# Near infrared spectroscopy - investigations in neurovascular diseases

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**The thesis is based on the following papers:**

1. Schytz HW, Wienecke T, Jensen LT, Selb J, Boas DA & Ashina M. Changes in cerebral blood flow after acetazolamide: an experimental study comparing near-infrared spectroscopy and SPECT. European Journal of Neurology 2009; 16: 461-467.
2. Phillip D, Schytz HW, Selb J, Payne S, Iversen HK, Skovgaard LT, Boas DA & Ashina M. Low frequency oscillations in cephalic vessels assessed by near infrared spectroscopy. European Journal of Clinical Investigations 2012; 42: 1180-1188.
3. Schytz HW, Ciftçi K, Akin A, Ashina M & Bolay H. Intact neurovascular coupling during executive function in migraine without aura: interictal near-infrared spectroscopy study. Cephalalgia 2010; 30: 457-466.
4. Schytz HW, Hansen JM, Phillip D, Selb J, Boas DA & Ashina M. Nitric oxide modulation of low-frequency oscillations in cortical vessels in FHM - a NIRS study. Headache 2012; 52: 1146-1154.
5. Schytz HW, Barløse M, Guo S, Selb J, Caparso A, Jensen R & Ashina M. Experimental activation of the sphenopalatine ganglion provokes cluster-like attacks in humans. Cephalalgia 2013; 33(10):831-41.
6. Schytz HW, Jensen BE, Jennum P, Selb J, Boas DA & Ashina M. Low-frequency oscillations and vasoreactivity of cortical vessels in obstructive sleep apnea during wakefulness: A near infrared spectroscopy study. Sleep Medicine 2013; 14(5):416-21.
7. Schytz HW, Guo S, Jensen LT, Kamar M, Nini A, Gress DR & Ashina M. A new technology for detecting cerebral blood flow: a comparative study of ultrasound tagged NIRS and 133Xe-SPECT. Neurocritical Care 2012; 17: 139-145.

## INTRODUCTION

The human brain depends on a fascinating intricate and complex interaction between neurons and the cerebral vasculature. Neurovascular disease encompasses a growing number of neurological diseases based on recent scientific explorations. As an example, neurovascular dysregulation has been demonstrated in Alzheimer´s disease (AD) and is likely to be involved in the disease mechanism of AD (1). In the primary headaches, especially within the field of migraine, there is an ongoing debate on possible neurovascular dysautoregulation, which is still not resolved (2-6). It is of obvious great interest to investigate brain function and vascular changes with different imaging modalities. Most imaging methods detect brain activity indirectly by monitoring hemodynamic changes and uptake of tracers consequent to neuronal activity, these include single photon emission computer tomography (SPECT), positron emission tomography (PET) and magnetic resonance imaging (MRI) (7). Near infrared spectroscopy (NIRS) is an emerging optical imaging method (8), which is non-invasive, easy to use, has a high patient-tolerability and does not expose patients to radiation. However, NIRS is largely unknown in clinical neurology, even at specialized centers, despite being an often used research tool (9). It is therefore of key interest to examine neurological diseases, which NIRS may be useful in, and to develop new NIRS methods to be applied in neurology. Recently, Obrig (9) reviewed the present state of NIRS in neurological diseases and identified neurovascular diseases, primary headache and epilepsy as the three major disease entities in which NIRS may become more than a promising tool. In stroke research NIRS would especially be of interest to apply as a non-invasive method to study changes in cerebral blood flow (CBF) and cerebral autoregulation, but NIRS has yet not proven to measure CBF reliably, and validation studies in cerebral autoregulation studies have not been performed yet. In headache research NIRS has been applied in a few studies in migraine with aura (MA) and migraine without aura (MO) to investigate vasoreactivity (10-12), functional neurovascular coupling (13) and vascular alterations during and between attacks (14). The studies are difficult to compare due to differences in design and migraine subtype, and other primary headache disorders have not been investigated with NIRS. Hence, further studies within the headache field are highly needed to explore headache pathophysiology and possibly find specific biological headache markers using NIRS. In obstructive sleep apnea (OSA), which may be coined a cerebrovascular disease based on the increased risk of heart attack and stroke (15-17), NIRS has been applied in studies measuring primarily vascular alteration during apnea episodes (18, 18-21), and further studies
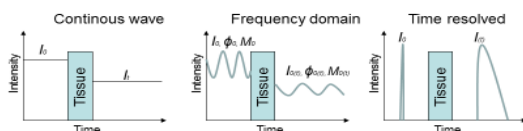
are in demand to investigate if there are other vascular changes that may explain the increased stroke risk.

The purpose of this thesis was to explore and develop methods, where NIRS can be applied in different neurovascular diseases, in order to find biological markers that may become useful in clinical neurology. The thesis specifically aimed to explore the ability of NIRS: A) To study changes in CBF, B) to reliably detect spontaneous cerebral autoregulation, C) to detect neurovascular coupling in MO patients, D) to study vasoreactivity in familial hemiplegic migraine (FHM) patients, E) to study cephalic vascular changes following neurostimulation of the sphenopalatine ganglion (SPG) in cluster headache (CH) patients, F) to study increased sympathetic tone in cortical vessels in OSA patients.

**NEAR INFRARED SPECTROSCOPY IN NEUROLOGY**
*Basic physical principles of near infrared spectroscopy*
In 1977 Frans F. Jöbsis at Duke University in the U.S. demonstrated that it was possible to measure changes in near infrared light attenuation from temple to temple in anesthetized cats during normoxia, anoxia and asphyxia (22), which thereby initiated the field of NIRS brain imaging. Then 8 years later, in 1985, the first human brain clinical studies were published by Brazy et al. (23, 24) and Ferrari et al. (25). Since then the utility of NIRS in neuroscience has been expanding (8). NIRS utilizes the absorption and scattering properties of light at wavelengths of approximately 650-950 nm, which is the so-called "optical window" into biological tissue (7, 22). The biological chromophores of primary interest are oxygenated (HbO) and deoxygenated hemoglobin (HbR), which absorb light in the near infrared spectrum. Thus, changes in detected light from a constant source reflect changes in HbO and HbR concentrations, assuming that the concentrations of other biological absorbing and scattering tissue are constant.

There are 3 technological approaches to NIRS (figure 1). Continuous-wave (CW)-NIRS, which measures the changes in attenuation of light from a constant light source (8). Frequency domain (FD)-NIRS uses light that is amplitude modulated at a high frequency (a few hundred MHz) and measures the reduction in amplitude and phase shift of the transmitted signal (26). Time-domain (TD)-NIRS measures the temporal distribution of photons produced when a short duration (a few picoseconds) pulse of light is transmitted through the object of interest (26). FD- and TD-NIRS are more complex systems, but can retrieve absolute values of oxygenation and cerebral blood value, whereas CW-NIRS can only retrieve relative concentrations of chromophores. Yet, CW-NIRS is the most widely used system in clinical neurology and neuroscience, including all studies in this thesis.



**Figure 1** Illustration of different near infrared spectroscopy approaches. I: intensity, φ: phase shift, M: modulation depth, t: time. Adapted from the PhD thesis "Development of a Time-Resolved Optical Tomography System for Neonatal Brain Imaging" by Florian E. W. Schmidt, 1999. Biomedical Optics Research Laboratory, University College London of Medical Physics and Bioengineering, England.

To quantify changes in HbO and HbR, the NIRS light intensity is converted to time series of variations in HbO and HbR concentrations using the modified Beer–Lambert law (MBLL) (27, 28):

$$\Delta OD(\lambda) = \Delta\mu a(\lambda)L\ell DPF(\lambda) = (\varepsilon HbO(\lambda)\Delta(HbO) + \varepsilon HbR(\lambda)\Delta(HbR)) \times L\ell DPF(\lambda)$$

$\Delta OD(\lambda)$ is change in optical density at a given wavelength, $\Delta\mu a(\lambda)$ is the corresponding change in tissue absorption, L is the separation between source and detector and $\ell DPF(\lambda)$ is the differential pathlength factor. The change in absorption is related to relative changes in concentrations of HbO and HbR by the extinction coefficients $\varepsilon HbO(\lambda)$ and $\varepsilon HbR(\lambda)$, which are wavelength dependent. If changes in optical density are measured with two different wavelengths, then the concentrations changes can be calculated based on two equations with two unknown.

When applying the MBLL in CW-NIRS in vivo in the human brain certain assumptions are violated, which affect the accuracy and quantification of HbO and HbR. The key assumptions are that monitored areas are homogeneous, and that changes are homogenous (8). These assumptions are wrong as the scalp, skull and brain are heterogeneous in composition, and since HbO changes predominate in the arterial compartment, whereas HbR changes predominate in the venous compartment. A theoretical and experimental study suggests that following a motor-task 76% of the entire ΔHbO signal and 19% of the entire ΔHbR signal arise from the cortical region (29). The remaining 24% and 81% of the ΔHbO and ΔHbR signals respectively originate from the pial veins located at the surface of the cortex, where a change in oxygen saturation takes place following brain activation (29). So when focal or global changes are elicited in the cortex by a specific stimulation, this will influence the magnitude of the change calculated and produce cross talk (30), which is an erroneous calculation of a change in one chromophore due to the real change in another. During functional NIRS studies it is also estimated, using source-detector distances between 2-5 cm, that the activated region only represents a 10 % fraction of the sampling region (31). These issues lead to a relative low spatial resolution for CW-NIRS compared to other vascular imaging, such as fMRI, PET and SPECT, which is contrasted by the excellent temporal resolution in CW-NIRS. It is also well known that the CW-NIRS signal is strongly contaminated by systemic interference occurring in the superficial layers of the head (32) and has in one study been estimated to account for 17% of the total NIRS signal (33). The contribution is dependent upon the experimental setting. Thus, in a quiet non-stressful environment functional changes in cortical HbO and HbR can be relatively easily measured via functional stimuli (34), but experiment inducing both systemic and cerebral effects may be very difficult to interpret. To minimize the skin contribution data from superficial 1 cm channel can be regressed out from the corresponding 3 cm channel (aiming at measuring only cortical vascular changes). Thus, experimental studies demonstrate that a single short separation channel produces an average reduction in noise of 33% for HbO, and by using two short separation measurements further increases the performance to an average noise reduction of 59% (35). NIRS is also very susceptible to signal artifacts caused by relative motion between the NIRS fibers and the scalp. These artifacts can be very damaging to the utility of NIRS, particularly in challenging patient groups where motion can be unavoidable. A number of approaches to the removal of motion artifacts from NIRS data have been suggested (36, 36, 37), and developments within this field are ongoing.
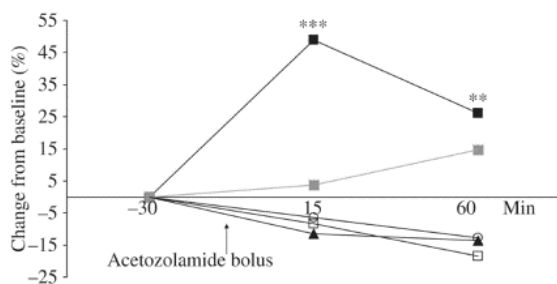
To sum up, when using CW-NIRS the degree of assumption violations and artifacts are very dependent upon the experimental setting, challenge and patient selection, which is very important to keep in mind when designing NIRS studies.

*NIRS and measurement of cerebral blood flow*
Given that NIRS is so easy to use, it would be of great benefit to use as a non-invasive system to estimate alterations in cerebral blood flow (CBF). CW-NIRS is currently being used in both routine surgical procedures and intensive care units, but there is insufficient data on the ability of NIRS to measure CBF in clinical settings (38). CW- NIRS does not directly measure CBF, and following acetazolamide only a small (4–5 %) increase in cerebral oxygenation is observed using NIRS (39-41). The primary problems with CW-NIRS are limitations based on the previous mentioned assumptions and artifacts, which deems it very difficult to measure CBF reliably.
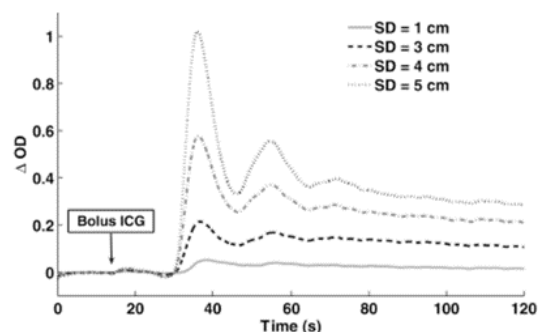
Alternative approaches have been used to obtain CBF markers with CW-NIRS. The use of the optical dye indocyanine green (ICG) as an intravascular tracer to measure relative changes in CBF with CW-NIRS has been suggested as an easy, safe and reliable method for serial bedside measurements of relative CBF changes (42, 43). The method is based on monitoring the first passage of an injected ICG bolus through the cerebral vasculature by NIRS, and is assumed to be a specific indicator of cerebral, not extracerebral, circulation (44). The accuracy of the method was confirmed in a piglet model, in which the NIRS blood flow index (BFI), a relative measure of CBF, significantly correlated with the gold standard microspheres technique (44). Human studies have shown that repeated measurements of BFI have an acceptable coefficient of variation during baseline recordings (43, 45, 46). However, no studies so far have correlated the BFI method to established methods of measuring CBF in humans, such as SPECT or PET. Furthermore, the vast majority of NIRS studies using an ICG bolus to estimate CBF were performed using CW systems and single source-detector (SD) distance separation configurations (43, 45, 47, 48). This method is known to be impaired by strong contamination of the cerebral signal from superficial tissue layers (49). As an alternative, Steinbrink et al (50) used a FD-NIRS system and Kohl-Bareis et al. (51) a TD-NIRS system to track the passage of an ICG bolus in healthy subjects and patients undergoing cardiopulmonary bypass respectively. By measuring modulation and time-of-flight of photons, they were able to get depth-resolved measurements of the bolus time course and therefore to distinguish between extra- and intracerebral signals. Both studies showed that a conventional single-distance CW analysis will fail to detect changes in CBF due to skin blood flow contamination.

To develop a new approach to detect changes in CBF using ICG as a tracer in combination with CW-NIRS Schytz et al. (52) investigated a multi-source detector separation configuration in order to assess a corrected BFI (BFIcorr) value, which attempts to eliminate contamination of skin blood flow. The BFIcorr method was compared simultaneously to the established method 133Xenon SPECT (133Xe-SPECT). Ten healthy subjects were investigated before and after a bolus of acetazolamide.

**Figure 2** Increase in percent from baseline for CBF of the middle cerebral artery (CBFMCA) (■) measured by single photon computer tomography (SPECT), blood flow index (BFI) (○), BFIcorr (□), indocyanine green (ICG) ICGmax (▲) and rise time (grey box) measured with continuous wave near infrared spectroscopy (CW-NIRS) after infusion of acetazolamide. A significant increase in CBFMCA was found 15 min (48.9 ±6.6, *** P < 0.001) and 60 min (26.0 ±6.8, ** P = 0.003) after the acetazolamide bolus.

The results showed no significant correlation between CBF changes measured by 133Xe-SPECT and BFIcorr (0.133, P = 0.732) (figure 2). After acetazolamide, a 49% increase in CBF was detected using the 133Xe-SPECT method, whereas no changes in any ICG variables were observed after acetazolamide. Thus, the study clearly showed, by comparing the ICG-NIRS method to the gold standard of measuring CBF, that it was not possible to obtain reliable BFI data with the ICG CW-NIRS method, even when using multi-source detectors to eliminate superficial contribution (52) . The NIRS results were not caused by technical problems as the quality of NIRS and dye-densitometer measurements recorded after every injection of ICG was high. Moreover, at longer SD separations, the BFI increased, and the absorption peak became narrower and arrived earlier (figure 3). Such a pattern is characteristic of the bolus time course in the brain, and is consistent with larger separations being more sensitive to brain, while the time course at short distance is more characteristic of ICG perfusion in the skin. One possible explanation for the failure of the ICG method could be a change in the relative contribution from skin and brain from one injection to the other, for instance through a change in the skin blood volume. It would be interesting to inject ICG into the internal carotid artery instead, but this would make the ICG method more invasive and difficult to apply in a clinical setting. Given that acetazolamide would also be expected to increase skin blood flow (53), it is very surprising that there were no changes in BFI at all. This also raises serious concerns about that ICG CW-NIRS method.



**Figure 3** Optical density curve after an indocyanine green (ICG) bolus. Colored lines show source detector pairs at different distances. Y axis: ΔOD = delta optical density.

The design by Schytz et al. (52) was different from previous studies using ICG dilution techniques, which measured baseline values without comparison with a conventional measurement technique and without inducing changes in blood flow (43, 45, 54). A recent NIRS study by Terborg et al. (55) in ischemic stroke patients showed a diminished BFI and increased time to peak (TTP) at the affected hemisphere. The authors also showed that TTP measured with NIRS correlated with TTP values assessed by perfusion weighted MRI (55). Oldag et al. (56) investigated cortical maps of TTP in patients unilaterally affected by severe stenosis or occlusion of the middle cerebral artery (MCA) acquired by multichannel NIRS after an ICG bolus. The authors demonstrated that TTP

was increased on the affected hemisphere in the distribution of the affected MCA in 9/10 patients (56). Again, a correlation between TTP NIRS values and MRI was found to be statistically significant (56). Thus, side to side comparisons using NIRS-ICG technique seems feasible and is also supported by Schytz et al. (52) who found no difference in ICG values between hemispheres within the healthy subjects, which suggests that the ICG values are not completely random and reflect brain perfusion. The Schytz et al. study was supported by Rothoerl et al. (57), who showed lack of correlation between ICG area under the curve and rise time compared with 133Xe-SPECT values found during baseline. Tachtsidis et al. (47) also failed to show any changes in ICG values after acetazolamide infusion using a single SD pair of 5 cm. In conclusion, ICG-CW-NIRS is not at present suitable as a method of detecting changes in CBF over time in a clinical setting, but may be used to detect hemisphere differences in stroke and carotid disease.

*NIRS and low frequency oscillations*

Over the last 10 years, an increasing number of studies have assessed spontaneous oscillations in velocity of the MCA (VMCA) using transcranial Doppler (TCD) (58). Spontaneous VMCA changes may be oscillations at respiratory and cardiac frequencies. However, numerous studies (59-67) have focused on changes in low frequency oscillations (LFOs), known as Mayer waves (68) centered around 0.1 Hz. Interventional studies have shown that neurogenic (69), metabolic (70) and myogenic (71) stimuli all affect LFOs. The etiology behind and physiological significance of spontaneous oscillations in cerebral vessels are not yet fully elucidated, but experimental studies indicate that spontaneous oscillations in cerebral blood flow reflect impaired cerebral autoregulation (58).

Measuring changes in VMCA is an indirect method of investigating oscillations, because fluctuations in CBF are caused by changes in small resistance vessels downstream from the MCA. As an alternative, some studies have demonstrated detection of LFOs in humans by NIRS (66, 72-75).  NIRS LFOs analysis have been investigated in different pathologies and shown that HbO LFOs amplitude are decreased in cerebral microangiopathy (74), upon hypercapnia (72) and declines with age (73, 76). During paced breathing in carotid disease the phase relation between the arterial blood pressure (ABP) and HbO is right shifted, which is identical to the ABP-VMCA phase relation (66). But whether ABP-VMCA and ABP-HbO are correlated during spontaneous breathing has not been investigated during.

The questions arises whether NIRS measurements of LFOs in the human brain is a reliable method to investigate cerebrovascular alterations? To answer this, the first step is to assess the variability between hemispheres and over time of NIRS LFOs. If the NIRS LFOs method is reliable, it may then become an important investigative tool in neurovascular diseases affecting one hemisphere, such as stroke and carotid artery disease, and by measuring changes in LFOs over time following disease development, aging or therapy. To investigate this, Phillip et al. (77) investigated hemispheric and day-to-day variations in 0.1 Hz LFOs using NIRS simultaneously with TCD during spontaneous and paced breathing (6 breaths per minute) on two separate days in 44 healthy subjects. The study aimed to determine the variations between hemispheres for phase shift and gain of both HbO and VMCA to the ABP (table 1).

| Phase | Hemispheres ABP | Day to day ABP | Hemispheres direct | Day to day direct |
|---|---|---|---|---|
| HbO | 3 (39) | -5 (61) | -4 (25) | -1 (32) |
| VMCA | -2 (57) | - 10 (45) | 5 (60) | 3 (46) |
| Paced HbO | -9 (70) | 2 (82) | -1 (55) | -7 (57) |
| Paced VMCA | 0 (19) | 1 (57) | 4 (42) | 2 (16) |
| Gain | Hemispheres ABP | Day to day ABP | Hemispheres direct | Day to day direct |
| HbO | -6 (69) | 2 (297) | 0.927 (44) | -2 (60) |
| VMCA | -8 (158) | -8 (166) | 0.828 (138) | 11 (141) |
| Paced HbO | -7 (120) | -3 (388) | 1.071 (105) | 9 (97) |
| Paced VMCA | -6 (83) | - 15 (235) | 0.890 (115) | 7 (77) |

**Table 1** Mean values and prediction intervals (±PI) between hemispheres and days in healthy subjects for spontaneous 0.1 Hz phase shift and gain between arterial blood pressure (ABP) and oxyhemoglobin (HbO) (measured by near infrared spectroscopy) as well as ABP and velocity of the middle cerebral artery (VMCA) (measured by transcranial Doppler). Mean values (±PI) are also shown for direct hemisphere HbO and VMCA phase shift and gain. The table also shows mean values (±PI) during paced breathing at 0.1 Hz for the same parameters. Mean values represent values measured on day 1 for between hemispheres and left side for ABP-HbO/VMCA between days. Mean gain values (±PI) are in percent.

The study also determined for both HbO and VMCA the direct left–right phase shifts (i.e. time difference between the two hemispheres LFOs) and gain (i.e. amplitude difference between the two hemispheres LFOs). The NIRS measurements were performed using CW-NIRS, and the optodes were placed bilateral on the forehead with one light source at two wavelengths (690 and 830 nm) and two detectors on each side, avoiding the midline sinus. The distance between source and detectors was 3 cm, with the detectors lateral to the source. Thus, the detectors measured the frontal cortex region in the territory supplied by the MCA. The study showed that spontaneous LFOs HbO phase shift and gain parameters varied less than LFOs VMCA, based on the prediction intervals, except between days for ABP-HbO/VMCA (table 1). The opposite pattern was seen for parameters at paced breathing, except that ABP-VMCA still showed less variation between days and the direct hemisphere variation was still discretely lower for HbO than VMCA. The study also demonstrated no linear relation between phase shift and gain of ABP-oxyHb and ABP-VMCA at rest, which was in contrast to paced breathing showing a significant linear relation for both phase and gain. The study did not show changes for any parameters in relation to age and gender.

At present there are no other studies comparing spontaneous LFOs variation between hemispheres and over days in healthy subjects using NIRS. Reinhard et al. (78) did show a significant correlation coefficient between days for VMCA LFOs phase and gain in patients with unilateral stenosis, which is difficult to directly compare to the Phillip et al. study in healthy subjects. Thus, the Phillip et al. study was important, as it showed that LFOs ABP-HbO vary less than LFOs ABP-VMCA when assessing between hemispheres, as well as HbO interhemispheric parameters directly and over time. The lack of linear relation between spontaneous ABP-VMCA and ABP-HbO may be due to local cortical autoregulatory mechanisms, which may be altered during disease. Paced breathing induces a linear correlation, which has also found by others (66). The linear relation is likely to be driven by $CO_2$ modulation (79) and changes in the intrathoracic pressure (66, 72). Furthermore, VMCA showed less variation during paced breathing, which also reflects that VMCA is more influenced than HbO by paced breathing. HbO LFOs amplitude has been shown to decline with age (76), and if ABP LFOs also decreases with age, the Philip et al. study would not be able to demonstrate a decrease of ABP-HbO gain with age, as the relation between ABP

and HbO amplitudes would remain constant. In support, Vermeij et al. (76), in a study published after the Philips et al. study, showed that both spontaneous HbO (measured with NIRS) and ABP LFOs amplitude are decreased in a group of older adults in comparison to younger adults. Furthermore, ABP-HbO LFOs phase and gain were not influenced by age. A caveat of the Phillips et al. study was that the superficial contribution to the LFOs was not assessed, which could have been obtained using a source detector distance of 1 cm as a control.

Overall, the Phillips et al. study was the first to investigate variations in NIRS LFOs oscillations in healthy subjects, and the results suggest that analysis of NIRS LFOs is a feasible tool upon assessing spontaneous LFOs in between groups and over time in neurological diseases. Further NIRS studies of spontaneous LFOs should therefore be applied in neurovascular diseases, and could also become a possible prognostic and treatment marker in cerebrovascular disease.

## NIRS IN MIGRAINE WITHOUT AURA
*Migraine – a neurovascular disorder?*
The underlying goal of much of the human research in primary headaches is to find a specific marker of the specific headache type and subtype. Migraine is suggested to be a neurovascular coupling disorder, but this is based on an assumption, derived from familial hemiplegic (FHM) mutations and animal models (4), and has not been fully proven in humans. So far NIRS has only been applied in the common migraine subtypes to investigate altered cortical vasomotor reactivity following different types of metabolic (10, 11, 80), physiological (12, 14), pharmacological (81) and functional tests (13).
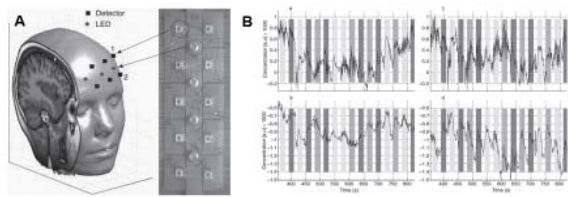
The breathhold test is a way to test vasoreactivity during changes in arterial $CO_2$ concentration (82). Using this, Akin et al. (10) showed that following breathhold tests in 6 migraine without aura (MO) patients interictally HbR, but not HbO, amplitude increases were smaller than in healthy controls. Liboni et al. (11) showed smaller HbO increases and lower HbR decreases in 30 MO patients than in healthy controls, but this result may have been biased by a shorter breath holding duration in MO patients compared to the healthy subjects. Another way of assessing vasomotor reactivity is via head down maneuver inducing elevated venous pressure and an increase in frontal lobe pressure, which usually leads to elevated CBF. This maneuver produced a significantly smaller increase in right-sided total hemoglobin (HbT, which is the product of HbO+HbR) in migraineurs when compared to volunteers (12). In migraineurs, the maneuver produced a significantly smaller increase in right-sided HbT than in left-sided HbT, but also produced a significantly greater increase in right-sided THb than in left-sided HbT in healthy volunteers (12). The patients were not coded as having MO and/or migraine with aura (MA) and the migraine attacks of some patients were mainly unilateral confined to the right or left, but the frequent side of the migraine attacks did not have any effect on the change in HbT. The lateralized vasoreactivity is difficult to understand, but could, speculatively, be attributed to a possible sympathetic lateralization in the right hemisphere and, conversely parasympathetic predominance in the left hemisphere (83, 84), even though it is not known if this may lead to asymmetric vasomotor responses. Another group (80) investigated within differences in MA patients in the interictal period patients showing an increased cerebral vasoreactivity to $CO_2$ inhalation measured by NIRS in the most frequent attack side. A recent study investigated 10 patients with MA, 10 with MO during the interictal period of migraine in comparison to healthy controls (85). The time-delay between the

R-wave of an electrocardiogram and the arterial pulse wave of cerebral microcirculation, detected by NIRS on the frontal cortex of both side, was determined to evaluate the presence of cerebral arteriolar vasoconstriction. Both migraine groups had a significantly longer time delay than the control subjects, but there were no differences between sides. The same group measured the amplitude of the arterial pulse wave in patients with prolonged aura (1 h to 7 days) and found during aura a significant decrease of the arterial pulse wave and an increase of cerebral tissue oxygen saturation ipsilateral to the pain side and contralateral to aura symptoms compared with the headache-free periods (14). Overall, the studies on vasomotor reactivity are few and the results do not all translate into one concept of altered vasomotor reactivity following metabolic and physiological changes in migraine patients. This is likely due to differences in design, NIRS system used and migraine subtype.

NIRS has also been used to detect pharmacological changes following sumatriptan, a 5HT1B-D agonist and effective in migraine treatment. Watanabe et al. (81) investigated 4 MO patients during attacks following subcutaneous sumatriptan injection in comparison to 4 subjects receiving sham injections. The authors showed following sumatriptan injection a simulatenous decrease in HbO measured by NIRS, which was correlated to changes in skin blood flow measured by laser Doppler flowmetry. In vivo human studies have not shown that sumatriptan constricts extracerebral arteries more than cerebral arteries (86-88), and the decrease found by Watanabe may therefore very likely be a systemic extracerebral effect. Thus, NIRS monitoring using scalp probes are probably not entirely brain specific, and the clinical value of this type of investigation, when systemic changes are expected, is questionable. Studies attempting to separate superficial and cerebral signals would be useful to further explore these types of pharmacological effects.
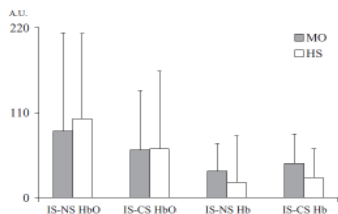
*Neurovascular investigation - Stroop test*
Migraine has been suggested to be a neurovascular coupling disorder (4, 89), and NIRS has an excellent temporal resolution that can detect specific cortical vascular changes during brain activation (34) and correlates with evoked potentials (90). Therefore, NIRS may be an ideal tool to investigate neurovascular coupling among migraine patients. To investigate if neurovascular coupling may be altered in migraine without aura in the interictal state, Schytz et al. (91) investigated 12 MO patients aura in comparison to 13 healthy controls during activation of the prefrontal cortex interictally. The activation consisted of neutral, congruent and incongruent Stroop tests, which is known to be an efficient and consistent activator of the prefrontal cortex (92, 93). CW-NIRS was used with light sources and detectors giving a total of 16 channels in an area of 20 × 7 cm of the forehead, and the center bottom light was placed on the Fz position of the international 10–20 EEG system.

**Figure 4**
A: Near-infrared spectroscopy (NIRS) light sources and detectors in 16-channel arrangement covering the forehead in a 20×7 cm area.  B: Representative Stroop data obtained from one subject. (a) Averaged oxy-haemoglobin (HbO) data from left lateral detectors (1–4). (b) Averaged deoxyhaemoglobin (HbR) data from left lateral detectors. (c) Averaged HbO data from right lateral detectors (13–16). (d) Averaged HbR data from right lateral detectors. Shaded areas denote the trial blocks, from dark (neutral) to light (incongruent).

The study showed no changes in error rate or reaction time between the groups and showed identical responses in terms of magnitude to changes in HbO and HbR (91) (figure 5). The latency was also identical, except on the right side for HbO for one parameter (incongruent task subtracted by the congruent task), in which the latency response was slower among healthy controls. The significant result was found in only one out of four HbO parameters concerning latency, and was therefore likely due to a type 1 error. Curiously, the response to the Stroop test showed an increase in HbO and HbR in both MO patients and healthy controls, which is not the usual response in functional NIRS (HbO ↑ and HbR ↓). This may have been related to cross-talk between HbO and HbR, since the NIRS system used 730 and 830 nm wavelength. Hence, Uludağ et al. (94) has shown that cross talk is low if one wavelength is below 720 nm, and this may explain the unusual hemodynamic response in the study. Overall, the study pointed to intact neurovascular coupling in the pre-frontal cortex interictally among MO patients (91).



**Figure 5** Magnitude (+1 SD error bar) in arbitrary units (A.U.) of oxyhemoglobin (HbO2) and deoxyhemoglobin (Hb) response to Stroop tasks in migraine without aura (MO) patients and healthy subjects (HS) on the right side of the frontal cortex. IS, incongruent Stroop task; NS, neutral Stroop task; CS, congruent Stroop task.

It is possible that neurovascular coupling alterations may be found in other brain areas, such as the visual cortex, which may be more relevant to investigate given that aura symptoms, and thereby likely CSD, are more frequent elicited from the occipital cortex. However, a recent NIRS study by Coutts et al. (13) characterized the hemodynamic response to visual stimuli in 15 MA, 5 MO patients and healthy controls. The authors found no differences between migraine and healthy controls for amplitude, but did show a decreased latency in healthy controls compared to migraine patients during checkerboard stimulation. However, latency differences were not seen when individually adapted color lenses were applied in order to attenuate visual discomfort. Thus, differences in time lag between migraine patients and controls do not suggest neurovascular coupling alterations in

migraine, but can be interpreted as a marker of photophobia in migraine patients.
To sum up, the present two NIRS studies investigating functional changes in migraine patients interictally showed no signs of a general neurovascular coupling disorder in frontal or occipital areas (13, 91). It would be of great interest to study changes in neurovascular coupling and/or neuronal excitation between the ictal and interictal phase, which could be done combining EEG and NIRS to truly measure the neurovascular coupling.

**NIRS IN FAMILIAL HEMIPLEGIC MIGRAINE**
Hemiplegic migraine is a rare migraine subtype in which the aura symptoms include transient hemiplegia, which may last up to 72 h (95). Hemiplegic migraine is further subdivided into familial hemiplegic migraine (FHM) and sporadic hemiplegic migraine (SHM). Genetic investigations has specified the mutations: in FHM1 there are mutations in the CACNA1A gene (coding for a calcium channel) on chromosome 19 (96); in FHM2 there are mutations in the ATP1A2 gene (coding for a K/Na-ATPase) on chromosome 1 (97); and in FHM3 there are mutations in the SCN1A gene (coding for a sodium channel) on chromosome 2 (98). Thus, all known FHM mutations encode proteins affecting ion transport in the brain, and analysis of these mutations have identified glutamatergic neurotransmission in migraine (99). FHM also exists in a form, where the FHM1-3 mutations are not found (95). Whether FHM without known mutations represents one or more FHM subtypes remain to be investigated.
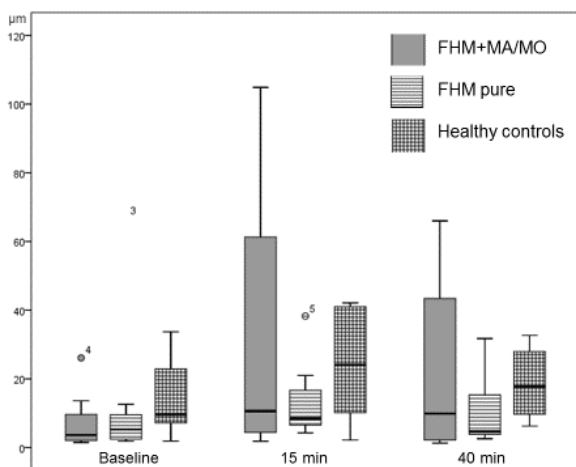
*Functional consequences of FHM mutations*
It is of great interest to further explore the functional consequences of the FHM mutations to gain more knowledge on the prolonged aura symptoms and to develop effective anti-migraine treatment. The functional consequences of FHM have been investigated in human provocation studies (100-102). The FHM studies with known mutations revealed that the pharmacological migraine trigger and nitric oxide (NO) donor glyceryl trinitrate (GTN) did not induce more migraine like attacks compared to controls (100, 101). This in contrast to previous experiments showing that GTN triggers migraine in a majority of patients with MA and MO (103-105). Interestingly, FHM patients without known mutation and with co-existing MA/MO are more sensitive to the migraine inducing effects of GTN infusion compared to controls, whereas the patients with the pure FHM phenotype are not (102). The difference in GTN responses could be explained by an arterial hypersensitivity to NO in FHM patients with coexisting MO/MA, which have previously been suggested to exist in MO patients (35).

*FHM and cortical vascular reactivity*
Using NIRS it is possible to study the cortical vascular reactivity in FHM, which is of interest given the prolonged aura symptoms in FHM. Analysis of spontaneous LFO offers the oppurtunity to investigate the FHM patients at rest with as few stressors as possible. In addition, LFOs are modulated by NO (70, 106). Thus, investigation of LFOs changes following NO-donor infusion in FHM is a way to explore the functional consequence of FHM. Schytz et al. (107) therefore explored changes in LFO following intravenous infusion of GTN in 9 healthy controls, 12 patients with pure FHM and then 11 FHM patients with co-occurring MO and/or MA (5 MA, 2 MO and 4 both MA & MO). The study used CW-NIRS with optodes placed bilaterally on the forehead with one source (2 wavelengths: 690 nm and 830 nm) and two detectors on each side with a source-detector distance of 3 cm. The study showed

that GTN increased the LFOs amplitude in FHM patients, but not healthy controls. Furthermore, only in FHM patients with coexisting common migraine types did GTN infusion induced changes in LFO amplitudes (P < .001), where post-hoc analysis revealed an increase in LFO amplitude 15 minutes (P = .003) and 40 (P = .013) minutes after start of infusion compared with baseline (figure 6). There were no correlation between LFOs amplitude changes and blood pressure or heart rate changes.

The findings by Schytz et al. support the concept that the sensitivity to NO resides within the common migraine phenotypes rather than the FHM phenotype. The study was the first to show a cortical vascular marker in line with the headache results (107). Thus, cortical LFOs may be a marker of NO hypersensitivity in MO/MA, but not FHM. This also suggests, that NO-inhibition is probably not effective as treatment in FHM. The study would have benefitted from having a control group with MO/MA patients without FHM, in order to show GTN induced LFOs changes as a marker of these common migraine subtypes. Another concern is that FHM without mutations is a heterogeneous group with potentially different mutations, which might have different functional consequences. Even though there were no correlation between systemic blood pressure and heart rate, it is possible that the changes found in the study reflect changes in systemic LFOs, and the study would have benefited from having a control source detector to control for changes in superficial skin LFOs. As the study was the only of its kind in humans there is no literature to confirm or challenge the findings. This was the first time a NO donor was given systemically to detect LFOs amplitude changes of cortical vessels in human. The results were in contrast to experimental animal models, where NO synthase inhibitors increase cerebral LFOs amplitude (70, 106). This difference may be due to NO synthase inhibitors constricting animal cephalic vessels, decreasing cerebral blood flow by 18% (108) and raising systemic blood pressure by 26% (70). The increase in LFOs amplitude among FHM patients with co-morbidity may also be explained by a reflex increase in sympathetic tone (109), though there were no significant correlation between changes in systemic hemodynamics and the LFOs amplitude changes (107).



**Figure 6** Boxplots during baseline, 15 min from infusion start and 40 min after infusion start for familial hemiplegic migraine (FHM) with co-occurring migraine with aura (MA) and migraine without aura (MO), abbreviated FHM+MA/MO (grey boxes), pure FHM (striped boxes) and healthy controls (dotted boxes). Band near the middle is the median value. The bottom and top of the boxes is the 25th and 75th percentile. The ends of the whiskers represent the maximum and minimum of the data. Y-axis units are in micromoles (μm). Outliers are shown except one

extreme outlier in FHM+MA/MO patients (15 min: 233 micromoles) and two extreme outliers in healthy subjects (15 min: 216 micromoles; 40 min: 130 micromoles). For FHM+MA/MO patients there were an increase in LFO amplitude 15 minutes (P = 0.003) and 40 (P = 0.013) minutes after start of infusion compared with baseline.

In conclusion, the study revealed interesting vascular differences within migraine subtypes, which were very much in line with headache results from the same population. As the design was novel, and the investigated population rare, it would be of great interest to investigate the model in other migraine subtypes to possibly confirm the results. Thus, further studies are very much warranted to investigate the effects of NO on LFOs measured with NIRS.

**NIRS IN CLUSTER HEADACHE**

Cluster headache (CH) is one of the most painful primary headaches characterized by attacks of severe or very severe unilateral orbital, supraorbital and/or temporal pain associated with ipsilateral cranial autonomic symptoms (95). The autonomic symptoms during attacks are due to outflow from the superior salivatory nucleus (SSN) via the sphenopalatine ganglion (SPG). It has also been shown that attacks involve activation in the posterior hypothalamus (110).
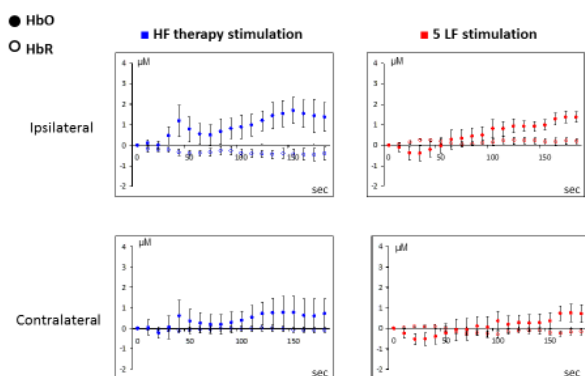
*A pronociceptive role of the parasympathetic nervous system in cluster headache*
The parasympathetic features define the cluster attacks and are easy to detect clinically, but the interesting question is whether parasympathetic outflow has a role in the development of pain during a cluster attack. There are experimental evidence for the SPG to induce pain; 1) activity in meningeal nociceptros are modulated by efferent parasympathetic SPG fibers (111), 2) acetylcholine, a parasympathetic neurotransmitter, can activate C-fibers (112), 3) NO, which is found in parasympathetic perivascular nervefibers (113), can induce cluster attacks and sensitize dural nociceptors (114), 4) SPG activation lead to dural plasma extravasation (115). In addition, a recent animal cluster headache model showed that stimulation of the SSN using frequencies between 10-50 Hz leads to firing of second order neurons in the trigeminal nucleus caudalis (TNC) in rats, which can be explained by an increased parasympathetic outflow via the SPG (116). Collectively, experimental data suggest that cranial parasympathetic outflow may in fact initiate and/or sustain head pain during CH attacks.

*Experimental studies of sphenopalatine ganglion stimulation*
Recently, Schoenen et al. (117) conducted a multicenter-trial to investigate the therapeutic response in chronic CH patients to high frequency (HF) SPG stimulation. The study reported that pain relief was achieved in 67 % of full stimulation-treated attacks compared to 7 % of sham-treated (117). HF SPG stimulation may primarily activate parasympathetic neurons or pre/post ganglionic parasympathetic nerve fibers well above the intrinsic firing rates, which may physiologically block parasympathetic outflow, and result in an acute effect on head pain and autonomic symptoms. The HF therapeutic SPG stimulation used in the multicenter study was applied for at least 15 minutes during an attack. In contrast, low frequency (LF) SPG stimulation may correspond to a more intrinsic pattern, which would possibly lead to increased parasympathetic outflow and subsequent head pain. The rationale for LF stimulation is based on animal studies demonstrating ipsilateral dilatation after SPG activation using electrical stimula-

tion (range 5 – 60 Hz) of the middle cerebral artery (MCA) (118), small pial arteries (119), internal carotid artery (ICA) (118), anterior carotid artery (ACA) (118) and increased ipsilateral cortical blood flow (120). Schytz et al. (121) therefore conducted a double-blind randomized cross-over study to record headache characteristics and autonomic symptoms during and after HF or LF SPG stimulation using the same neurostimulation system as Schoenen et al. (117). To record changes in scalp and cortical vessels NIRS optodes were placed bilaterally on the forehead with one source (two wavelengths: 690 nm and 830nm) and two detectors on each side, carefully avoiding the midline sinus. The two detectors were placed with a distance of 1 and 3 cm lateral from the source. Thus, the 1 cm detector was measuring frontal scalp territory primarily supplied by the superficial temporal artery, while the 3 cm detector was measuring mixed contribution from the scalp territory and the frontal cortex territory supplied by the anterior cerebral artery (ACA) and middle cerebral artery (MCA). To minimize contribution of the scalp physiology, the data from each 1 cm channel were regressed out from the corresponding measurement at 3 cm. The study demonstrated that three out of six patients (50 %) reported ipsilateral cluster-like attacks during or within 30 min of LF SPG stimulation, whereas one out of six reported a cluster-like attack with 3 min HF SPG stimulation. The superficial NIRS recordings showed an increase (3.0 ± 1.1 μM) (mean ± SEM) for HbO following stimulation, which was most pronounced on the ipsilateral side following HF experimental stimulation. The NIRS recordings primarily assessing cortical vascular changes showed a moderate increase, which was most pronounced (1.7 ± 0.6 μM) for HbO on the ipsilateral side following HF experimental stimulation. For all compartments HbR tended to stay constant or decrease following both HF and LF stimulation (figure 7).



**Figure 7** Continuous wave near infrared spectroscopy changes with normalized relative oxygenated hemoglobin (HbO) and deoxygenated hemoglobin (HbR) values during 3 min high frequency (HF) therapy stimulation (range 80–120 Hz) and low frequency (LF) stimulation at 5 Hz. 3 cm optodes measuring cortical changes in which the signal from 1 cm optodes have been regressed out. Y-axis is micromolar (μM) concentration. Error bars show SEM. There was a moderate increase, which was most pronounced (1.7 (±0.6)) on the ipsilateral side following HF stimulation.

The study also showed a decrease in VMCA following both HF and LF stimulation, which was most pronounced on the ipsilateral side and following LF stimulation. The NIRS recordings showed changes, which would be expected to be seen during an increase in CBF (HbO↑ and HbR↓) and are therefore in line with the experimental animal data. The study has some limitations as the population, although unique, was very small. The vascular data are therefore descriptive and needs to be reproduced in larger

studies, where repeated measurements are applied. Future studies should attempt to focus on demonstrating frequency-response curves for the hemodynamic parameters. The individual changes in HbO and HbR did not have a distinct pattern, which could explain why some patient experienced pain. It was surprising that HF and LF stimulation caused similar vascular changes. This may reflect that 3 min stimulation causes depletion of parasympathetic neurotransmitters irrespective of HF or LF stimulation, whereas HF stimulation has to be ongoing for at least 10 minutes to lead into a physiological block. It is of great interest to investigate objective vascular markers following SPG stimulation, as a reliable marker can be used during surgery to confirm the correct placement and to find the best possible treatment setting. In summary, the study by Schytz et al. (121) demonstrated for the first time the feasibility of NIRS recordings following SPG neurostimulation in humans and showed hemodynamic changes, which were similar to experimental animal studies.

**NIRS IN OBSTRUCTIVE SLEEP APNEA**
Obstructive sleep apnea (OSA) is characterized by repetitive episodes of partial or complete upper airway obstruction during sleep associated with intermittent oxygen desaturation, snoring and sleep fragmentation (122). The prevalence of OSA has been reported up to 33 % (123) in the general population and is strongly associated with transitory ischemic attacks (TIA) (15) and cerebral infarction (15-17) independent of other risk factors. During apnea periods, NIRS has been used to measure changes in relative hemoglobin parameters and cerebral oxygenation. HbO decreases during obstructive apnea periods and is correlated to peripheral oxygen concentrations (18-20), whereas both HbT and HbR increase (18, 21). Outside of the apnea brain tissue oxygenation is decreased during general sleep compared to the awake state, and oxygen levels are lower in patients with severe OSA compared to those with mild and moderate OSA (124). Overall, the NIRS studies in the OSA field are congruent and point to a deficient autoregulatory mechanisms during apneas, in which CBF fails to compensate for the lack of respiration, resulting in a decreased HbO and increased in HbR. This is also in line with the fact that brain activity decreases during OSA (125). These findings demonstrate the utility of NIRS and may point to the increased susceptibility to ischemic stroke during sleep. In fact, one study demonstrated that patients with wake-up stroke had larger apnea hypopnea index (AHI) than non wake-up stroke patients (126). However, the above OSA NIRS studies are limited to apnea periods, and do not explore changes such as arterial stiffness and sympathetic tone in the awake phase.

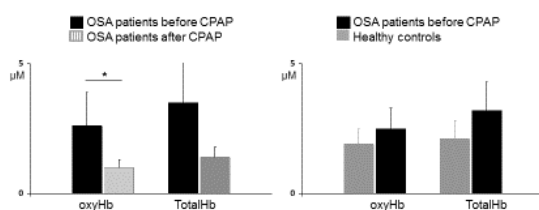*Obstructive sleep apnea and the autonomic nervous system*
OSA is associated with increased sympathetic activity, which has been shown in animal and human models (127). In rats hypoxia for 7 h per day for 30 days leads to increased blood pressure, which can be blocked by chemical denervation of the peripheral sympathetic nervous system (128). Mice exposed to hypoxia for 8 h per day leads to increased levels of urine catecholamine after 14 days (129). In OSA patients urine catecholamine levels are elevated throughout the day as compared to controls (130) and awake OSA patients show elevated muscle sympathetic nerve activity and plasma norepinephrine compared to controls (131). Effective nasal continuous positive airway therapy (CPAP) reduces the cardiovascular outcomes associated with OSA (17), but the mechanism behind this effect is unclear. It could be speculated that the effect may be due to a reduction in sympathetic nerve activity. Thus, randomized controlled studies have reported re-

duced 24 h catecholamine excretion in OSA patients after CPAP therapy for 4-6 months (132, 133). A possible linkage between stroke risk and sympathetic activity is not yet clear, but may be related to increased systemic arterial stiffness correlated to increased sympathetic tone. Arterial stiffness is obtained via augmentation index, which measures how fast a pulse wave is returned from the periphery, the faster the pulse wave, the stiffer is the artery. A randomized trial with 4 weeks of CPAP therapy showed reduced systemic arterial stiffness (133), which was also shown in two other trial with 4 (132) and 6 (134) months of CPAP treatment. The changes in arterial stiffens found in the above studies are found in systemic vessels, which raises the question if cerebral vessels might also be affected in OSA patients?

*Sympathetic alteration in cerebral vessels following CPAP therapy*
Experimental animal models have shown increased cortical LFOs amplitude following sympathetic alterations (135, 136). In humans, Tachtsidis et al. (75) measured increased HbO LFOs amplitude with NIRS upon posture-dependent activation of the autonomic reflex. Furthermore, Pierro et al. (137) investigated LFOs amplitude in healthy subjects and showed that HbO and HbR LFOs amplitude decreased during non-rapid eye movement (non-REM) sleep as opposed to the awake and REM sleep states. This can be interpreted as a sign of decreased sympathetic activity during non-REM sleep, which is accordance with heart rate variability spectral analysis showing higher parasympathetic drive during non-REM sleep as opposed to REM sleep (138). Thus, NIRS may a tool to investigate changes in sympathetic activity using LFOs amplitude analysis. Based on this, Schytz et al. (139) investigated if OSA patients showed signs of increased sympathetic activity and decreased vasoreactivity in cerebral cortical vessels as measured with NIRS, and if this may be reversed by CPAP treatment. In total 23 OSA patients naive to CPAP were included in a prospective interventional study, where the OSA patients received CPAP therapy for at least two months. HbO LFOs amplitude at rest and HbO vasoreactivity during a breath hold test were measured with NIRS and compared between baseline, after CPAP treatment and to 13 healthy controls The NIRS optodes were placed bilaterally on the forehead with one source (two wavelengths: 690 nm and 830 nm) and two detectors on each side, avoiding the frontal sinus. The distance between sources and detectors were three cm with the detectors lateral to the source. The study showed a decrease in LFO amplitude after CPAP therapy (P = 0.022) in OSA patients, but no differences in LFO amplitudes between untreated OSA patients and healthy controls (P = 0.934) (139) (figure 8). In addition, there were no differences in peak vascular response following breath hold tests in OSA patients before or after CPAP therapy or compared to healthy controls (139).



**Figure 8** Low frequency oscillation amplitudes of oxygenated hemoglobin (oxyHb) and total hemoglobin (totalHb) for 14 obstructive sleep apnea (OSA) patients before and after continuous positive airway pressure (CPAP) treatment for at least two months. 23 OSA patients before CPAP treatment were also compared to 13 healthy controls, right sided figure. Error bars are standard error of the mean. μM = micromolar oxyHb or totalHb concentration. Black boxes are OSA patients before CPAP treat-

ment, striped grey boxes are OSA patients after CPAP treatment, and grey boxes are healthy subjects. *Indicates a P value = 0.022.

Interestingly, the study showed no differences between healthy subjects and untreated OSA patients (139). It is possible that sympathetic activity and arterial stiffness have opposite effect on LFOs amplitude (sympathetic activity increases, while stiffness decreases LFOs amplitude), which may hide the pathological effect in OSA patients in comparison to healthy subjects. The study showed no change in vasoreactivity following CPAP, as measured with the breathhold test, which suggests that cortical arterial stiffness was either not present or not modulated by CPAP. The study did not control for the superficial contribution to the changes in LFOs, and it is possible that the LFO alterations are actually systemic (139). In fact, the LFOs amplitude between different sleep and awake states by Pierro et al. (138) was measured with 4 source-detector pair, in which the HbO amplitude did not vary between different source-detector distances, a finding also noticed by Obrig et al. (72). However, as suggested by Obrig et al. (72), LFOs at 3 cm are modulated following specific cortical activation, and if the oscillations were to solely stem from the skin, their magnitude should be smaller when sampling with a larger probe separation, due to a partial volume effect. The study showed quite large variations between sessions and groups, which may blunt smaller alterations between groups and treatment. Furthermore, it would have been desirable to have a non-compliant OSA group to show that LFOs amplitude is unchanged over time, when CPAP therapy is not administered.
In summary, Schytz et al. (139) showed for the first time in awake OSA patients a decrease in HbO LFOs amplitude following successful CPAP treatment measured with NIRS. This may be a marker of decreased sympathetic activity in cortical vessels and is a potential source of increased stroke risk in OSA patients. Further studies are needed to elucidate this novel finding further.

**NIRS - NEW TECHNOLOGIES**
So far, NIRS cannot measure changes in CBF in a reliable way, which may be due to the limitations of CW-NIRS. Schytz et al. (52), as previously described, failed to detect obvious changes in CBF using ICG after acetazolamide injection. Even TD- or FD-NIRS techniques have not yet been able to reliably detect changes in CBF over time in a clinical setting. The need for non-invasive CBF measurements are of obvious interest and new technological approaches must be pursued to reach this.
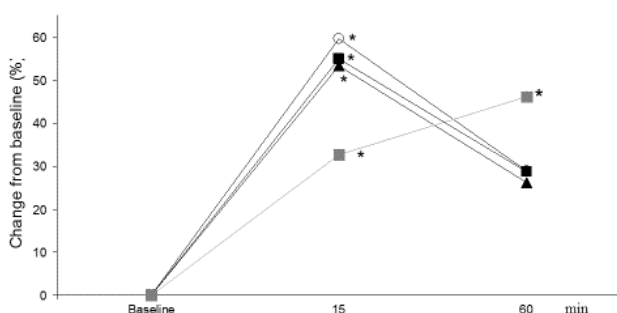
*Ultrasound tagged near infrared spectroscopy*
 In 1998 Mahan et al. (140) presented a new non-invasive hybrid technology employing ultrasound tagged NIRS (UT-NIRS). This technique may estimate changes in CBF using a cerebral flow index (CFI) without the need of an injected tracer, but the technology has not yet been used clinically or in human experiments. Recently, a commercial available system was FDA and CE cleared (CerOx, Ornim Medical Ltd., Israel). This system consists of a single probe, which illuminates the tissue with coherent light at three wavelength between 780-830nm and collects the scattered light back to the detector, placed at a distance of 12 mm from the source. The probe also incorporates a small ultrasound transducer that provides low-power waves for inducing the UT-NIRS signal.  UT-NIRS is a hybrid technology based on locally modulating coherent light (laser) with a localized low power ultrasound wave via the acousto-optic effect (140, 141). The ultrasound signal is a sequence of phase modulated waves with a central frequency at 1 MHz. This frequency is similar to the frequency (2

MHz) of transcranial Doppler ultrasound (TCD) used to monitor blood flow velocity through the skull, and is less attenuated by the bone than 2 MHz. The ultrasound signal induces a modulation in the detected light intensity, which is correlated with the pattern of the transmitted ultrasound wave as a function of the time of propagation of the ultrasound wave. In biological tissue the changes in the cross correlation signal is assumed to be due to movement of scattering particles (namely blood cells), in which an increase in blood flow results in poorer correlation. By measuring the degree of local correlation over the random fluctuations, UT-NIRS flowmetry is assumed to measure blood flow in the microcirculation in deep tissue in volumes of about 1cm3. The blood flow signal is independent of the specific wavelength of light, and consequently is independent of the oxygen saturation signal. The UT-NIRS probe collects light from all depths and the signal processing algorithm selects a certain segment of the signal (corresponding to a predefined distance from the skin) for calculating the regional CFI. Consequently, the UT-NIRS signal does not require as large a source-detector separation as conventional NIRS systems to reach gray matter vasculature, and a 12 mm separation provides brain tissue perfusion (primarily grey matter).

*The first experimental human study of cerebral blood flow using ultrasound tagged NIRS*
The CerOx is a new system based on a novel principle. It is highly needed to test the system in a clinical setting, and compare with known and established technologies to measure CBF. Therefore, Schytz et al. (142) tested if this new hybrid technique, combining NIRS and ultrasound, could detect CBF changes in healthy subjects. The study investigated changes over time for UT-NIRS CFI and 133XE-SPECT CBF data assessed in 10 healthy volunteers after an intravenous bolus of acetazolamide. UT-NIRS CFI was measured continuously, whereas SPECT CBF was measured at baseline, 15 and 60 min after acetazolamide. CFI showed a significant increase of 33 % at 15 min and 43% at 60 min after acetazolamide injection (142). The global CBF measured with SPECT (which closely followed local CBF changes in the MCA and ACA regions) showed a significant increase of 53% at 15 min and 36% at 60 min after acetazolamide injection (figure 9) (142). There was a significant correlation between CFI and SPECT CBF values (r = 0.67 & P < 0.033) at 15 min, but not at 60 min (P ≥ 0.777) (142). Thus, the study showed an increase in CFI, which correlated with CBF measured with 133Xe-SPECT at 15 min, but not 60 min (142).



**Figure 9** Relative percent changes in cerebral flow index (CFI) measured with ultrasound tagged NIRS and CBF measured with single photon computer tomography (SPECT) 15 and 60 min after acetazolamide. Mean increase from baseline (%) for CFI (grey square), CBFglobal (black triangle), CBF of the anterior cerebral artery CBFACA (open circle) and CBF of the middle cerebral artery (CBFMCA) (black square). Values represent average for all subjects. *P < 0.011 after acetazolamide as compared to baseline.

The Schytz et al. study (142) was the first controlled study to explore the clinical feasibility of measuring a CFI using UT-NIRS technique, which can be used as a marker of cortical CBF below the probes. There are no human or animal studies to compare the obtained results with. One caveat of the study is that it was necessary to remove movement artifacts post-hoc, which is not practical and feasible to do in a clinical setting. This issue may be overcome by developing probes less sensitive to movement. It was unexpected that CFI values increased between 15 and 60 min, and based on the present and previous study it would be expected that CBF decreased between 15 and 60 min post-injection (52, 142). As the use of 133XE-SPECT mandates a wash-out period of the tracer, it is a shortcoming that there is a lack of a comparator, which can track CBF changes over time noninvasively. In future studies it would be of clear benefit to investigate CBF for more than 60 min based on the present findings. It may be a technical issue, such as drift of the signal or undetected motion artifact (as it becomes increasingly difficult for the subjects to lie still for up to at least 60 min). Other explanations may also account for the discrepancy between SPECT and UT-NIRS values at 60 min. Thus, normal CBF in the human brain is approximately 50 mL/100 g/min averaged over the brain volume, with blood flow to the grey matter higher (80 mL/100 g/min) than to the white matter (20 mL/100 g/min). SPECT slices encompass a larger region of interest, while the UT-NIRS CFI signal is derived from a much smaller tissue volume of about 1cm3 encompassing mostly grey matter (cortex). Bruhn et al. (143) demonstrated, using MRI imaging, that post acetazolamide the changes in their signal in the white matter were different from those observed in the grey matter (cortex). So it is possible that the discrepancy is caused by the magnitude or time course of blood flow differ between grey and white matter following acetazolamide. Furthermore, SPECT measures CBF in the overall vascular regions of interest from transactional slices, whereas UT-NIRS measures microcirculatory blood flow within the interrogated volume of 1cm3. This quantity is affected by the diameter of the mixed vessels, the velocity of the scattering particles (blood cells) and their concentration. Therefore, changes in vessel diameter and flow velocity may be reflected differently by the two methods. Nevertheless, the obvious mismatch at 60 min between CFI and SPECT CBF values cannot be clearly explained (142), and further studies are highly needed to investigate this.

**CONCLUDING REMARKS AND FUTURE PERSPECTIVES**
New imaging methods are needed to further explore pathophysiology and treatment effects in neurology. Near infrared spectroscopy (NIRS) has a number of advantages that makes it an attractive investigate tool in clinical neurology. NIRS also has some inherent limitations, and it is therefore crucial that NIRS is used and interpreted correctly. This thesis shows that continuous wave NIRS (CW-NIRS) cannot reliably be used as a marker of cerebral blood flow (CBF), even with the use of multi-source detectors and indocyanine green (ICG) as an intravenous contrast agent. Instead, side to side comparisons using NIRS-ICG technique is a possible approach in neurovascular diseases with unilateral affection such as stroke and carotid artery disease. The study on low frequency oscillations (LFOs) in cortical vessels suggests that analysis of NIRS LFOs is a feasible tool upon assessing LFOs in neurological diseases between hemispheres and over time. In addition, detection of a linear relationship between velocity of the middle cerebral artery (VMCA) and oxygenated hemoglobin (HbO) in the LFOs spectrum may be a marker of defect local autoregulatory function. The study on neurovascular coupling in

migraine without aura (MO) patients reveals no apparent inter-ictal neurovascular coupling disorder. Future studies are needed to further explore this, and whether there might be changes ictally. The NIRS study in familial hemiplegic migraine (FHM) reveals a different cortical vascular response to nitric oxide (NO) depending on the specific subtypes, which supports differences in headache sensitivity to NO within primary headache subtypes. Thus, the study indicates that LFOs alterations in cortical vessels following infusion of vasoactive substances are a marker of an underlying difference in pathophysiology between migraine sub-types. For the first time a study is presented, which indicates that NIRS can measure cephalic vascular changes following stimulation of a parasympathetic ganglion. Future studies should attempt, with larger subject numbers, to examine other differences in headache and vascular function to explore the underlying patho-physiological mechanisms. Obstructive sleep apnea (OSA) pa-tients have increased sympathetic tone that decreases following continuous positive airway pressure (CPAP) treatment, and it is shown that this state might also be detected in cortical LFOs measured with NIRS. Future studies could investigate if the de-gree of OSA severity is correlated to the LFOs amplitude, and if LFOs amplitude may be a stroke predictor. The study using the hybrid technique ultrasound tagged NIRS (UT-NIRS) suggests that CBF estimation may be possible, but as the study is the first hu-man clinical study using UT-NIRS further methodological and clinical and explorative studies are necessarily needed. The thesis demonstrates various approaches to study neurovascular dis-eases using NIRS. It is possible to perform these studies, as NIRS is an easy non-invasive technique to apply and analyze in human experiments. The thesis also demonstrates technical and theo-retical challenges upon using NIRS. NIRS could increasingly be-come a more valuable tool in clinical neurology and research if future studies carefully deals with these limitations and chal-lenges. If so, NIRS will possibly lead to a new and deeper under-standing of the human brain and neurovascular diseases. Overall, the present thesis contributes to our understanding of disease states in clinical neurology and shows several novel approaches by which NIRS may be used in clinical neurology.

## SUMMARY

The purpose of this thesis was to explore and develop methods, where continuous wave near infrared spectroscopy (CW-NIRS) can be applied in different neurovascular diseases, in order to find biological markers that are useful in clinical neurology. To develop a new method to detect changes in cerebral blood flow (CBF), the first study investigated a multi-source detector separa-tion configuration and indocyanine green (ICG) as a tracer to calculate a corrected blood flow index (BFI) value. The study showed no correlation between CBF changes measured by 133Xenon single photon emission computer tomography (133Xe-SPECT) and the corrected BFI value. It was concluded, that it was not possible to obtain reliable BFI data with the ICG CW-NIRS method. NIRS measurements of low frequency oscillations (LFOs) may be a reliable method to investigate vascular alterations in neurovascular diseases, but this requires an acceptable LFOs variation between hemispheres and over time in the healthy brain. The second study therefore investigated day-to-day and hemispheric variations in LFOs with NIRS. It was shown that NIRS might be useful in assessing LFOs between hemispheres, as well as interhemispheric phase and gain directly and over time. Mi-graine may be associated with persistent impairment of neurovascular coupling, but there is no experimental evidence to support this. The third study therefore investigated interictal neurovascular coupling during a mental task by a Stroop test in migraine without aura (MO) patients, which is the most common type of migraine. The study showed intact neurovascular coupling in the prefrontal cortex outside of attacks in patients with MO. The fourth study aimed to investigate possible changes in LFOs amplitude following nitric oxide (NO) donor infusion in familial hemiplegic migraine (FHM), which is a rare Mendelian subtype of migraine with aura. This study showed increased LFOs amplitude only in FHM patients with co-existing common type of migraine, but not in patients with pure FHM phenotype. This suggests that the sensitivity to NO resides within the common migraine pheno-types rather than the FHM phenotype. Stimulation of the sphenopalatine ganglion (SPG) may lead to parasympathetic outflow and cause pain in cluster headache (CH). The fifth study therefore investigated pain and autonomic symptoms in relation to high or low SPG frequency stimulation in chronic CH patients. Cortical changes in oxygenated hemoglobin (HbO) were also recorded with NIRS and showed a moderate HbO increase, which was most pronounced on the ipsilateral CH side following high frequency stimulation. A possible application of NIRS to assess cerebral vascular changes due to sympathetic activity was investi-gated in obstructive sleep apnea (OSA) patients, who have in-creased sympathetic activity and risk of stroke. Following success-ful continuous positive airway pressure (CPAP) therapy, OSA patients decreased their LFOs amplitude, which was interpreted as a marker of decreased sympathetic activity in cortical vessels. Finally, a novel hybrid technique, combining NIRS and ultrasound, was tested to detect CBF changes after acetazolamide injection in healthy volunteers using a cerebral flow index (CFI). The study showed an increase in CFI, which correlated with CBF measured with 133Xe-SPECT at 15 min, but not 60 min. Further methodo-logical and explorative clinical studies are needed to assess the feasibility of ultrasound tagged NIRS in clinical neurology. In summary, the thesis presents several novel approaches, by which NIRS may be used in clinical neurology, and potentials of NIRS to investigate complex mechanisms in neurovascular diseases.

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