to  $7.31 \pm 1.15 \text{ mm}^2$ , see Figure 6.3. The change in cross-sectional area was  $-2.11 \pm 0.81\%$  (95% confidence interval: -3.74%, -0.49%,  $p = 0.01$ ), see Figure 6.4, corresponding to a decrease in diameter of  $1.1 \pm 0.4\%$  (0.033 mm). Repeated exercise challenges (time-effect) did not have an effect on this relation ( $p = 0.07$ ).

The age of the participants was strongly clustered into two groups. Therefore, the effect of age was assessed by splitting the population into a group below- (3 males, 8 females) and above 40 years of age (5 males, 3 females). There was no relation between age and the change in cross-sectional area in response to exercise ( $p = 0.76$ ). The change in  $\text{PetCO}_2$  did not have a relation to the change in cross-sectional area ( $p = 0.51$ ). There was a larger reduction in cross-sectional area in males compared to females ( $\beta_{\text{male}} = -3.46 \pm 1.47$ ,  $p = 0.02$ ).



**Figure 6.3** | Effect of dynamic handgrip exercise on the average MCA cross-sectional area. Black error-bars indicate mean and standard deviation. Squares represent males and circles females. Corresponding measurements of a subject are connected by a line. \*  $p < 0.05$  rest vs. handgrip.



**Figure 6.4** | Scatterplot of the cross-sectional area during rest and handgrip. All 57 data pairs are plotted for male (square) and female (circle) subjects. The Mixed Model Estimate is indicated by the dotted line with 95% confidence intervals (dashed lines). The line of identify (dotted line) is added for visual reference.

## DISCUSSION

The main finding of the present study is that a 2% reduction in the contralateral MCA cross-sectional area in response to rhythmic handgrip. This suggests that vasoconstriction occurs in a large brain artery during exercise induced sympathetic activation. This falsifies the hitherto assumed constancy of cerebral artery diameter.

Regarding the evaluation of cerebral perfusion with TCD, the concern has been raised whether the diameter of the basal cerebral arteries remains constant during sympathetic activation, specifically exercise [8,9,25]. To our knowledge this is the first study to directly observe a MCA lumen change during exercise with high resolution MRI. To date a single study estimated the change in MCA cross-sectional area specifically during rhythmic handgrip [8]. Their estimate of 21% change was indirect, i.e. by comparing the regular TCD signal with the spectrally averaged Doppler signal, and therefore not easily comparable with the present findings. A study, assessing the effect of sympathetic activation on arterial diameter did not find a change in a small peripheral artery of the foot as assessed with ultrasound [7]. Moreover, direct MRI observations of the MCA during sympathetic stimulation by lower body negative pressure [26] also did not find a diameter change. The absence of observable changes in these studies suggest that, the magnitude of any changes can be considered minor. Moreover, changes in MCA cross-sectional during CO<sub>2</sub> inhalation and pressure pulsatility can also be considered small [19,20,22]. This is in line with the current finding of 2% vasoconstriction, indicate that MCA cross-sectional area changes are small when induced by sympathetic activation by moderate exercise.

The extent in MCA diameter response to exercise and sympathetic activation could differ depending on factors such as age and gender. Findings of the present study suggest a larger change in MCA cross-sectional area in males compared to females in response to rhythmic handgrip. This finding coincides with the observation that premenopausal females exhibit lower sympathetic outflow in response to static handgrip exercise [27] and lower cerebrovascular reactivity [28,29] as a marker for brain vessel dilatatory capacity. Contrary, with age, no relation with the degree of MCA vasoconstriction was observed in present study. This is in line with preserved sympathetic response to autonomic challenges [30,31] and preserved carbon dioxide cerebrovascular reactivity [32,33] in aging. We consider that the limited sample size of the gender and age groups precludes proper inference of possible differences.

Small changes in vessel lumen can have profound influence on the estimation of CBF by relative velocity measurements. A decrease in vessel lumen proportionally increases the flow velocity even at constant blood flow. However, with exercise both the lumen and blood flow are changing. For example, during handgrip the CBF<sub>v</sub> increases from 50 cm/s to 55 cm/s, corresponding to an apparent 10% increase in CBF. However, a concurrent decrease in cross-sectional area of 2.1% reduces the underlying CBF change to 7.9% rather than the 10%, as would be estimated from the velocity only. Moreover, the variation in the cross-sectional area change ranged (mean±STD)

from  $-10.4 \pm 4.7\%$  (subject 3, male,  $n = 3$ ) to  $4.3 \pm 5.3\%$  (subject 16, female,  $n = 2$ ), respectively overestimating and underestimating the underlying CBF response to handgrip exercise by 10.5% and by 5.3% in these subjects. Given a measured 10% change in velocity, the corresponding change in CBF, corrected for the change in lumen area of these subjects, would be +0.5% and +15.3%, respectively. While the change in lumen seems slight, it constitutes a sizable part of the TCD determined cerebral hemodynamic response during handgrip exercise.

Measuring small changes in vessel lumen requires high precision measurements. The acquisition resolution of the employed MCA-scan was  $0.2 \times 0.2 \text{ mm}^2$ , therefore the average change in MCA cross-sectional area of  $0.16 \text{ mm}^2$  constitutes  $\sim 4$  pixels, providing for sufficient statistical power in the current study to infer these changes. The determination of the cross-sectional area can be dependent on the effective contrast of the acquisition as well as on the observer accuracy. Reliability of the cross-sectional area assessments was augmented by excluding scans with poorly discernable MCA vessel wall (e.g. subject motion), as well as by averaging the observations of two observers, who were blinded to participant number and condition.

High resolution scanning was complemented with cardiac triggering that stabilized the imaging by timing the acquisition with respect to the cardiac cycle. Although this approach prolongs scan time, it mitigates blurring effects and imaging artifacts. In addition, the sensitivity of the MCA-scan to variations in blood flow has been minimized by the application of dummy echoes, thereby creating a clear signal void within the vessel lumen [19]. Although some dependency of the intravascular signal on the blood flow velocity may persist, an increase in flow velocity near the vessel wall would make the lumen appear larger rather than smaller. This effect can therefore not explain the observed decrease in cross-sectional area as found in the current study, it might only have resulted in an underestimation of the actual decrease in MCA cross-sectional area.

A potential limitation of the present study is the application of concurrent visual feedback to maintain the desired exercise level. Stimulation of the visual cortex by the visual feedback is associated regional increases in CBF [34]. However, the visual cortex is supplied by the posterior cerebral circulation rather than by the MCA, rendering an effect of visual stimulation on the MCA cross-sectional area negligible. Another limitation of the present study is that the level of sympathetic activation was not directly determined. This could have contributed to the large between-subject variation. However, the exercise protocol was setup to provide a consistent response within- and between-subjects, by scaling the prescribed force to the individually maximal voluntary contraction. Currently no data exist how the level of (rhythmic handgrip) exercise impacts sympathetic activation, and we can therefore not exclude an effect of the level of sympathetic activation on the large between-subject variation. In the present study arterial pressure was not monitored during exercise, as brachial pressure measurements would occlude either the handgripping arm or disrupt blood flow in the finger used for triggering the MCA-scan. However, it has been well documented that the arterial pressure increases in response to rhythmic handgrip exercise [35,36]. This increase in pressure is required to provide the work to counteract

the higher vascular resistance and actually increase the blood flow velocity. Without an increase in pressure the blood flow would decrease with a constant flow velocity. Therefore, given that the arterial pressure increases during handgrip exercise, then the observed vasoconstriction in the MCA can be considered to modulate the blood flow velocity and lead to an overestimation when using TCD to assess CBF changes.

## CONCLUSION

In conclusion, the present study observed a 2% decrease in MCA cross-sectional area with MRI during rhythmic handgrip exercise in healthy subjects. This further strengthens the current concept of sympathetic control of large cerebral arteries, showing in-vivo vasoconstriction during exercise induced sympathetic activation. Finally, the assumption of constancy vessel diameter is falsified. Therefore, care must be taken when interpreting TCD measurements of the cerebral circulation in exercise studies.

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