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Non-invasive MRI Measurement of the Absolute CBV-CBF Relationship during Visual Stimulation in Healthy Humans

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Abstract

Purpose—The relationship between cerebral blood volume (CBV) and cerebral blood flow (CBF) underlies blood oxygenation level dependent functional MRI signal. This study investigates the potential for improved characterization of the CBV-CBF relationship in humans, and examines gender effects as well as spatial variations in the CBV-CBF relationship.

Methods—Healthy subjects were imaged non-invasively at rest and during visual stimulation, constituting the first MRI measurement of the absolute CBV-CBF relationship in humans with complete coverage of the functional areas of interest.

Results—CBV and CBF estimates were consistent with literature, and their relationship varied both spatially and with gender. In a region of interest (ROI) with stimulus induced activation in CBV and CBF at the $P < 0.05$ significance level, a power function fit resulted in $CBV = 2.1 CBF^{0.32}$ across all subjects, $CBV = 0.8 CBF^{0.51}$ in females and $CBV = 4.4 CBF^{0.15}$ in males. Exponents decreased in both genders as ROIs were expanded to include less significantly activated regions.

Conclusion—Consideration for potential gender related differences, as well as regional variations under a range of physiological states, may reconcile some of the variation across literature and advance our understanding of the underlying cerebrovascular physiology.

Keywords

Cerebral blood volume; Cerebral Blood Flow; Brain activation; Blood oxygenation level dependent; Functional MRI; Grubb's Relationship; Visual Stimulation

Introduction

Blood oxygenation level dependent (BOLD) functional MRI (fMRI) is primarily sensitive to changes in deoxyhemoglobin concentration with activation; and calibrated fMRI aims to dissociate changes in $CMRO_2$, the cerebral metabolic rate of oxygen, from changes in cerebral blood volume (CBV) and cerebral blood flow (CBF) (1,2). In lieu of CBV measurements, many studies have assumed that CBV is related to CBF^α (1,3–7), with $\alpha=0.38$ based on the absolute CBV-CBF relationship obtained by Grubb et al. in macaque brains under hypo and hypercapnia using PET (8), although, α may differ across functional challenges, brain regions and species (2,8–12).

The CBV-CBF relationship has been studied extensively in rats under anesthesia, and published exponents range from 0.18 to 0.64 across respiratory manipulations and functional activation, with spatial and temporal variations (11–16). Few results are available in humans: exponents of 0.29 and 0.64 during respiratory manipulation (17,18), and 0.3 during functional activation (10,19) were reported using PET measurements of absolute total CBV and CBF. Changes in arterial, capillary and venous compartments impact BOLD differently as well. Venous CBV is expected to have the largest impact on BOLD due to large oxygenation changes on the venous side (20). Optical measurements in animals indicate a complicated mechanism involving small venous CBV changes (21) as also supported by MRI in animals (14,22) and humans (23). Oxygenation changes have even been shown on the arterial side, accompanying large fractional volume changes (21,24). Significant capillary CBV changes have also been suggested (21,25–27), given that capillaries are the major source of oxygen extraction and closer to the activation site (27). Smaller exponents of the CBV-CBF relationship, of 0.18 during respiratory manipulation (28) and 0.23 during functional activation (23), were reported based on MRI measurements of relative contributions of venous blood to CBV. Although underlying hemodynamic parameters have been shown to vary (29–38), potential variations in the CBV-CBF relationship, spatially, with gender, and age for example have not yet been considered.

This study presents the first MRI measurement of the absolute CBV-CBF relationship in humans with complete multi-slice coverage of the functional areas of interest. Healthy subjects were non-invasively imaged at rest and during visual stimulation. Absolute total CBV and CBF were quantified in the steady-state using an Inversion Recovery (IR) based method with extended slice coverage (39), and Arterial Spin Labeling (ASL), respectively. Grubb et al. and a number of PET studies have reported exponents based on absolute total CBV measurements (8,10,17–19). The current non-invasive method with sensitivity to total CBV may not only enable more direct comparisons, but may also complement existing arterial or venous CBV weighted methods to improve our understanding of compartmental changes in different brain regions, stimulus types and durations in humans. Improved characterization of the CBV-CBF relationship in humans under various metabolic or functional challenges and conditions may have significant implications for fMRI calibration; advance physiological interpretation of BOLD and our understanding of the fundamental physiological relationship between neuronal activity, hemodynamic regulation and metabolism.

Methods

CBV Measurement

Absolute CBV was quantified non-invasively using a previously described method (39), as summarized below. A novel multi-slice IR-EPI pulse sequence (Figure 1a (39)) with varying contrast weightings and an efficient rotating slice acquisition order was used to acquire data at rest and during visual activation. Non-selective inversion is followed by the acquisition of multiple slices at inversion time TI. The slice acquisition order is shifted over successive TRs until each slice is acquired at each TI. Spoilers and a global saturation pulse ensure steady-state by enforcing identical recovery durations for all slices. For this multi-slice inversion-recovery experiment with inversion time TI (s), repetition time TR (s), an additional saturation pulse at time TS (saturation time, s) and longitudinal relaxation time constant T_1 (s), longitudinal magnetization M_z at time TI is:

$$M_z(TI^-) = M_0(1 - 2\exp(-TI/T_1) + \exp(-(TR - TS + TI)/T_1)) \quad \text{Eq. [1]}$$

where M_0 is the equilibrium magnetization (A/m) established by the external magnetic field B_0 (T). The signal vs. TI curve differs between rest and activation conditions as summarized below.

In a voxel containing CSF and brain parenchyma, where CBV is the blood fraction in brain parenchyma, MR signal magnitude includes the following contributions:

$$S = K \cdot \text{abs}(S_{CSF} + S_{OBV} + S_{DBV} + S_{TISSUE}) \quad \text{Eq. [2]}$$

where K is a calibration factor accounting for equilibrium magnetization with transmit and receive sensitivity effects assuming uniform coil profiles and M_0 (a.u.), OBV and DBV are the oxygenated and deoxygenated blood volumes, respectively. Each compartment contributes signal according to its volume fraction F in the voxel (dimensionless), water proton density C (dimensionless), effective transverse relaxation time constant T_2^* (s), and longitudinal magnetization, M_z , at the time of excitation including longitudinal relaxation effects. Signal at inversion time TI is:

$$S_i(TI) = F_i \cdot C_i \cdot M_{z,i}(TI^-) \cdot \exp(-TE/T_{2,i}^*) \quad \text{Eq. [3]}$$

for $i = \text{CSF, OBV, and DBV}$, where TE is the echo time (s), with:

$$M_{z,i}(TI^-) = 1 - 2\exp(-TI/T_{1,i}) + \exp(-(TR - TS + TI)/T_{1,i}) \quad \text{Eq. [4]}$$

for $i = \text{CSF, OBV, DBV, and TISSUE}$. CSF fraction changes with activation were found to be negligible in the occipital cortex (40,41). This allows limiting the number of parameters in the current study, however, CSF may have larger effects in other brain areas (41,42) and it would be desirable to extend the model to consider such changes elsewhere (39). Extravascular tissue signal is modified by the influence of deoxygenated blood and its oxygenation level (43–45), as follows:

$$S_{TISSUE}(TI) = F_{TISSUE} \cdot C_{TISSUE} M_{z, TISSUE}(TI^-) \cdot \exp(-TE/T_{2, TISSUE} - F_{DBV} \cdot f(Y_{DBV}, Hct)) \quad \text{Eq. [5]}$$

$$f(Y_{DBV}, Hct) = \frac{1}{3} \int_0^1 (2+u) \sqrt{1-u} \left(\frac{1 - J_0(2\gamma \cdot B_0 \cdot \pi \cdot \Delta\chi \cdot TE \cdot Hct \cdot (1 - Y_{DBV}) \cdot u)}{u^2} \right) du \quad \text{Eq. [6]}$$

where $T_{2, TISSUE}$ is the transverse relaxation time constant (s), F_{DBV} is the fraction of DBV in the voxel (%), Y_{DBV} is the DBV oxygenation fraction (%), Hct is the microvascular hematocrit estimate (%), J_0 is the zero-order Bessel function, γ is the gyromagnetic ratio (42.576 MHz/T), $\Delta\chi$ is the susceptibility difference between fully oxygenated and deoxygenated blood (ppm).

T_1 of blood varies slightly with hematocrit and blood oxygenation, decreasing with increased hematocrit and reduced oxygenation (46,47), as follows:

$$T_{1, blood}(Y_b, Hct) = 1 / (a \cdot Hct + b \cdot Y_b + c \cdot Y_b \cdot Hct + d) \quad \text{Eq. [7]}$$

where Y_b is the average blood oxygenation fraction (%), $Y_b = 98\%$ for OBV, $Y_b = Y_{DBV}$ for DBV, $Y_b = (Y_{OBV} \cdot F_{OBV} + Y_{DBV} \cdot F_{DBV}) / (F_{OBV} + F_{DBV})$ for CBV, $a = 2.4084 \text{ (s}^{-1}\text{)}$, $b = 0.708 \text{ (s}^{-1}\text{)}$, $c = -1.9998 \text{ (s}^{-1}\text{)}$, $d = -0.2892 \text{ (s}^{-1}\text{)}$, based on interpolation of published results (46–48). For instance, the commonly used blood T_1 s of 1624–1627ms correspond to $Y_b = 81\%$ (average of measurements at arterial $Y_b = 92\%$ and venous $Y_b = 69\%$), and a T_1 of 1612ms corresponds to $Y_b = 77\%$, approximately, both at Hct of 42% (average of male and female macrovascular Hct). Considering a lower microvascular Hct of 37.4% (85% of macrovascular Hct), blood T_1 s of 1747ms and 1703ms correspond to an arterial oxygenation fraction of $Y_b = 98\%$ and venous $Y_b = 61\%$, respectively (48).

T_2^* of blood also varies with hematocrit and blood oxygenation, decreasing with increasing hematocrit and decreasing oxygenation (49,50). At 3T under physiological conditions (49):

$$T_{2, blood}^*(Y_b, Hct) = 1 / (a^*(Hct) + b^*(Hct) \cdot (1 - Y_b) + c^*(Hct) \cdot (1 - Y_b)^2) \quad \text{Eq. [8]}$$

where A^* , B^* , and C^* depend on hematocrit. For $Hct = 34\%$, (females, for 85% of macrovascular $Hct = 40\%$) $a^* = 16.1957 \text{ (s}^{-1}\text{)}$, $b^* = 36.5348 \text{ (s}^{-1}\text{)}$, $c^* = 91.3478 \text{ (s}^{-1}\text{)}$, and for $Hct = 38.25\%$ (males, for 85% of macrovascular $Hct = 45\%$) $a^* = 16.75 \text{ (s}^{-1}\text{)}$, $b^* = 37.625 \text{ (s}^{-1}\text{)}$, $c^* = 103.1 \text{ (s}^{-1}\text{)}$ based on interpolation of published results (48,49).

Functional challenges influence the voxel signal S through changes in compartment fractions F (Eq. [3] and [5]) as well as blood oxygenation Y_b (Eq. [3] and [5–8]). A slight curve shift occurs upon stimulation (19,51), and CBV:

$$CBV = (F_{OBV} + F_{DBV}) / (1 - F_{CSF}) \quad \text{Eq. [9]}$$

is estimated by fitting the fractional signal change between rest and activation, $(S_{act} - S_{rest}) / S_{rest}$, over multiple TI times.

CBF Measurement

Absolute CBF was quantified using Q2TIPS Pulsed ASL (PASL) imaging (52) at baseline and during stimulation. The EPISTAR (echo-planar imaging with signal targeting using alternating radio frequency) sequence was modified by adding a train of thin-slice saturation RF pulses, applied at a post-labeling delay inversion time after the inversion RF pulse, to control the bolus delivery and suppress intravascular signal from large vessels (52). Interleaved labeling and control images were acquired using gradient echo-planar imaging in an ascending order. A slab-selective hypersecant inversion RF pulse was applied to slabs inferior and superior to the imaging slab for ASL (inferior slab) and as control (superior slab) for magnetization transfer effects, respectively. A bipolar gradient was applied to the imaging slices to suppress signal contamination from labeled arterial water within large vessels.

MRI Experiments

Twelve healthy subjects provided written informed consent and participated in this Yale University Institutional Review Board approved, Health Insurance Portability and Accountability Act compliant study (mean age $\pm$ sd: 31.8 $\pm$ 6 years, 5 females). Previously acquired data (39) has been re-analyzed to evaluate regional and gender related variations in CBV, CBF, and the CBV-CBF relationship. Experiments were performed on a 3T whole body scanner (Tim Trio, Siemens Medical Systems, Erlangen Germany) with a 32-channel receive-only phased-array head coil and body coil transmission. 3D high-resolution (MPRAGE, 1mm isotropic, 176 $\times$ 202 $\times$ 179mm FOV, TR/TI/TE:1500ms/800ms/2.83ms) acquisition was followed by multi-slice 2D high-resolution (FLASH, 1mm in-plane, TR/TE: 300/3.69ms), T_1 mapping, PD-weighted, CBV and CBF sequences with the same slice prescription. Multi-slice prescriptions consisted of 20 transverse slices covering the whole brain including the calcarine fissure with 4mm slice thickness and 2mm gap. The CBV sequence parameters were: TE/TS/TR=11ms/1.2s/3s, gradient-echo EPI, 192 $\times$ 256mm FOV, 4 $\times$ 4mm in-plane. Images were acquired at the following 60 TI values: $TIs(n,s) = TI_{start} + (n-1) \times TI_{shift} + (s-1) \times TI_{gap}$, where $TI_{start}=400ms$, $TI_{shift}=13ms$, $TI_{gap}=38.51ms$ (acquisition duration for one slice), $n=1$ to 3, and $s=1$ to 20, covering the TI range of 400–1158ms with 13ms resolution using 3 sets of 20 TI values. For T_1 mapping, TI values covering the TI range of 120–2400ms were acquired in steps of 120ms with TR/TE/TS =6s/11ms/2.5s and 3 repetitions. The CBF parameters were: TR/TE/TI1/sliceTR: 3s/20ms/1.4s/52.3ms, post-labeling delays $TI(s) = 1400ms + 52.3ms \times (s-0.5)$ for slices $s=1$ to 20, 10cm adiabatic inversion of slabs 2cm inferior and superior to the imaging slab for labeling and control, a bipolar gradient of 5cm/s was applied to suppress signal contamination from labeled arterial water within large vessels. PD parameters were the same as the CBF parameters except TR/ TI/ TD: 8s/ 6.05s/ 0.

A visual stimulation paradigm consisting of a full-field black-and-white flashing checkerboard (frequency 10Hz) was presented on a back-projection screen viewed from a mirror mounted on the head coil. A block paradigm was generated in Eprime (Psychology Software Tools, Sharpsburg PA) with ON and OFF blocks of 78s duration each, where a generous 18s after each ON/OFF transition was allowed for settling of the hemodynamic response. CBV data were acquired on 12 subjects (5 female, 7 male) with three OFF/ON

cycles such that each CBV acquisition lasted 7min 48sec (6 blocks of 78 s), which was repeated for three sets of TI values each with three repetitions. CBF data were acquired on 10 subjects (5 female, 5 male) with four OFF/ON cycles such that each CBF acquisition lasted 10min 24sec (8 blocks of 78 s).

Data Processing

Time series images were grouped into volumes with the same contrast (same TI times), and motion corrected using SPM (Statistical Parametric Mapping, www.fil.ion.ucl.ac.uk/spm/). Motion correction involved registering all images via a six-parameter affine transformation initially to the middle image in each time series, finding the average of this registration, and then registering to this average. Linear drift correction was applied, and data were averaged over blocks and repetitions.

As customary, absolute CBF was calculated from the difference between interleaved labeled and control image pairs, averaged over multiple acquisitions (53–55). Hematocrit differences between males and females (8,56) were taken into account in CBF calculations by assuming a linear decrease in hematocrit from macro to microvasculature (from 45% in males and 40% in females to 85% of these values (8,56)) over five pre-capillary compartments of equal volume (57), leading to arterial blood T_1 of 1728 ms in females and 1673 ms in males.

Data were transformed to a common whole-brain template defined by the Montreal Neurological Institute (MNI) using BioimageSuite (www.bioimagesuite.org) using a combination of three transformations: linear transformations that co-register each subject's functional images (average over TI values) to the same subject's high-resolution 2D, then 3D images, and a non-linear transformation that co-registers these 3D images to the MNI brain. Tri-linear interpolation was employed for re-gridding and all subsequent analyses were performed in MNI space at 4×4×4mm resolution.

CBV was calculated following the procedure described in (39), by fitting the relative signal change between rest and activation, $(S_{act}-S_{rest})/S_{rest}$, to the biophysical model: CSF and tissue T_1 s were measured while previous publications and models provided estimates of the T_1 and T_2^* of blood, and T_2^* of tissue as functions of oxygenation and compartment fractions (Eq. [3] and [5–8], Table 1), enabling estimation of oxygenation during activation, CSF fractions, CBV at rest and during activation. CSF and tissue T_1 s for each subject were obtained from T_1 maps generated by least-squares fitting to signal over multiple TIs, leading to CSF T_1 of 4166 ± 0.377 ms (mean $\pm$ sd across subjects, vs. published values of 4300 ms (58) and 3700 ± 500 ms (59)), and occipital GM T_1 of 1267 ± 52 ms (mean $\pm$ sd across subjects, vs. published values of 1283 ± 37 ms to 1356 ± 26 ms (60) and 1122 ± 117 ms (58)). Microvascular hematocrit was assumed to be 85% of macrovascular hematocrit (8) of 45% in males and 40% in females (56). OBV was assumed to be nearly fully oxygenated ($Y_{OBV} = 98\%$), occupying 21% of CBV at rest (considering baseline CBV consisting of 21% arterial/arteriolar, 33% capillary, 46% venous contributions based on microvascular morphometry (57,61)). DBV consists of the remaining capillaries and venules at rest, with $Y_{capillary}=77\%$ and $Y_{venule}=61\%$ typically assumed at rest considering an exponential drop in oxygen saturation from arterioles to venules (49,62), such that $Y_{DBV,rest}=68.78\%$ (vs.

published values of $Y_{v,rest}=68.7\%$ (23) and $Y_{v,rest}=69\%$ (63)). No assumptions were made regarding OBV vs. DBV fractions, or Y_{DBV} , during activation. Parameters used in CBV fitting are listed in Table 1. Errors in the assumed parameters (i.e. of -10% to 10%) have been shown to result in a comparable range of errors in the fitted parameters: CBV estimates were most strongly influenced by errors in Hct (up to 15%) and resting oxygenation (up to 12%), while errors in T2 and T2* values had less influence on all estimates (up to 6%). Overestimation of CBV resulted from overestimation of Hct or underestimation of resting oxygenation. Over and underestimation of parameters had fairly symmetric effects on error, except for resting oxygenation whose overestimation resulted in slightly smaller errors in all parameters other than CSF fraction (39).

The CBV-CBF relationship was evaluated across nine region-of-interest (ROI) masks covering a range of sizes (Figure 1). Areas with significant activation in the primary visual cortex (Brodmann areas 17 and 18 defined on the MNI brain (64)) were identified using t-tests of task-induced changes between rest and activation in CBV and CBF, at four levels of significance ($P<0.01$, $P<0.05$, $P<0.1$, and $P<0.2$). ROI masks were generated from areas showing activation in both CBV and CBF (four intersections, ROI masks 1–4), from areas showing activation in CBV or CBF (four unions, ROI masks 5–8), and the Brodmann area definition (one anatomical, ROI mask 9). Mean CBV and CBF values were calculated within each ROI mask (voxels with $CBV>30$ mL/100 g were excluded as vessels). This resulted in one data point for each subject at baseline and activation states in each ROI. The CBV-CBF relationship was estimated by fitting to a power function ($CBV = a \cdot CBF^b$) as well as a linear function ($CBV = c + d \cdot CBF$). Matlab (Mathworks, Natick MA) functions for linear and nonlinear regression were used for fitting, student's t-tests (two-tailed) were used for comparisons (across rest vs. activation states, ROIs, ages and gender), analysis of covariance (ANCOVA, separate lines for each gender, no constraints) and multiple comparison tests were used for comparisons across genders over multiple ROIs.

Results

Stimulation resulted in bilateral activation in all subjects. Increases in CBV and CBF in the occipital cortex are shown in Figure 1 in a composite image across all subjects, along with the ROI masks used in the analyses. ROIs with highly significant activation were smaller as expected ($ROI1<ROI2<ROI3<ROI4$ & $ROI5<ROI6<ROI7<ROI8$). Intersection ROIs (ROIs 1–4), only including voxels activated in both methods were generally smaller; union ROIs (ROIs 5–8), also including voxels activated in only one of the methods were generally larger. Only the largest intersection ROI (ROI 4) was slightly larger than the smallest union ROI (ROI 5), resulting in an ascending size order of: ROI1, ROI2, ROI3, ROI5, ROI4, ROI6, ROI7, ROI8, ROI9. CBV and CBF values at rest and activation across all ROIs are provided in Table 2. Smaller ROIs were associated with larger CBV values and larger activation induced increases in CBV. The smallest CBV increase was observed in the largest ROI (ROI 9, anatomical mask) where CBV increased by 22.2%, from 5.4 mL/100 mL at rest to 6.7 mL/100 mL during activation. The largest CBV increase was observed in the smallest ROI (ROI 1, activation mask, $P<0.01$ significance level in both CBV and CBF) where CBV increased by 39.4%, from 7.9 mL/100 mL at rest to 11 mL/100 mL during activation. CSF fractions ranged from 6% to 18%, covering approximately 9% of ROIs on average and with

slightly larger CSF fractions in smaller ROIs (ranging from 7–18% in the smallest ROI, to 6–12% in the largest ROI). Smaller ROIs were also associated with larger increases in CBF, and CBF responses were higher than CBV responses overall. The smallest CBF increase was observed in the largest ROI (ROI 9, anatomical mask) where CBF increased by 23.1%, from 72.8 mL/min/100 mL at rest to 88.9 mL/min/100 mL during activation. The largest CBF increase was observed in the smallest ROI (ROI 1, activation mask, $P < 0.01$ significance level in both CBV and CBF) where CBF increased by 65.6%, from 72.9 mL/min/100 mL at rest to 120.7 mL/min/100 mL during activation. Within group standard deviations were smaller at rest (0.5 to 1 mL/100 mL in CBV; 22.9–24.5 mL/min/100 mL in CBF) than during activation (0.7–1.3 mL/100 mL in CBV; 26–35.7 mL/min/100 mL in CBF), and decreased with increasing ROI volume (1–1.3 mL/100 mL to 0.5–0.7 mL/100 mL in CBV; 24.5–35.7 to 22.9–26 mL/min/100 mL in CBF).

CBV and CBF at rest and during activation are also provided in Table 2 across all ROIs for each gender. Gender data generally showed the same trends as the combined data: Smaller ROIs were associated with larger CBV values and larger CBV increases. The smallest CBV increase was in the largest ROI (ROI 9, anatomical mask) where CBV increased by 24.6% in females (from 5.6 mL/100 mL to 7.0 mL/100 mL) and by 21.5% in males (from 5.2 mL/100 mL to 6.4 mL/100 mL). The largest CBV increase was in the smallest ROI (ROI 1) where CBV increased by 44.5% in females (from 8.1 mL/100 mL at rest to 11.6 mL/100 mL during activation) and by 35.1% in males (from 7.7 mL/100 mL at rest to 10.4 mL/100 mL during activation). Smaller ROIs were also associated with larger CBF increases. The smallest CBF increase was in the largest ROI (ROI 9) where CBF increased by 20% in females (from 86.9 mL/min/100 mL to 103.9 mL/min/100 mL) and by 27.2% in males (from 58.6 mL/min/100 mL to 73.9 mL/min/100 mL). The largest CBF increase was in the smallest ROI (ROI 1) where CBF increased by 60.1% in females (from 90.1 mL/min/100 mL to 143.9 mL/min/100 mL) and by 83.9% in males (from 55.6 mL/min/100 mL to 97.6 mL/min/100 mL). CBF increases were higher than CBV increases in all ROIs for all subjects, in all ROIs for males, and in smaller ROIs in females (except for larger ROIs in females, where CBV increases exceeded CBF increases).

CBV and CBF values at rest and during activation, and their activation induced increases were compared across genders, and ROIs, as summarized in Table 3. In all ROIs, CBV at rest, CBV during activation, CBV increases, CBF at rest and CBF during activation were higher in females than in males, whereas CBF increases were higher in males than in females. With this small cohort, differences between genders did not reach significance for CBV at rest ($P > 0.05$), with only a slight trend for differences in CBV during activation and CBV increases ($P \sim 0.2$). On the other hand, differences between genders were significant for CBF both at rest and during activation ($P < 0.05$), with only a slight trend for differences in CBF increases ($P \sim 0.2$). All possible combinations of ROIs were compared to evaluate differences between CBV and CBF values across ROIs. There were significant differences in CBV at rest and during activation ($P < 0.05$) in 97% of comparisons across ROIs. CBF during activation also varied significantly ($P < 0.05$) in 94% of comparisons across ROIs, while CBF at rest, CBV increases and CBF increases showed significant differences

($P < 0.05$) in 19%, 31% and 39% of comparisons across ROIs, respectively. CBV and CBF values across three sample ROIs are shown in Figure 2.

Based on these underlying differences, the CBV-CBF relationship was fitted to both a power function, $CBV = a CBF^b$, and a linear function, $CBV = c + d \cdot CBF$, in all subjects as well as in females and males separately, in each ROI. Fitting to a power function resulted in the expression $CBV = 2.1 CBF^{0.32}$ while fitting to a linear function resulted in the expression $CBV = 5.7 + 0.03 CBF$ in ROI 2, which corresponds to stimulus induced activation in CBV and CBF at the $P < 0.05$ significance level. Both power and linear fits showed spatial variation, with power exponents decreasing from 0.31 ± 0.08 to 0.14 ± 0.10 , and slopes decreasing from 0.031 ± 0.008 to 0.012 ± 0.007 , going from the smallest ROI to the largest ROI. Power and linear fitting results across all ROIs and subjects are depicted in Figure 3. Fitting results across all ROIs using all subjects, as well as male and female subjects separately are provided in Table 4.

Both power and linear function fits showed variations with gender, as well as across ROIs, consistent with the observed differences in the underlying hemodynamic parameters. Fitting to a power function resulted in the expression $CBV = 0.8 CBF^{0.51}$ in females vs. the expression $CBV = 4.4 CBF^{0.15}$ in males, in ROI 2 (stimulus induced activation in CBV and CBF at the $P < 0.05$ significance level). Fitting to a linear function resulted in the expression $CBV = 4.5 + 0.04 CBF$ in females vs. the expression $CBV = 7.2 + 0.014 CBF$ in males, in the same ROI. Both power and linear fits continued to show spatial variation within individual genders, with power exponents decreasing from 0.57 ± 0.18 to 0.17 ± 0.19 in females, and from 0.23 ± 0.13 to 0.00 ± 0.17 in males, going from the smallest ROI to the largest ROI. Slopes from linear fits decreased from 0.046 ± 0.01 to 0.013 ± 0.01 in females, and from 0.023 ± 0.02 to 0.002 ± 0.02 in males, across the same ROI range. Power fits to the CBF-CBV relationship across all ROIs, for female and male subjects are depicted in Figure 4.

The left side of Figure 5 depicts experimental data and power fits in a sample ROI (ROI 2, stimulus induced activation in CBV and CBF at the $P < 0.05$ significance level). Substantial inter-subject variations and small sample sizes resulted in large standard errors and wide confidence intervals (i.e. in ROI 2, exponents across all volunteers, females and males were 0.32, 0.51, and 0.15, with standard errors of 0.09, 0.17 and 0.16, and 90% confidence intervals of 0.17 to 0.47, 0.23 to 0.79 and -0.11 to 0.41, respectively). Despite these limitations, gender related variations in the CBV-CBF relationship were statistically significant when data over multiple ROIs was considered. Analysis of the covariance of the power exponents over multiple ROI sizes for females vs. males led to significantly different slopes ($P < 0.05$), intercepts ($P < 0.05$), and population marginal means ($P < 0.05$) in multiple comparison tests, as well as significantly different exponents in females vs. males after removal of trends across ROI sizes ($P < 0.05$). The right side of Figure 5 shows the CBV-CBF relationship power exponent estimates as a function of ROI size, for all subjects, for females and for males, demonstrating higher exponents in females and decreasing exponents with increasing ROI size.

Discussion

Absolute CBV and CBF were quantified non-invasively in healthy subjects at rest and during visual stimulation. CBV and CBF estimates agree well with the literature showing both spatial and gender related variations.

Average CBV across all subjects and ROIs was 6.6 ± 0.75 mL/100 mL (with a range of 5.4 ± 0.5 to 7.9 ± 1.0 mL/100 mL across ROIs) at rest. Resting GM arterial CBV (CBVa) was previously reported as 1.605 mL/100 mL (65) and 2.04 ± 0.27 to 0.76 ± 0.17 mL/100 mL (66) using iVASO. Considering 21% arterial/arteriolar contribution to baseline CBV (57,61), our resting CBV corresponds to CBVa of 1.386 mL/100 mL, well within the range of iVASO results. Values near the upper end of our range were reported in occipital cortical GM of 6.67 ± 1.07 mL/100 g of tissue (7mL/100 mL with a brain tissue density of 1.05 g/mL) using bolus tracking (67), as well as 7.8 ± 1.1 mL/100 mL in the visual area using PET (68,69). Values near the lower end of our range, of 5.0 ± 1.5 , 5.5 ± 0.2 and 5.6 ± 0.3 mL/100 mL were reported in (19,51,70), respectively (note that, in (51), one of the five subjects had a resting CBV of only 2.5 mL/100 mL while others averaged 5.7 mL/100 mL). Our observation of variations in resting CBV across ROIs is also in good agreement with the range of results from different approaches for ROI selection (anatomical descriptions, freehand tracings, circles, ellipses, activation based ROIs, T1 or SNR based thresholds (10,19,51,65,67–73) ...) and spatial heterogeneities seen in (17,67,74,75). During activation, average CBV across all subjects and ROIs was 8.7 ± 1.3 mL/100 mL (with a range of 6.7 ± 0.7 to 11 ± 1.3 mL/100 mL across ROIs), with activation induced CBV increases ranging between 22.2% in the Brodmann ROI (ROI 9), to 37.6% and 39.4 % in the smallest most significantly activated ROIs (CBV and CBF activation, ROI 2: $P < 0.05$, ROI 1: $P < 0.01$). CBV increases during visual activation in humans have previously been reported as 19 ± 3 % and 22 ± 4 % to 35 ± 4 % (73) using bolus tracking; 32 ± 12 % (51) and 42 ± 5 % (19) using a biophysical model; 56 ± 1 % using multi-echo VASO (76). CBV increases in our study are well within the range of previous publications and in closer agreement with bolus tracking results.

Average CBF across all subjects and ROIs was 72.5 ± 1 mL/min/100 mL (with a range of 71.2 ± 25 to 73.9 ± 25 mL/100 mL across ROIs) at rest. More pronounced inter-subject variability was observed in CBF as evidenced by large inter subject variations, consistent with reports of age and gender related variations in CBF. CBF slowly decreases with age, by ~ 0.5 –1% per year (33,34,37,38). Although there was a significant difference in age, of ~ 10 years, between the younger and older halves of our subjects ($P = 0.0014$, younger half: 27.1 ± 2.1 vs. older half: 37.2 ± 4.2 years); this difference was not large enough to observe any significant age related CBF or CBV differences across our subjects ($P > 0.05$). Higher CBF has been reported in females across PET, SPECT, and MRI methods (29–36), with largest differences observed in the occipital lobe (31), in good agreement with the current study. We were able to readily observe these gender differences (with 50% female subjects, and no difference in age between genders, $P = 0.897$), and our resting CBF measurements agree well with reports of increasing CBF values with increasing fractions of female subjects, including: whole-brain GM PET results of 65.1 ± 18.9 mL/min/100 mL with 27% females ($n = 11$, ages: 23–29) (18) and continuous ASL (CASL) results of 92.0 ± 15.1 mL/min/100 mL

with 69% females ($n=26$, ages: 26 ± 3) (77), 64.4 ± 6.5 ml/min/100 mL with all males ($n=28$), and 76.3 ± 10.7 mL/min/100 mL with all females ($n=28$) (31). During activation, average CBF across all subjects and ROIs was 102 ± 10 mL/min/100 mL (with a range of 88.9 ± 26 to 120.7 ± 35.7 mL/100 mL across ROIs). This corresponds to an activation induced CBF increase of $40.4 \pm 13\%$ on average (with a range of 23.1 to 65.5% across ROIs) in close agreement with literature on visual activations, including: $37 \pm 2.5\%$ with FAIR and $39 \pm 2.6\%$ with PET (78), $38.5 \pm 7.6\%$ with FAIR and $36.9 \pm 8.8\%$ with bolus tracking (72). Our observation of larger CBF increases with smaller activation areas also agrees well with literature: $55 \pm 6\%$ (23) and $55 \pm 0.6\%$ (79) increases in CBF were observed within an activation mask at the $P < 0.05$ significance level using PASL, similar to our $P < 0.05$ activation ROI CBF increase of 54.1%, and 58% to 64% increases using FAIR (32). Larger increases of $83 \pm 4\%$ (from a baseline value of 98.7 ± 13.6 mL/min/100 mL) using a combination of FAIR and Look-Locker EPI (80), and of 68% using PET (10) were reported using smaller ROIs, and these studies are in good agreement with our smallest ROI CBF increase of 65.6%.

The variations in CBV and CBF values translated into spatial and gender related variations in both power and linear function representations of the CBV-CBF relationship. The exponents or slopes of the CBV-CBF relationship reflect the degree to which rising perfusion is accommodated through blood velocity increases vs. volume increases (diameter changes or recruitment effects). Spatial variation of the CBV-CBF relationship is driven by the combination of spatial variations in CBV at rest and during activation, as well as CBF during activation (Table 3). Fairly uniform perfusion appears to be maintained across the brain at rest despite variations in vascular density, while areas of higher vascular density respond to activation with larger volume responses (Figure 2, Table 4). In addition to publications supporting variations in the underlying CBV and CBF values in humans, experiments on anesthetized rat models also support spatial variations of the CBV-CBF relationship (12,81). The limited human literature available appears to also support our observation of gender related variations in the CBV-CBF relationship. Fitting to PET measurements of absolute CBV and CBF during respiratory manipulation yielded power exponents of 0.29 using all male subjects (17) while higher power exponents of 0.46–0.64 were found when 27% of the subjects were female (18), in line with our observation of a steeper curve or line representing females. Similarly, during activation, a power exponent of 0.16 was suggested as appropriate for BOLD modeling of the venous contributions to total CBV in a cohort where 55% of the subjects were female (79), while the relationship was described by a higher power exponent of 0.23 in a cohort where 87.5% of the subjects were female (23) using the VERVE (venous refocusing for volume estimation) method. While exponents reported in these papers reflect the relationship between relative changes in CBF and relative changes in the venous contribution to CBV, preventing a direct comparison with our findings or PET results, these smaller values are consistent with an expected 36% to 46% venous contribution to CBV (14,61), and our observation of a steeper CBV-CBF relationship in females compared to males. Fitting the absolute CBV-CBF relationship during visual stimulation in males produced an exponent of 0.30 in a manually traced ROI of $\sim 0.7 \text{ cm}^3$ using PET (Ito 2001), while it produced an exponent of 0.23 in our smallest ROI

of 4cm^3 . Although a direct comparison is difficult without matching ROIs, this agrees well with our findings of decreasing exponents with increasing ROI volume.

The reasons for the observed gender differences are unclear, and there is little evidence for gender-based anatomical differences in the visual system but some of the proposed explanations include differences in visual physiology, differences in the vascular response to focal brain activation (i.e. to vasoactive substances that may be part of the neuronal activity and blood flow coupling mechanism), and hormonal influences (32). Hematocrit levels differ between males and females (~12% lower in females) (56). Lower hematocrit has been suggested to result in reduced oxygen carrying capacity, such that an adequate supply of oxygen to the brain may require higher CBF in females (33). Lower hematocrit also increases blood relaxation times (46,47,49,50) and has been considered in the current study (see Eqs. [3] and [5]). Our use of gender specific T_1 s (instead of an average value for both genders) decreased CBF in females and increased CBF in males by 1–2%, slightly de-emphasizing the observed gender differences. Lower hematocrit also corresponds to reduced blood viscosity, which is consistent with the observation of shorter transit times in women (33,82). The largest transit time gender differences were seen in younger subjects and diminished with age. Considering this worst case scenario, failing to account for ~110ms transit time difference between males and females would lead to CBF overestimation in females and underestimation in males by 7–8%. CBF gender differences persist not only after accounting for these differences in MRI, but also across other modalities (29–36), and more studies are required to better understand these differences in brain hemodynamics.

Areas showing activation in both CBV and CBF (intersection ROIs) corresponded generally to smaller ROIs, while areas showing activation in either CBV or CBF (union ROIs) corresponded generally to larger ROIs. As a result, spatial effects resulting from the use of intersection vs. union ROIs cannot be distinguished from spatial effects resulting from differences in ROI sizes. Both the power and linear functions appear equally descriptive within this physiological range of changes in normal humans, given the limited number of subjects, limited SNR and the described intrinsic inter-subject variability, in accord with the conclusions of (23,83).

One of the primary utilities of the CBV-CBF relationship exponent, α , is in calibrated fMRI, and altering the assumed value of α could have a notable impact on the estimation of CMRO_2 . In simulations, its overestimation by ~50% resulted in ~30% underestimation of CMRO_2 and its underestimation by ~50% resulted in up to ~40% overestimation of CMRO_2 (23). Since the CBV-CBF relationship is nonlinear, the exact severity of the errors depends on the true value of α . When the intermediate parameter M , the maximum achievable BOLD signal, was also estimated using calibrated fMRI (i.e. hypercapnic calibration) instead of assumed based on literature, similar biases resulted in the estimation of M ; these reduced the final bias in CMRO_2 , however, at the cost of introducing a dependency of error magnitude on the CBF change (larger errors with larger changes in CBF) (23). Further studies are desirable to better characterize the CBV-CBF relationship with more subjects across more brain regions, species, gender, or functional challenges. In the meantime, benefits of increased accuracy from measuring each parameter on each subject or such additional calibration scans should be carefully weighed against the requirements of increased scan

time and potential reductions in the number of subjects, as the answer may differ across studies.

Hemodynamic parameters have been reported using many different approaches for ROI selection, including manually placed circles or ellipses of various diameters on one or more slices or averaged across distinct regions (10,17,37); circles centered on significant CBF activation regions (84); freehand tracings on average BOLD, CBF and CBV activation maps (85); a fixed number of pixels centered over anatomical definitions (86); as well as other activation, T1 or SNR based thresholds (19,51,65,67–73). Our use of activation based ROIs may align well with functional areas across subjects, however, could also bias measurements towards higher values, with potentially larger contributions from small arteries. To limit signal contribution from small arteries, we used a modified QUIPSS II ASL implementation (Q2TIPS) which was proposed to suppress in-flowing arterial signal (52). Additionally, a bi-polar gradient was inserted between RF excitation and EPI data acquisition as a crusher to further suppress the confounding effects from larger vessels. Both CBF and CBV measurements were based on this sequence. Furthermore, CBV increases in different vascular compartments have been shown to depend on stimulus duration among other factors (87): arterial CBV changes dominate during shorter stimuli (21,22,24), while capillary (25) and venous contributions (88) develop more slowly, such that longer stimulus durations, such as the 78s stimulus used in this study, may be more appropriate for quantification of BOLD (88) which has more vein bias. However, despite such efforts to limit potential confounds, caution should always be used in interpretation of results and comparisons across methods.

Conclusion

We report on the first MRI measurement of the absolute CBV-CBF relationship in healthy human subjects with complete multi-slice coverage of the functional areas of interest. Estimates of absolute CBV and CBF at rest and during visual stimulation were consistent with prior publications. The CBV-CBF relationship varied both spatially and with gender, with $CBV = 2.1 CBF^{0.32}$ across all volunteers, $CBV = 0.8 CBF^{0.51}$ in females and $CBV = 4.4 CBF^{0.15}$ in males, in a commonly used ROI corresponding to stimulus induced activation in CBV and CBF at the $P < 0.05$ significance level. Exponents also decreased in both genders as ROIs were expanded to include less significantly activated regions, reconciling some of the variation across prior publications. Such improved characterization of the CBV-CBF relationship in humans under various metabolic or functional challenges may have significant implications for fMRI calibration; and advance our understanding of the fundamental physiological relationship between neuronal activity, hemodynamic regulation and metabolism under normal, pathological and neuronally active conditions. These findings highlight the importance of considering potential gender related differences, as well as regional variations under a range of physiological states in the interpretation of results.

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Figure 1.

Increases in CBV (mL/100 mL) and CBF (mL/100 mL/min) in the occipital cortex with visual stimulation (composite image over all subjects); and ROI masks used in analyses (Masks 1–4 are the intersections and masks 5–8 are the unions of CBV and CBF activations at significance levels $P < 0.01$, $P < 0.05$, $P < 0.1$, and $P < 0.2$, respectively, while mask 9 is the anatomical Brodmann area definition of the primary occipital cortex. Masks 1–9 cover approximately 4, 16, 27, 41, 29, 45, 55, 69, and 89 cm³, respectively.)

Figure 2.

CBV (mL/100 mL) and CBF (mL/100 mL/min) across ROIs 1, 5, and 9. Error bars represent one standard deviation; green: rest; red: activation.

Figure 3.

Power and linear function fits to the CBF-CBV relationship across all ROIs. Each curve or line was obtained from 20 data points (10 subjects in 2 conditions, rest or activation, each).

Both power exponents and slopes consistently decrease with increasing ROI sizes.

Figure 4.

Power fits to the CBF-CBV relationship across all ROIs, for female and male subjects. Each gender curve was obtained from 10 data points (5 subjects of each gender, in 2 conditions, rest or activation, each).

Figure 5.

Left: Sample data and power fits across all subjects, female subjects or male subjects in ROI 2 (stimulus induced activation in CBV and CBF at the $P < 0.05$ significance level). Right: Power exponents of the CBV-CBF relationship as a function of ROI size, for all subjects, female subject and male subjects (vertical bars represent standard errors of the estimates).

Table 1

Parameters used in CBV model fitting

Parameter	Description	Value
Hct _{MALE}	Microvascular hematocrit in males (%)	45%×85% (8,56)
Hct _{FEMALE}	Microvascular hematocrit in females (%)	40%×85% (8,56)
C _{BLOOD}	Blood water proton density (mL water / mL blood)	0.95–0.22×Hct (42)
C _{CSF}	CSF water proton density (mL water / mL CSF)	1 (42)
C _{GM}	GM water proton density (mL water / mL GM)	0.89 (42)
χ	Susceptibility difference between oxygenated and deoxygenated blood (ppm)	0.2 (43)
CSF T ₂ [*]	CSF effective transverse relaxation time constant (ms)	1442 (66)
GM T ₂	GM transverse relaxation time constant (ms)	71.1 (40,49)
(OBV/CBV) _{REST}	Oxygenated blood volume fraction at rest (%)	21% (57,61)
Y _{OBV}	OBV oxygenation fraction (%)	98% (89)
Y _{DBV,REST}	DBV oxygenation fraction at rest (%)	68.78% (49,57,61,62)

Table 2

CBV and CBF values at rest, during activation, and percentage changes with activation across all ROIs, over all subjects, female subjects, or male subjects.

ROI	CBV			CBF			
	Rest	Activation	Change	Rest	Activation	Change	
All	1	7.9 ± 1.0	11.0 ± 1.3	39.4	72.9 ± 24.5	120.7 ± 35.7	65.6
	2	7.3 ± 0.8	10.0 ± 1.2	37.6	72.8 ± 25.1	112.1 ± 35.2	54.1
	3	6.9 ± 0.7	9.3 ± 1.1	34.7	71.2 ± 25.2	105.0 ± 33.9	47.4
	4	6.7 ± 0.7	8.8 ± 1.0	31.1	71.2 ± 25.0	98.9 ± 31.6	39.0
	5	6.7 ± 0.5	9.0 ± 0.9	33.9	71.5 ± 24.9	100.5 ± 32.5	40.6
	6	6.5 ± 0.6	8.4 ± 0.9	30.7	73.9 ± 25.0	99.5 ± 31.0	34.6
	7	6.2 ± 0.6	8.0 ± 0.8	28.7	73.6 ± 24.6	96.6 ± 29.8	31.2
	8	5.8 ± 0.5	7.3 ± 0.7	26.4	72.7 ± 23.7	92.8 ± 28.1	27.8
	9	5.4 ± 0.5	6.7 ± 0.7	22.2	72.8 ± 22.9	88.9 ± 26.0	23.1
Female	1	8.1 ± 1.2	11.6 ± 1.4	44.5	90.1 ± 15.1	143.9 ± 29.0	60.1
	2	7.5 ± 0.9	10.6 ± 1.2	41.2	90.3 ± 17.2	134.2 ± 31.1	48.5
	3	7.1 ± 0.8	9.8 ± 1.0	37.8	88.8 ± 18.5	126.5 ± 31.0	42.2
	4	6.8 ± 0.7	9.1 ± 1.0	33.7	88.3 ± 19.8	118.6 ± 29.6	34.4
	5	6.8 ± 0.5	9.3 ± 0.8	36.9	87.9 ± 20.1	120.0 ± 30.3	36.7
	6	6.6 ± 0.6	8.8 ± 0.8	33.3	90.6 ± 20.8	118.4 ± 29.2	30.9
	7	6.3 ± 0.6	8.3 ± 0.8	31.0	89.8 ± 20.9	114.7 ± 28.4	27.8
	8	6.0 ± 0.6	7.7 ± 0.7	28.5	88.0 ± 20.9	109.6 ± 27.1	24.8
	9	5.6 ± 0.5	7.0 ± 0.7	24.6	86.9 ± 21.3	103.9 ± 25.3	20.0
Male	1	7.7 ± 0.7	10.4 ± 1.0	35.1	55.6 ± 19.5	97.6 ± 25.2	83.9
	2	7.0 ± 0.7	9.4 ± 1.0	34.2	55.3 ± 18.9	90.0 ± 23.0	69.4
	3	6.7 ± 0.7	8.9 ± 0.9	31.7	53.6 ± 17.6	83.5 ± 20.5	61.4
	4	6.5 ± 0.6	8.4 ± 0.8	28.5	54.1 ± 16.4	79.1 ± 18.7	50.5
	5	6.6 ± 0.6	8.6 ± 0.8	31.0	55.0 ± 17.1	80.9 ± 20.7	51.3
	6	6.3 ± 0.5	8.1 ± 0.7	28.2	57.2 ± 16.2	80.5 ± 18.9	43.6
	7	6.0 ± 0.5	7.6 ± 0.7	26.4	57.5 ± 15.7	78.6 ± 17.9	39.1

ROI	CBV			CBF		
	Rest	Activation	Change	Rest	Activation	Change
8	5.6 ± 0.4	7.0 ± 0.6	24.4	57.3 ± 14.8	76.1 ± 16.9	34.6
9	5.2 ± 0.4	6.4 ± 0.5	21.5	58.6 ± 13.8	73.9 ± 16.9	27.2

CBV values are in mL/100 mL; CBF values are in mL/min/100 mL; Change refers to increase (or decrease) relative to rest as a percentage (%).

Table 3

Summary of comparisons across genders and ROIs, of CBV and CBF values at rest, during activation, and percentage changes with activation.

Comparison	CBV		CBF	
	Rest	Activation	Rest	Activation
Gender	NS	$P \sim 0.2$	$P < 0.05$	$P \sim 0.2$
Spatial	$P < 0.05^1$	NS ²	NS ³	NS ⁴

(NS: not significant;
Spatial: across all ROI combinations, $P < 0.05$ in 197%, 231%, 319%, 494%, 39% of cases);
Age difference between genders was NS, $P = 0.897$).

Table 4

Results of fitting the CBV-CBF relationship to power and linear functions over different ROIs, over all subjects, female subjects, or male subjects. ROI 2 exponents, corresponding to stimulus induced activation in CBV and CBF at the $P < 0.05$ significance level, are highlighted.

ROI	Power Fit: $CBV = a CBF^b$				Linear Fit: $CBV = c + d \cdot CBF$				
	a	b	RMSE	R	c	d	RMSE	R	
All	1	2.3 ± 0.9	0.31 ± 0.08	1.36	0.68	6.1 ± 0.8	0.031 ± 0.008	1.29	0.72
	2	2.1 ± 0.9	0.32 ± 0.09	1.37	0.63	5.7 ± 0.8	0.030 ± 0.008	1.32	0.66
	3	2.8 ± 1.2	0.24 ± 0.10	1.39	0.50	5.7 ± 0.8	0.026 ± 0.008	1.20	0.63
	4	3.1 ± 1.3	0.21 ± 0.10	1.25	0.47	6.0 ± 0.8	0.020 ± 0.009	1.23	0.47
	5	3.1 ± 1.4	0.21 ± 0.10	1.27	0.45	6.1 ± 0.8	0.020 ± 0.009	1.25	0.48
	6	3.1 ± 1.4	0.20 ± 0.10	1.18	0.42	5.9 ± 0.8	0.018 ± 0.008	1.16	0.44
	7	3.3 ± 1.5	0.17 ± 0.10	1.10	0.37	5.8 ± 0.7	0.015 ± 0.008	1.08	0.40
	8	3.2 ± 1.4	0.16 ± 0.10	0.98	0.35	5.5 ± 0.7	0.013 ± 0.008	0.97	0.38
	9	3.2 ± 1.5	0.14 ± 0.10	0.86	0.31	5.1 ± 0.6	0.012 ± 0.007	0.85	0.34
Female	1	0.6 ± 0.6	0.57 ± 0.18	0.29	0.74	4.2 ± 1.7	0.046 ± 0.01	0.28	0.74
	2	0.8 ± 0.7	0.51 ± 0.17	1.43	0.72	4.5 ± 1.6	0.040 ± 0.01	1.43	0.72
	3	1.0 ± 0.8	0.47 ± 0.17	1.31	0.69	4.6 ± 1.5	0.035 ± 0.01	1.30	0.69
	4	1.3 ± 1.1	0.40 ± 0.18	1.25	0.61	4.9 ± 1.4	0.030 ± 0.01	1.24	0.62
	5	1.3 ± 1.0	0.40 ± 0.17	1.23	0.63	5.0 ± 1.4	0.030 ± 0.01	1.22	0.64
	6	1.5 ± 1.3	0.35 ± 0.18	1.22	0.54	5.1 ± 1.4	0.025 ± 0.01	1.21	0.56
	7	2.0 ± 1.7	0.29 ± 0.19	1.18	0.46	5.2 ± 1.4	0.021 ± 0.01	1.16	0.48
	8	2.2 ± 2.0	0.25 ± 0.19	1.09	0.40	5.1 ± 1.3	0.017 ± 0.01	1.07	0.43
	9	2.9 ± 2.5	0.17 ± 0.19	0.98	0.30	5.1 ± 1.2	0.013 ± 0.01	0.96	0.35
Male	1	3.4 ± 1.9	0.23 ± 0.13	0.28	0.53	7.3 ± 1.4	0.023 ± 0.02	1.62	0.44
	2	4.4 ± 3.0	0.15 ± 0.16	1.57	0.32	7.2 ± 1.4	0.014 ± 0.02	1.60	0.26
	3	4.6 ± 3.2	0.13 ± 0.16	1.45	0.27	7.0 ± 1.4	0.012 ± 0.02	1.47	0.21
	4	4.9 ± 3.4	0.10 ± 0.17	1.28	0.21	6.9 ± 1.3	0.009 ± 0.02	1.30	0.16

ROI	Power Fit: CBV = a CBF ^b				Linear Fit: CBV = c + d, CBF			
	a	b	RMSE	R	c	d	RMSE	R
5	3.8 ± 2.3	0.17 ± 0.15	1.18	0.42	6.9 ± 1.3	0.010 ± 0.02	1.34	0.18
6	4.8 ± 3.5	0.10 ± 0.17	1.20	0.20	6.7 ± 1.3	0.008 ± 0.02	1.21	0.15
7	3.7 ± 2.5	0.15 ± 0.16	0.98	0.35	6.5 ± 1.2	0.005 ± 0.02	1.10	0.10
8	5.0 ± 3.7	0.06 ± 0.18	0.96	0.12	6.1 ± 1.1	0.003 ± 0.02	0.96	0.07
9	5.9 ± 4.3	0.00 ± 0.17	0.80	0.00	5.9 ± 1.0	0.002 ± 0.02	0.80	0.04

RMSE: root mean squared error; R²: Coefficient of determination; ROIs 1–4: intersections and ROIs 5–8: unions of CBV and CBV activations at significance levels $P<0.01$, $P<0.05$, $P<0.1$ and $P<0.2$, respectively; ROI 9: Brodmann area definition.