File S3: Results for studies without within-subject comparisons with reference techniques

In sixty-six sub-studies in 16 articles no reference techniques were used or NIRS-measurements were compared with reference techniques only on a group level (group mean (SD) or median (IQR) values), but not on an individual level. To obtain an overview of the results from these studies, the results were first summarised. Changes in the NIRS-measurements and (if applicable) reference technique measurements were classified as 'increased', 'decreased', or 'no change'. Changes in NIRS-measurements or reference technique measurements were classified as 'increased' (↑) or 'decreased (↓)' when the study reported a 'significant' change, either as 1) $p<0.05$, 2) 95% confidence interval of change does not contain zero, 3) interquartile range of change does not contain zero. When this was not the case, it was classified as 'no change' (=). If a study reported only raw data, the non-parametric distribution was calculated post-hoc and decision was based on this distribution.

The studies were subsequently classified as 1) 'Supports intracerebral origin of NIRS-signals', when NIRS-measurements changed (increased or decreased) upon intracerebral perfusion modification or when the NIRS-measurements changed in the same direction as intracerebral reference technique measurements. Similarly, studies were classified as 2) 'Supports extracerebral origin of NIRS-signals' when NIRS-measurements changed during an extracerebral perfusion modification or when the NIRS-measurements changed in the same direction as extracerebral reference technique measurements. If both of the previous results were found, the study was classified as 3) 'Supports both the intra- and extracerebral origin of the NIRS-signals'. If results were unclear or no changes NIRS-measurements were found, the study was classified as 4) 'Inconclusive results'. Classification is shown in Supplementary File S3– Table 2 and Supplementary File S3 – Table 3.

Classification results are shown in Supplementary File S3 – Table 1. Studies on Hb concentrations and oxygen saturation indices generally supported the intracerebral origin of the NIRS-measurement when an intracerebral perfusion modification protocol was applied. Similarly, when the extracerebral perfusion was changed, these studies supported the extracerebral origin of the NIRS-signal. Two studies on Hb concentrations in which both the intra- and extracerebral perfusion was selectively modified, showed support for both the intra- and extracerebral origin of the NIRS-signal. For oxygen saturation indices, two studies supported the intracerebral origin. Results were inconclusive for four articles in total.

Supplementary File S3—Table S1: Classification of articles without within-subject comparisons of NIRS-measurements with reference technique measurements.
Results are divided into haemoglobin concentrations and tissue oxygen saturation indices. Rows represent the perfusion modification paradigms that were applied in each article. Columns represent the classification. Results of 16 articles are listed. In three articles both Hb concentrations and rSO_2 were studied, so the classification for Hb concentrations and rSO_2 are provided separately. Numbers indicate the number of studies in that class.

		Supports intracerebral origin	**Supports extracerebral origin**	**Supports both**	**Inconclusive**
Haemoglobin concentrations	Intracerebral perfusion modification	1 [58]	-	-	1 [47]
	Extracerebral perfusion modification	-	2 [59,60]	-	-
	Intra- and extracerebral perfusion modification	-	-	2 [56,57]	1 [49]
Tissue oxygen saturation indices	Intracerebral perfusion modification	3 [45–47]	-	-	-
	Extracerebral perfusion modification	-	5 [50–54,60]	-	-
	Intra- and extracerebral perfusion modification	2 [48,49]	-	-	2 [40,55]

Supplementary File S3—Table S2: Haemoglobin concentrations. Changes in haemoglobin concentrations and intracerebral and extracerebral reference techniques. Changes in NIRS-measurements or reference technique measurements were classified as 'increased' (↑) or 'decreased (↓)' or 'no change' (=). The classification per study is shown in the last column. 1 Intra: Supports intracerebral origin. 2 Extra: Supports extracerebral origin. 3 Both: Supports both intra- and extracerebral origin. 4 Inconclusive: Inconclusive results.
ECA, external carotid artery; extra, extracerebral; ICA, internal carotid artery; Hb, haemoglobin; HHb, Deoxygenated Hb; intra, intracerebral; LDF, Laser Doppler Flowmetry; OxyHb, Oxygenated Hb; OxyHb*, Oxygenated Hb corrected for scalp blood flow; $SjvO_2$, jugular venous oxygen saturation; tHb, total Hb; TCD, Transcranial Doppler.

Articles	Perfusion modification	Study protocol	Hb			Reference technique intracerebral	Reference technique extracerebral	Classification
Tateishi 1995 [58]	Intra	Hypo- to hyper- to hypocapnia	OxyHb ↑	HHb ↓		$SjvO_2$ ↑		1 Intra
Hirasawa 2016-1 [59]	Extra	Cuff inflation	OxyHb ↓ OxyHb* =				LDF ↓	2 Extra
Moerman 2021-1 [60]	Extra	Phenylephrine infusion			tHb ↓			2 Extra
Germon 1999-1-16 [56]	Intra and extra	Hyper- to hypocapnia Cuff release	OxyHb ↓ OxyHb ↑	HHb ↑ HHb ↓	tHb ↓ tHb =	TCD ↓		3 Both
Germon 1998-1-4 [57]	Intra and extra	Hyper- to hypocapnia Cuff release	OxyHb ↓ OxyHb ↑	HHb ↑ HHb ↓	tHb ↓ tHb =	TCD ↓		3 Both
Grubhofer 1999-1 [47]	Intra	Hypocapnia	OxyHb =					4 Inconclusive
Cho 1998-3+4 [49]	Intra and extra	ICA-unclamping ECA-unclamping	OxyHb↑ OxyHb =	HHb = HHb =	tHb = tHb =			4 Inconclusive

Supplementary File S3—Table S3: Oxygen saturation indices (rSO_2). Changes in rSO_2 and intracerebral and extracerebral reference techniques. Changes in NIRS-measurements or reference technique measurements were classified as 'increased' (↑) or 'decreased (↓)' or 'no change' (=). The classification per study is shown in the last column. 1 Intra: Supports intracerebral origin. 2 Extra: Supports extracerebral origin. 4 Inconclusive: Inconclusive results. ECA, external carotid artery; extra, extracerebral; $etCO_2$, end-tidal carbon dioxide; ICA, internal carotid artery; inf, infusion; intra, intracerebral; INVOS, INVOS 4100 (Somanetics); LDF, Laser Doppler Flowmetry; NIRO, NIRO-200NX (Hamamatsu); rSO_2, regional tissue saturation of oxygen; $S_{cap}O_2$, cerebral capillary oxygen saturation; $SjvO_2$, jugular venous oxygen saturation; TCD, Transcranial Doppler.

Article	Perfusion modification	Study protocol	rSO_2			Reference technique intracerebral		Reference technique extracerebral			Classification
Yoshitani 2002-1 [45]	Intra	Hypocapnia	↓			TCD ↓					1 Intra
Henson 1998 [46]	Intra	Hypercapnia	↑			$SjvO_2$ ↑					1 Intra
Grubhofer 1999-2 [47]	Intra	Hypocapnia	↓								1 Intra
Samra 1999-1+2 [48]	Intra and extra	ICA and ECA clamping	ICA-clamping↓ ECA-clamping =								1 Intra
Cho 1998-1+2 [49]	Intra and extra	ICA and ECA unclamping	ICA-unclamping ↑ ECA-unclamping =								1 Intra
Kato 2017-1/2/3/4 [50]	Extra	Cuff inflation	↓					Scalp pulse oximetry ↓			2 Extra
Germon 1994-1 [51]	Extra	Cuff inflation and Frontalis muscle contraction	↓								2 Extra
Greenberg 2016-1/2 [52,53]	Extra	Cuff inflation	↓								2 Extra
Davie 2012-1/2/3 [54]	Extra	Cuff inflation	↓								2 Extra
Moerman 2021-2 [60]	Extra	Phenylephrine infusion	↓								2 Extra
Canova 2011-4-6 [40]	Intra and extra	Multiple	Protocol:			TCD		PPG	NIRS cheek	LDF	4 Inconclusive
			Hypocapnia ↓			↓		=	=	=	
			Valsalva maneuvre ↓			↓		=	↑	=	
			Head-up tilt ↓			↓		=	=	↓	
Sørensen 2015-1-14 [55]	Intra and extra	Multiple	Protocol:	INVOS	NIRO	TCD	$S_{cap}O_2$	LDF			4 Inconclusive
			Hypocapnia	=	=	↓	↓	=			
			Phenylephrine infusion	↓	↓	=	=	↓			
			Whole body heating	↑	=	=	=	↑			
			Noradrenaline infusion	↓	=	=	=	=			
			Noradrenaline infusion + $etCO_2$ stabilisation	↓	=	=	=	=			
			40° head-up tilt	=	=	=	=	=			
			40° head-up tilt + phenylephrine infusion	↓	↓	=	=	=			