Appendix 1

Further Discussion on CVR Measurement Using BOLD

The CVR results measured by BOLD MRI revealed a large variability due to several factors including the spatial resolution of the BOLD MRI data, modeling techniques, and signal contamination in large vessels. Among the CVR studies that applied BOLD MRI, the spatial resolution of ranged between 5 × 5 x 7 mm^3 and 3 × 3 x 3 mm^3, with a slice thickness of 4 mm being the most widely used. The BOLD data in a study that applied CO_2 as the vasodilator included 3 different spatial resolutions (3.2 × 3.2 × 3.5 mm^3, 3.4 × 3.4 × 3.8 mm^3, and 3.4 × 3.4 × 3.5 mm^3) [1]; however, although the study reported a negative relationship between the baseline end-tidal CO_2 and CVR, no direct comparison was made on the CVR measurements among different image resolutions. Another factor that affects BOLD CVR measurements is the analysis model, and the best modeling approach for the accurate quantification of CVR maps remains an open question. Among the studies reviewed in this work, the 3 most widely used data analysis techniques included general linear model implemented in FSL [2], linear regression model implemented in AFNI [3], and multi-linear regression implemented in SPM [4]. Only one study compared the different implementations of the general linear model in FSL, and using the 3rd order complexity showed highest reproducibility (ICC = 0.75 vs 0.54 for the 3rd and 0th order respectively) [5]. BOLD signal artifact due to signal contamination in large vessels impedes the localization of localization and accurate quantification of CVR. The high mean-CVR values observed in our review were likely driven by large vessel effects in BOLD, which were visible in the parenchymal CVR maps in several studies [6–8]. The

artifact may be suppressed using ROI-based outlier exclusion strategies or assessing the phase angle of the fMRI complex data [9,10].

For both BOLD and ASL MRI, the speed of data acquisition can be enhanced using multi-slice methods to increase the number of images collected during a hypercapnic challenge. In essence, the acceleration factor of the multi-slice method depends on the multi-band radiofrequency pulses and the simultaneous image refocusing factors [11]. Among the studies reviewed, only one study applied the multiband multi-echo method (multiband-factor = 4; number of excitations = 11, total slices = 44) to acquire simultaneous ASL/BOLD data from 14 normal subjects using BH as the vasodilator [12]. The results from this study suggested that combining the echoes resulted in slightly higher reproducibility and CVR values, making the multi-echo method advantageous for measuring CVR and BOLD activation. The limited data on multiband and multi-echo CVR studies in the current literature do not allow any specific recommendations regarding the use of such technique to improve CVR reproducibility. Future studies using multi-slice and multi-echo BOLD data with a higher temporal resolution and SNR may provide better insights on the impact of acceleration factors on CVR measurements.

Among the studies that employed hypercapnic gas as the vasodilator, the gas mixture was delivered either using a computer-controlled system or in an uncontrolled manner with a fixed gas mixture, where the rate of gas inhalation differed among individuals. There were 4 studied that explicitly stated to have applied the computer-controlled system and the CVR measurement modalities included TCD and BOLD MRI [6,13–15]. These

automated gas delivery systems included RespirAct™ (used in 3 studies) and an in-house developed device connected with SideTrak® 840 of Sierra Instruments (used in 1 study). Due to the insufficient information about the exact gas delivery mechanism in most studies (controlled or uncontrolled), the data were inconclusive to determine the impact of the gas delivery method on CVR reproducibility. Among the studies that used hypercapnia gas challenge, 14 studies included N_2 (or room air) while the gas mixture in 7 studies consisted of only CO_2 and O_2 (or carbogen). No clear implication can be found on the impact of including N_2 on the reproducibility of CVR measurements (the range of wsCV of CVR with and without N_2 was 13.3% - 46.9% and 3.1% - 78.0%).

It should be noted that the variations in individual hemoglobin levels directly affect the observed BOLD signal at the site of activation [16]. Specifically, since the BOLD signal reflects changes in concentrations of oxy- and deoxy-hemoglobin, inter-subject variations in hemoglobin levels influence the BOLD signal independent of the amount of neural activity [17]. The concentration of deoxyhemoglobin that gives rise of BOLD signal is determined by both the volume of the voxel occupied by blood and the level of hemoglobin and hematocrit [18]. In a study that investigated the relationship between functional connectivity and hemoglobin levels between males and females (N=518 healthy elderly subjects, 259 males), significant differences were observed in BOLD signal change between the high and low hemoglobin groups [19]. Such BOLD signal variations due to individual blood characteristics should be distinguished from those induced by vasodilators in CVR measurements. These limitations in BOLD techniques should be

considered when large variations in hemoglobin and hematocrit levels are expected in the study cohort.

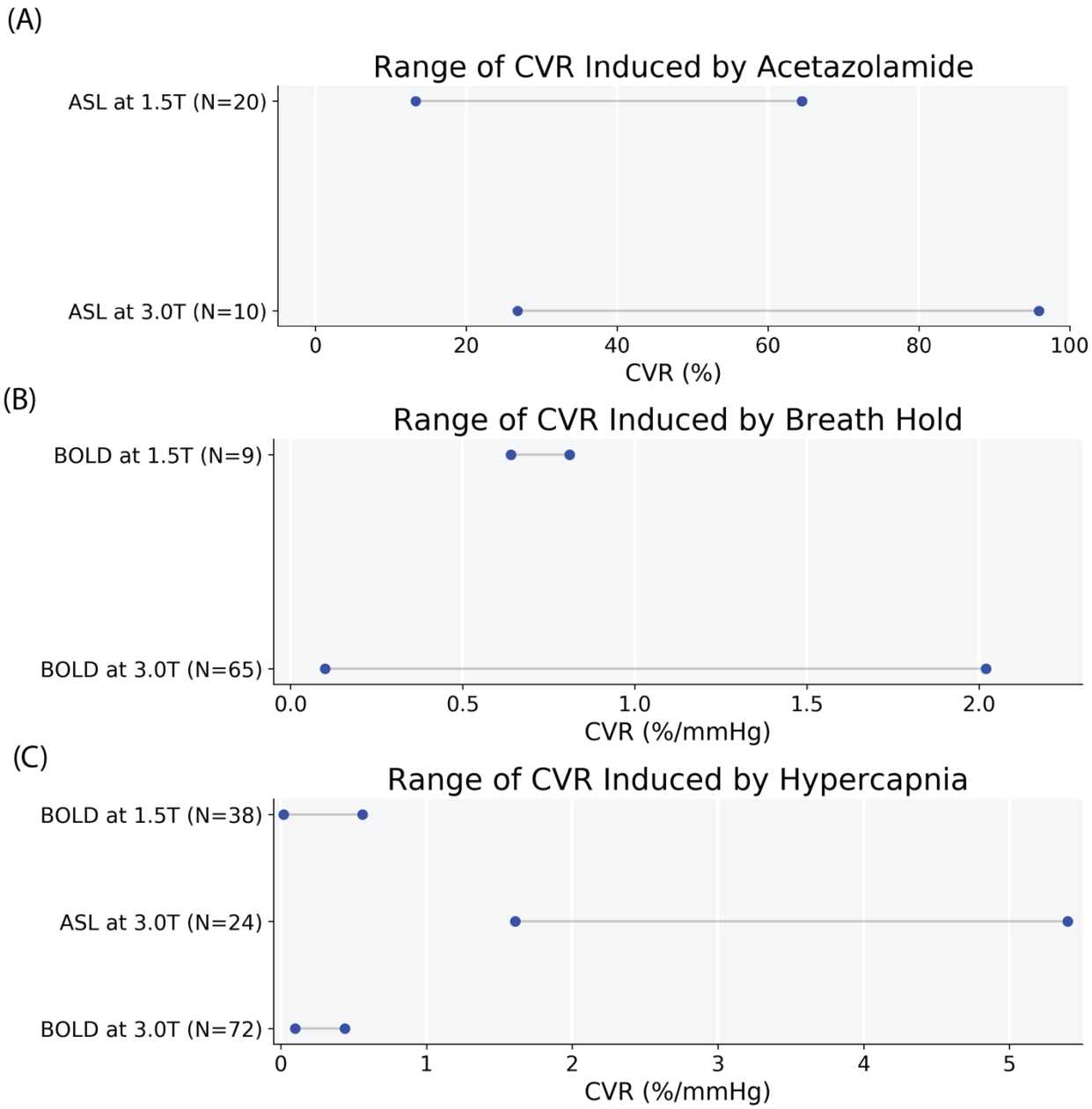

Figure A1: The range of CVR values using ASL and BOLD MRI at 1.5 and 3.0T. Modalities that were not used in any studies (N=0) were excluded.

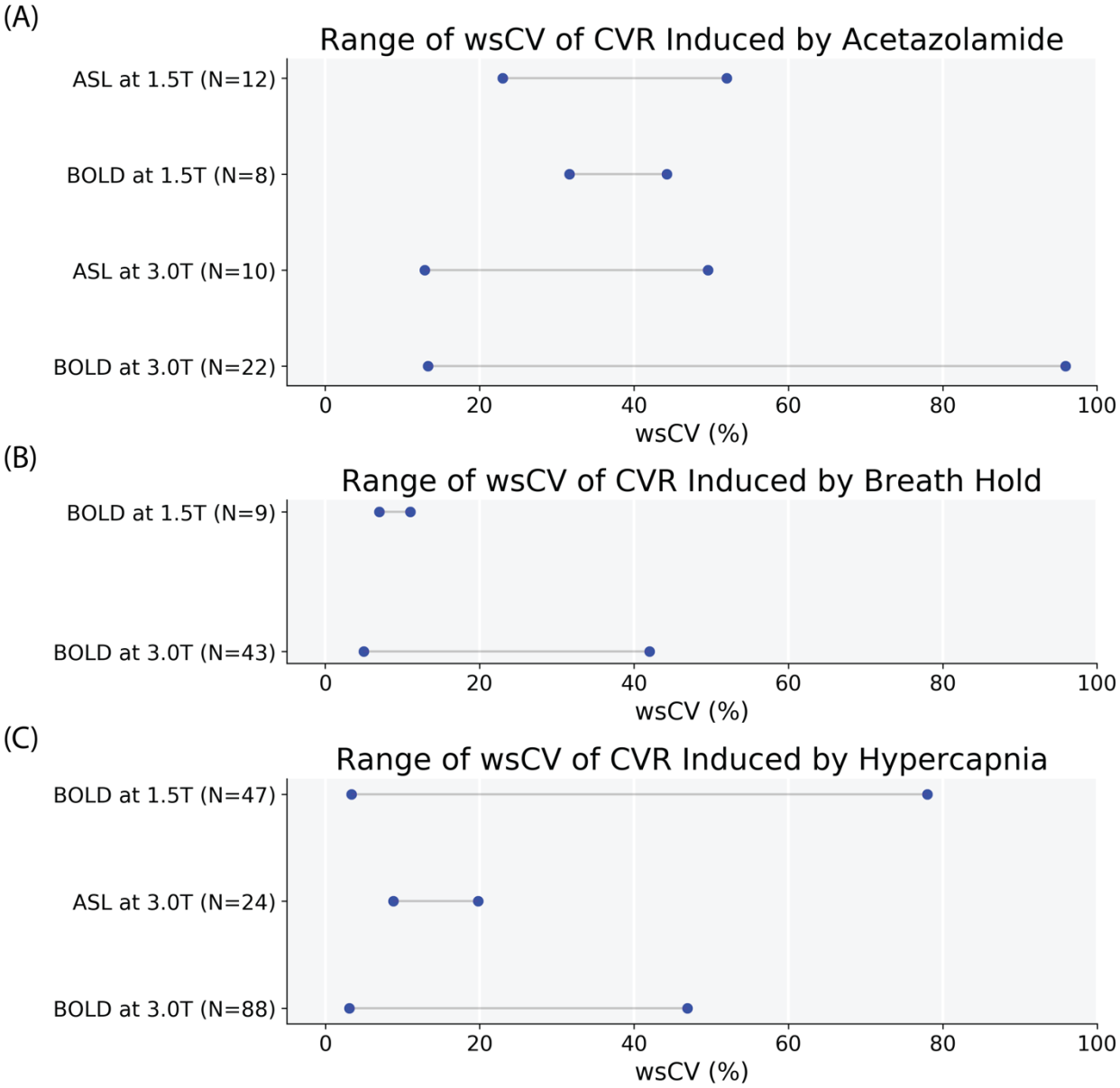

Figure A2: The range of wsCV values using ASL and BOLD MRI at 1.5 and 3.0T. Modalities that were not used in any studies (N=0) were excluded.

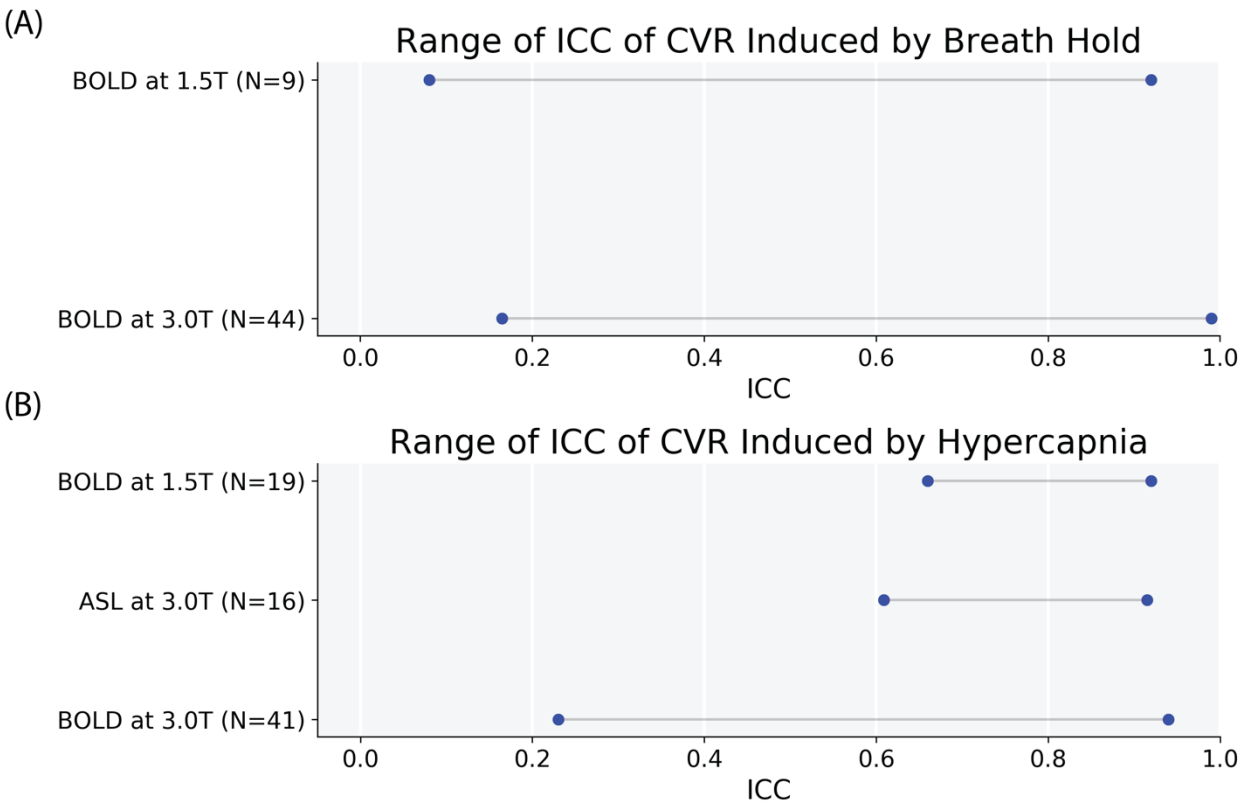

Figure A3: The range of ICC values using ASL and BOLD MRI at 1.5 and 3.0T. ICC was not applied as the reproducibility metric in any studies that used ACZ as the vasodilator. Modalities that were not used in any studies (N=0) were excluded.

PubMed Search

Date of search: 4/21/20

Search #	Search Query	Results
1	“Cerebrovascular circulation” [mesh] OR “cerebrovascular reserve” [tw] OR CVR [tw] OR “cerebrovascular reactivity” [tw] OR “vascular reactivity” [tw] OR vasoreactivity [tw] OR “Cerebral blood flow” [tw] OR CBF [tw] OR “brain perfusion” [tw] OR “cerebral perfusion” [tw]	83,831
2	“Magnetic resonance imaging” [mesh] OR “Perfusion imaging” [mesh] OR "Positron-Emission Tomography"[Mesh] OR MRI [tw] OR “Magnetic resonance imaging” [tw] OR “Arterial spin labeling” [tw] OR “Blood oxygenation level dependent” [tw] OR BOLD [tw] OR “Perfusion imaging” [tw] OR “Positron emission tomography” [tw] OR PET [tw] OR “Single-photon emission computed tomography” [tw] OR SPECT [tw] OR “Computerized Tomography” [tw] OR “Transcranial Doppler Ultrasound” [tw] OR “TCD Ultrasound” [tw] OR “Transcranial Doppler Ultrasonography” [tw] OR “TCD Ultrasonography” [tw]	740,365
3	Acetazolamide [mesh] OR “Breath holding” [mesh] OR Hypocapnia [mesh] OR "Hypercapnia"[Mesh] OR "Carbon Dioxide"[Mesh] OR "Vasodilation"[Mesh] OR Vasodilator [tw] OR Vasoconstrictor [tw] OR “vasoactive stimulus” [tw] OR vasodilation [tw] OR Vasorelaxation [tw] OR Acetazolamide [tw] OR Diamox [tw] OR “Breath holding” [tw] OR “breath hold” [tw] OR Hypocapnia [tw] OR Hypercapnia [tw] OR “CO2 challenges” [tw] OR “gas challenges” [tw] OR “carbon dioxide” [tw]	252,929

4	"reproducibility of results"[mesh] or reproduc* [tw] or repeat* [tw] or reliabl* [tw] or "test-retest" [tw] or valid* [tw] or variance [tw] OR Accuracy [tw] OR accurate [tw]	2,966,309
5	#1 AND #2 AND #3 AND #4	678
6	#5 AND English [lang]	637
7	#6 NOT ("Animals"[Mesh] NOT ("Animals"[Mesh] AND "Humans"[Mesh]))	573

PubMed Search Syntax:

((((((((((("Cerebrovascular circulation"[MeSH Terms] OR "cerebrovascular reserve"[Text Word]) OR "CVR"[Text Word]) OR "cerebrovascular reactivity"[Text Word]) OR "vascular reactivity"[Text Word]) OR "vasoreactivity"[Text Word]) OR "Cerebral blood flow"[Text Word]) OR "CBF"[Text Word]) OR "brain perfusion"[Text Word]) OR "cerebral perfusion"[Text Word]) AND ((((((((((((((("Magnetic resonance imaging"[MeSH Terms] OR "Perfusion imaging"[MeSH Terms]) OR "positron-emission tomography"[MeSH Terms]) OR "MRI"[Text Word]) OR "Magnetic resonance imaging"[Text Word]) OR "Arterial spin labeling"[Text Word]) OR "Blood oxygenation level dependent"[Text Word]) OR "BOLD"[Text Word]) OR "Perfusion imaging"[Text Word]) OR "positron emission tomography"[Text Word]) OR "PET"[Text Word]) OR "Single-photon emission computed tomography"[Text Word]) OR "SPECT"[Text Word]) OR "Computerized Tomography"[Text Word]) OR "Transcranial Doppler Ultrasound"[Text Word]) OR "TCD Ultrasound"[Text Word]) OR "Transcranial Doppler Ultrasonography"[Text Word]) OR "TCD Ultrasonography"[Text Word])) AND ((((((((((((((((("Acetazolamide"[MeSH Terms] OR "Breath holding"[MeSH Terms]) OR "Hypocapnia"[MeSH Terms]) OR "Hypercapnia"[MeSH Terms]) OR "Carbon Dioxide"[MeSH Terms]) OR "Vasodilation"[MeSH Terms]) OR "Vasodilator"[Text Word]) OR "Vasoconstrictor"[Text Word]) OR "vasoactive stimulus"[Text Word]) OR "Vasodilation"[Text Word]) OR "Vasorelaxation"[Text Word]) OR "Acetazolamide"[Text Word]) OR "Diamox"[Text Word]) OR "Breath holding"[Text Word]) OR "breath hold"[Text Word]) OR "Hypocapnia"[Text Word]) OR "Hypercapnia"[Text Word]) OR "CO2 challenges"[Text Word]) OR "gas challenges"[Text Word]) OR "Carbon Dioxide"[Text Word])) AND ((((((((("reproducibility of results"[MeSH Terms] OR "reproduc*"[Text Word]) OR "repeat*"[Text Word]) OR "reliabl*"[Text Word]) OR "test-retest"[Text Word]) OR "valid*"[Text Word]) OR "variance"[Text Word]) OR "Accuracy"[Text Word]) OR "accurate"[Text Word])) AND "English"[Language]) NOT ("Animals"[MeSH Terms] NOT ("Animals"[MeSH Terms] AND "Humans"[MeSH Terms]))

Embase Search

Date of search: 4/21/20

Search #	Search Query	Results
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2	'nuclear magnetic resonance imaging'/exp OR 'scintigraphy'/exp OR 'positron emission tomography'/exp OR MRI:ti,ab OR ‘Magnetic resonance imaging’:ti,ab OR ‘Arterial spin labeling’:ti,ab OR ‘Blood oxygenation level dependent’:ti,ab OR BOLD:ti,ab OR ‘Perfusion imaging’:ti,ab OR ‘Positron emission tomography’:ti,ab OR PET:ti,ab OR ‘Single-photon emission computed tomography’:ti,ab OR SPECT:ti,ab OR ‘Computerized Tomography’:ti,ab OR ‘Transcranial Doppler Ultrasound’:ti,ab OR ‘TCD Ultrasound’:ti,ab OR ‘Transcranial Doppler Ultrasonography’:ti,ab OR ‘TCD Ultrasonography’:ti,ab	1,256,498
3	'acetazolamide'/exp OR 'breath holding'/exp OR 'hypocapnia'/exp OR 'hypercapnia'/exp OR 'hypercapnia'/exp OR 'vasodilatation'/exp OR Vasodilator:ti,ab OR Vasoconstrictor:ti,ab OR ‘vasoactive stimulus’:ti,ab OR vasodilation:ti,ab OR Vasorelaxation:ti,ab OR Acetazolamide:ti,ab OR Diamox:ti,ab OR ‘Breath holding’:ti,ab OR ‘breath hold’:ti,ab OR Hypocapnia:ti,ab OR Hypercapnia:ti,ab OR ‘CO2 challenges’:ti,ab OR ‘gas challenges’:ti,ab OR ‘carbon dioxide’:ti,ab	243,160

4	'reproducibility'/exp OR reproduc*:ti,ab OR repeat*:ti,ab OR reliabl*:ti,ab OR 'test-retest':ti,ab OR valid*:ti,ab OR variance:ti,ab OR Accuracy:ti,ab OR accurate:ti,ab	3,458,793
5	#1 AND #2 AND #3 AND #4	613
6	#5 AND [english]/lim	583
7	#6 NOT ([animals]/lim NOT [humans]/lim)	528
8	#7 NOT ([conference abstract]/lim OR [conference paper]/lim OR [conference review]/lim)	344

Scopus Search

Date of search: 4/21/20

Search #	Search Query	Results
1	TITLE-ABS-KEY("Cerebrovascular circulation" OR "cerebrovascular reserve" OR CVR OR "cerebrovascular reactivity" OR "vascular reactivity" OR vasoreactivity OR "Cerebral blood flow" OR CBF OR "brain perfusion" OR "cerebral perfusion")	101,247
2	TITLE-ABS-KEY("Magnetic resonance imaging" OR "Perfusion imaging" OR "Positron-Emission Tomography" OR MRI OR "Arterial spin labeling" OR "Blood oxygenation level dependent" OR BOLD OR PET OR "Single-photon emission computed tomography" OR SPECT OR "Computerized Tomography" OR "Transcranial Doppler Ultrasound" OR "TCD Ultrasound" OR "Transcranial Doppler Ultrasonography" OR "TCD Ultrasonography")	1,181,462

3	TITLE-ABS-KEY(Acetazolamide OR “Breath holding” OR Hypocapnia OR "Hypercapnia" OR "Carbon Dioxide" OR "Vasodilation" OR Vasodilator OR Vasoconstrictor OR “vasoactive stimulus” OR vasodilation OR Vasorelaxation OR Diamox OR “breath hold” OR “CO2 challenges” OR “gas challenges”)	614,855
4	TITLE-ABS-KEY("reproducibility of results" or reproduc* OR repeat* OR reliabl* OR "test-retest" OR valid* OR variance OR Accuracy OR accurate)	7,999,924
5	#1 AND #2 AND #3 AND #4	766
6	#5 AND (LIMIT-TO (LANGUAGE , "English")	725
7	#6 AND (EXCLUDE (DOCTYPE , "cp"))	708

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