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TAKE NOTICE TECHNOLOGY

A New Technology for Detecting Cerebral Blood Flow: A Comparative Study of Ultrasound Tagged NIRS and ^{133}Xe-SPECT

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Abstract There is a need for real-time non-invasive, continuous monitoring of cerebral blood flow (CBF) during surgery, in intensive care units and clinical research. We investigated a new non-invasive hybrid technology employing ultrasound tagged near infrared spectroscopy (UT-NIRS) that may estimate changes in CBF using a cerebral blood flow index (CFI). Changes over time for UT-NIRS CFI and ^{133}Xenon single photon emission computer tomography (^{133}Xe-SPECT) CBF data were assessed in 10 healthy volunteers after an intravenous bolus of acetazolamide. UT-NIRS CFI was measured continuously and SPECT CBF was measured at baseline, 15 and 60 min after acetazolamide. We found significant changes over time in CFI by UT-NIRS and CBF by SPECT after acetazolamide ($P \leq 0.001$). Post hoc tests showed a significant increase in CFI ($P = 0.011$) and SPECT CBF ($P < 0.001$) at 15 min after acetazolamide injection. There was a significant correlation between CFI and SPECT CBF values ($r = 0.67$ and $P \leq 0.033$) at 15 min, but not at 60 min ($P \geq 0.777$). UT-NIRS detected an increase in CFI following an acetazolamide bolus, which correlated with CBF measured with ^{133}Xe-SPECT. This study demonstrates that UT-NIRS technology may be a promising new technique for non-invasive and real-time bedside CBF monitoring.

Keywords Cerebral blood flow · Ultrasound tagged near infrared spectroscopy · ^{133}Xenon single photon emission computer tomography · Acetazolamide · Monitoring

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Introduction

Non-invasive, continuous and accurate monitoring of cerebral blood flow (CBF) is a major challenge in clinical practice and particularly in neurocritical care [1, 2]. As changes in CBF may alter brain perfusion and lead to either ischemia or hemorrhage, it is important to monitor these changes bed side, especially when deliberate and non-deliberate hemodynamic changes occur. These changes can have detrimental effects in traumatic brain injury, stroke and SAH patients, and high risk patients undergoing surgery. Conventional methods for assessing CBF in humans are single photon emission computer tomography (SPECT), positron emission tomography (PET), computed tomography (CT) perfusion imaging, and cerebral magnetic resonance (MR) perfusion imaging [3]. These non-continuous methods may expose patients to radiation; have limited availability and considerable cost. Continuous methods such as thermal diffusion probes require invasive procedures to access the cerebral parenchyma directly.

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Therefore new technologies, which overcome these disadvantages, need to be developed and tested in human experimental models.

Near infrared spectroscopy (NIRS) is a non-invasive technique which provides a unique opportunity for continuous and bedside recording of brain oxygenation [4, 5]. The NIRS method has an excellent temporal resolution, whereas the spatial resolution is limited depending on the source–detector distance and number of detectors [6, 7]. NIRS oximetry is currently 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 [8]. Conventional NIRS does not directly measure CBF, and following acetazolamide only a small (4–5 %) increase in cerebral oxygenation was observed using NIRS [9–11]. We have previously attempted to estimate a CBF index with NIRS using indocyanine green as an intravascular tracer, but failed to obtain values reflecting changes in CBF [12].

In this study, we examined a new non-invasive hybrid technology employing ultrasound tagged near infrared spectroscopy (UT-NIRS) [13] that may estimate changes in CBF using a cerebral blood flow index (CFI) without the need of an injected tracer. For comparison, we simultaneously measured CBF with the ^{133}Xe-SPECT method, which is considered a gold standard of regional and global CBF measurements [14]. We examined changes over time for UT-NIRS CFI and ^{133}Xe-SPECT CBF data in healthy volunteers after an intravenous bolus of acetazolamide. In addition, we investigated the correlation between CFI and SPECT CBF data following the acetazolamide challenge and evaluated the ability of CFI for detecting an increase in CBF following injection of acetazolamide.

Materials and Methods

We enrolled 12 healthy volunteers (7 females and 5 males), mean age 22 (SD 3.5; range 19–30 years). Exclusion criteria were: Any daily medication apart from oral contraceptives, or prescribed medications for somatic or psychiatric disease. The study was approved by the Ethics Committee of the County of Copenhagen (H1-2010-136). All subjects gave informed consent to participate in the study. The study was followed in accordance with the Helsinki Declaration of 1964, as revised in Edinburgh in 2000.

Before the experiment each subject underwent a general physical and neurological examination. The intake of coffee, tea, cocoa, or other methylxanthine-containing foods or beverages was not allowed for the preceding 8 h before the start of the study. All procedures were performed in a quiet room at a temperature of 25 °C. The subjects were placed in the supine position and a venous catheter was inserted into the left antecubital vein for drug injection.

A single probe (see below for description of the experimental system) was placed on the right side of the forehead and held in position with elastic bands. All subjects then rested for at least 15 min before baseline measurements. CFI was measured by UT-NIRS continuously from 30 min (T_{-30}) before until 60 min (T_{60}) after an intravenous bolus of acetazolamide. Using ^{133}Xe-SPECT, we measured CBF at baseline (T_{-30}), as well as 15 min (T_{15}) and 60 min (T_{60}) after acetazolamide injection. The time intervals were chosen due to a safety margin of 45 min washout time of ^{133}Xe. Acetazolamide is a reversible inhibitor of the enzyme carbonic anhydrase and increases CBF by dilatation of arterioles [15]. UT-NIRS as well as SPECT data were coded and processed blindly with respect to time of drug administration. Acetazolamide (Diamox®, Goldshield Pharmaceuticals Ltd., Croydon, Surrey, England), 1 g dissolved in 10 mL of sterile water, was given as a bolus over 2 min through the intravenous catheter.

A CerOx monitor (Ornim Medical Ltd., Israel) was used to measure blood flow and tissue oxygen saturation using UT-NIRS technology. The experimental system consisted of a single probe, which illuminates the tissue with coherent light at three wavelength between 780 and 830 nm, 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 [13, 16]. The ultrasound waves induce a local, artificial modulation in the detected light intensity. This artificial modulation is correlated with the pattern of the transmitted ultrasound wave as a function of the time of propagation of the ultrasound wave.

UT-NIRS signals are derived from the correlation of the detected light intensity $I(t)$ (where t is the time of measurement) with the generated ultrasound signal $U(t)$. 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.

UT-NIRS flowmetry measures the effect of Doppler shifts in the signal, due to movement of scattering particles, (blood cells and thus blood flow) [17, 18]. The principle of operation is similar to laser Doppler flowmetry, however, UT-NIRS flowmetry is able to measure blood flow

non-invasively in the microcirculation in deep tissue in volumes of about 1 cm^3. It should be noted that the blood flow signal is independent of the specific wavelength of light, and consequently is independent of the oxygen saturation signal.

In UT-NIRS oximetry, the regional oxygen saturation is extracted independently by analyzing the spatial decay of the signal at three different wavelengths of NIR light. From the ratio between the signals at each wavelength the local absorption is calculated, and brain tissue oxygen saturation is calculated.

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 oxygen saturation and 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 gray matter).

During recording, cerebral flow index (CFI) and tissue oxygen saturation (StO_2) values were calculated and displayed on a screen, visible only to the operating technician. Significant movements of the subjects were recorded in case report forms (CRF). Movement artifacts were accounted for by deleting points with a significant deviation from the mean (larger than 3 standard deviations over a 5 min window) and significant shifts in the immediate intensity of light, with corresponding movement times recorded in the CRF. Data points were averaged over 5 min.

SPECT was performed using a brain-dedicated gamma camera (Ceraspect; DSI, Waltham, MA, USA). The system uses a stationary annular NaI crystal and a fast rotating collimator. Inhaled ^{133}Xe-gas was used as a flow tracer, calculated in each pixel based on the clearance curve, output was the k_i value [19]. To obtain CBF SPECT values in absolute terms (mL/min 100 g), a partition coefficient (λ) of 0.85 was used. Calculation of flow in the perfusion territories of each hemisphere (CBF_{HEM}), middle cerebral artery (CBF_{MCA}), anterior cerebral artery (CBF_{ACA}) and global CBF (CBF_{GLOBAL}) were performed by fitting standard vascular regions of interest (ROI) on five transactional slices (OM-plane) of the brain. Four marks with ink pen were drawn on the skin to ensure accurate re-positioning of the subjects for each SPECT acquisition.

For safety reasons heart rate (HR), mean arterial blood pressure (MAP), and end tidal CO_2 pressure ($P_{et}CO_2$) were monitored continuously. Vital signs data were recorded at baseline, 15 and 60 min after acetazolamide bolus. MAP was monitored using an auto-inflatable cuff and $P_{et}CO_2$ was monitored via an open mask that caused no respiratory difficulty (ProPac Encore®; Welch Allyn Protocol). 10 lead ECG was monitored on a LCD screen and recorded on paper every 10 min (Cardiofax V; Nihon-Koden, Shinju-ku, Tokyo, Japan).

Statistical Analysis

All values are presented as mean values (±SEM). CFI and StO_2 values were averaged over 15 min (20−5 min) before acetazolamide and defined as baseline. We defined the CBF SPECT measurement at 30 min before acetazolamide (T_{-30}) as baseline. Differences between CBF_{HEM} (SPECT) from left and right were analyzed by Wilcoxon test.

The primary endpoints were changes in UT-NIRS CFI, SPECT CBF_{MCA}, CBF_{ACA}, and CBF_{Global} after acetazolamide. The variables were analyzed for changes over time at baseline, 15 min (T_{15}) and 60 min (T_{60}) after acetazolamide using Friedman test. If Friedman test revealed statistical significance at the level of $P = 0.05$, we performed a post hoc analysis to test difference between baseline and after acetazolamide by Dunn's Bonferroni test.

The secondary endpoints were: (1) Correlation between SPECT CBF and UT-NIRS CFI variables for changes over time at T_{15} and T_{60}, where UT-NIRS CFI and SPECT CBF variables were detected simultaneously and could be compared to baseline values. All variables were converted to percent changes from baseline for correlation analysis. The correlation was evaluated using Spearman's rank correlation coefficient. (2) Receiver operating characteristic (ROC) analysis [20] was applied to evaluate the detection ability of changes by continuous CFI signals following injection of acetazolamide. (3) Changes over time with Friedman test for StO_2at baseline, 15 and 60 min after acetazolamide.

Calculation of sample size was based on the detection of a difference between baseline and after acetazolamide injection at 5 % significance with 90 % power. We assumed that the analyses of variables would show 10 % intraindividual variation. A 10 % difference between two experimental conditions was taken to be clinically significant. We estimated that 11 subjects should be included for within-person study [21]. All analyses were performed using SPSS for Windows 19.01 (Chicago, IL, USA). Five percent ($P < 0.05$) was accepted as the level of statistical significance.

Results

Ten subjects completed the study. One subject did not attend on the experimental day, whereas one subject was excluded due to strong motion artifacts in the continuous CFI acquisition.

Table 1 Dynamic CFI and CBF changes following acetazolamide

	Baseline to 15 min	15–60 min
CFI	32.6* (±6.4)	10.0 (±4.4)
CBF_{Global}	53.3* (±7.2)	−17.5 (±3.5)#
CBF_{ACA}	59.6* (±9.0)	−18.6 (±5.3)
CBF_{MCA}	54.9* (±7.2)	−16.8 (±3.5)

Mean changes (±SEM) from baseline (%) after acetazolamide for values of CFI and cerebral blood flow (CBF) globally (CBF_{Global}), anterior cerebral artery (CBF_{ACA}) and middle cerebral artery (CBF_{MCA})

* $P \leq 0.011$; # ($P = 0.043$)

SPECT recordings revealed a significant difference between left and right CBF_{HEM}, which was likely due to visible attenuation effect of the right sided UT-NIRS probe, which obscured the signal from the right ACA territory, resulting in right CBF_{HEM} data to be different at baseline than that of the left CBF_{HEM} ($P = 0.013$). Since the probe was placed over the right forehead, corresponding to ACA and possibly ACA/MCA watershed areas, we chose to present and analyze SPECT data from $CBF_{ACAleft}$, $CBF_{MCAleft}$ (contralateral to the probe), and CBF_{Global}. There were no statistical changes over time in StO_2 values ($P = 0.169$) following acetazolamide.

We found significant changes in CBF after acetazolamide injection measured by both UT-NIRS CFI and SPECT CBF data ($P \leq 0.001$, Friedman test) (Table 1; Figs. 1, 2). Post hoc analysis (Dunn's Bonferroni) revealed a significant increase from baseline 15 min after acetazolamide by both CFI ($P = 0.011$) and SPECT CBF (all regions, $P < 0.001$). We also found a significant increase from baseline in CFI ($P < 0.001$) 60 min after acetazolamide and no statistical significant changes in SPECT CBF (all regions) at 60 min relative to baseline ($P > 0.076$). CFI, SPECT CBF_{ACA} and CBF_{MCA} values remained unchanged from 15 to 60 min ($P = 1.000$, $P = 0.353$, and $P = 0.133$, respectively). The SPECT-derived global CBF showed a significant decrease from 15 to 60 min ($P = 0.043$). See Table 1 and Figs. 1, 2.

There was significant correlation between CFI and SPECT CBF values 15 min after acetazolamide ($P \leq 0.033$, Spearman's rank correlation), but not at 60 min ($P \geq 0.777$). See Table 2.

Specificity and sensitivity for detecting an increase in CFI following acetazolamide injection were calculated using a ROC curve. For ROC curve, we found that the area under the curve (AUC) for CFI was 0.95 (±0.001), (Fig. 3).

Though it was not a goal of this study, as the CerOx monitor also measures the regional oxygen saturation underneath the sensor, we examined the dynamics of the oxygen saturation readings post acetazolamide. We found no statistically significant changes over time in StO_2 values ($P = 0.169$) following acetazolamide, relative to baseline.

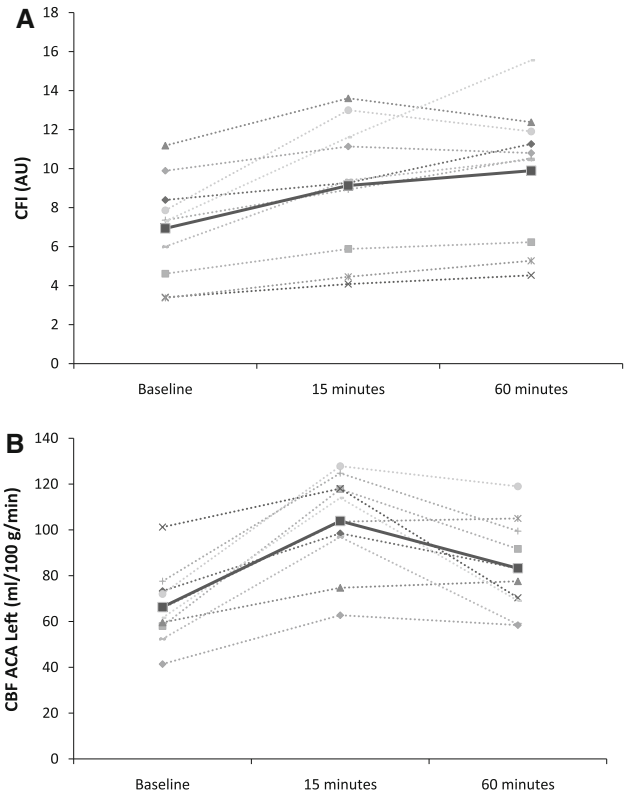

Fig. 1 CFI and CBF_{ACA} measurements Individual (*gray*) and mean (*thick black square*) values for CFI (**a**) and CBF_{ACA} (**b**) at baseline, 15 and 60 min after administration of acetazolamide. A significant increase from baseline was detected at 15 and 60 min by CFI ($P \leq 0.011$). A significant increase from baseline was detected at 15 min by CBF_{ACA} ($P < 0.001$)

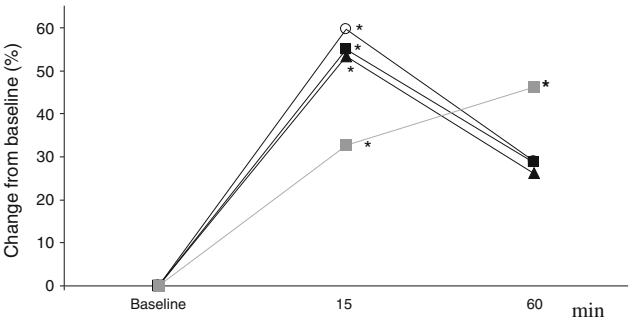

Fig. 2 Relative percent changes in CFI and SPECT CBF 15 and 60 min after acetazolamide Mean increase from baseline (%) for CFI (*gray square*), CBF_{global} (*black triangle*), CBF_{ACA} (*open circle*) and CBF_{MCA} (*black square*). *Values* represent average for all subjects. $P < 0.011$ after acetazolamide as compared to baseline

Discussion

This is the first controlled human study to explore the UT-NIRS technique in measuring in vivo CBF changes. The major findings were that UT-NIRS detected an increase in

Table 2 Correlation between CFI and CBF

Spearman's rank correlation	15 min	60 min
	CFI	CFI
CBF_{Global}	0.73 ($P = 0.016$)	−0.07 ($P = 0.855$)
CBF_{ACA}	0.67 ($P = 0.033$)	−0.06 ($P = 0.881$)
CBF_{MCA}	0.75 ($P = 0.013$)	−0.10 ($P = 0.777$)

Spearman's rank correlation 15 and 60 min after acetazolamide between CFI and cerebral blood flow (CBF) globally (CBF_{Global}), left anterior cerebral artery (CBF_{ACA}) and left middle cerebral artery (CBF_{MCA})

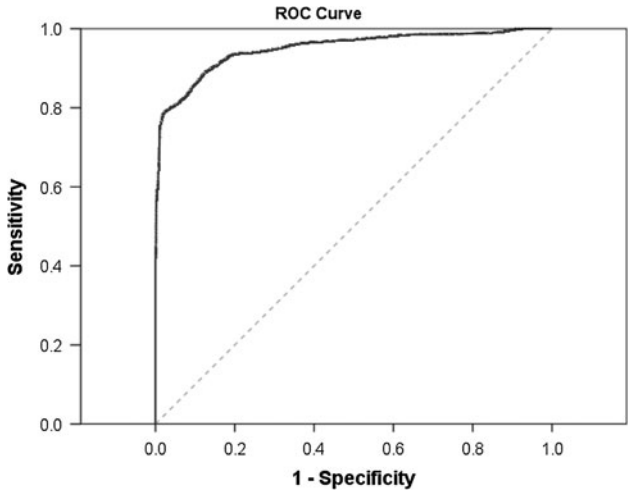

Fig. 3 CFI ROC curve specificity and sensitivity for detecting an increase in CFI following acetazolamide displayed in a ROC curve. The area under the curve (AUC) was 0.95 (±SEM 0.001)

CFI following an acetazolamide bolus. CFI changes correlated with CBF measured with ^{133}Xe-SPECT at 15 min, but not 60 min after acetazolamide. In addition, ROC curve analysis for detecting an increase in CFI following acetazolamide demonstrated a very good discrimination power.

The concept of using NIRS is well established for monitoring tissue oximetry and that of cerebral tissue in particular [22, 23], but conventional NIRS does not directly measure changes in CBF [24, 25]. This study was designed to track CBF changes as a result of an acetazolamide challenge, a drug known to cause cerebral arteriolar dilation and increase CBF [26]. Previous SPECT studies administrating acetazolamide, similar to this study, showed a maximal increase in CBF, ranging from 28 to 70 % and peaking 10 to 55 min after administration [12, 27, 28]. In this study, we showed that CFI increased by 33 % from baseline at 15 min after acetazolamide, which correlated with the maximal increase (53–60 %) at 15 min for ^{133}Xe-SPECT for all CBF regions. We found that CFI and SPECT CBF_{ACA}, CBF_{MCA} values remained unchanged from 15 to 60 min, but not SPECT-derived global CBF. Yet it was apparent that the vectors diverged and that CFI values differed significantly from baseline 60 min after acetazolamide (46 %). This difference could be explained by: (1) *The volume of interrogation*: normal CBF in the human brain is ~50 mL/100 g min averaged over the brain volume, with blood flow to the gray 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 1 cm^3 encompassing mostly gray matter (cortex). Bruhn et al. [29] demonstrated, using MRI imaging, that post acetazolamide the changes in their signal in the white matter were different from those observed in the gray matter (cortex). Thus, it is possible that the magnitude or time course of blood flow differ between gray and white matter following acetazolamide. (2) *The physical quantity measured by both technologies:* SPECT measures CBF in the overall vascular ROI from transactional slices, whereas UT-NIRS measures microcirculatory blood flow within the interrogated volume of 1 cm^3. 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 explained by this study. Thus, further studies are needed to investigate this. It would be of obvious interest to monitor CFI for more than 60 min after acetazolamide injection to clarify if the CFI values actually have a late-onset peak as compared to CBF SPECT.

This study shows the sensitivity of UT-NIRS to detect changes in CBF in response to acetazolamide, a drug known for its effect on intracerebral blood flow. The study followed a previous attempt to detect changes in intracerebral blood flow using a similar protocol, with continuous wave NIRS and indocyanine green as a contrast agent [12]. The study showed, despite correcting for extracerebral contamination, that no changes in any ICG variables were observed after acetazolamide [12]. This study demonstrated sensitivity for intracerebral changes, however, it did not control for extracerebral blood flow changes [30], which might have affected the results.

Simultaneous recording of CFI and StO_2 offers important complementary information on brain perfusion and oxygen saturation. It has been shown that acetazolamide injection induces a rapid and marked increase in CBF, leaving $CMRO_2$ unchanged [28]. In this study, we found no changes in saturation post acetazolamide, although a rise in saturation may have been expected due to increased perfusion and oxygen delivery. Under normal physiological conditions the magnitude of this change may be quite small, and other studies have shown only a small increase

(below 5 %) in oxygen saturation levels after acetazolamide [10, 11]. The fact that an increase was not observed in this study, may be attributed to several possible mechanism: a preferential increase in venous blood volume [11], thereby changing the ratio of oxygenated to de-oxygenated blood in the sampled volume; a right shift in oxygen–hemoglobin dissociation curve due to the effect of acetazolamide on blood acidity, resulting in a decreased oxygen saturation when oxygen partial pressure is constant [31] and thereby compensating for the increase in blood flow; or a lack of sensitivity of NIRS oximetry to the small increase in saturation.

NIRS-based measurements rely on a good coupling of the optodes to the skin and underlying tissue. In addition, a good acoustic coupling is required for good ultrasound transmission to the tissue. The probes used in this experiment were coupled using transparent ultrasound gel and secured using an elastic band. However, as the subjects were awake, significant movements of the head (due to head lifting or extensive coughing) have affected the signals and were filtered out in the analysis. As UT-NIRS signals do not rely solely on the DC intensity of the light for determining both CFI and StO_2, the effect of movements is reduced compared to conventional NIRS devices.

Routine patient monitoring is mainly systemic in nature, assuming a similar effect across all organ systems. Yet it is known that different organs respond differently to changes in blood pressure, regional blood flow and oxygen delivery. The brain has an autoregulation mechanism for preserving constant CBF independent of systemic pressure changes [32]. This mechanism is frequently disturbed in acute brain injury, and also by common critical care drugs and anesthetic agents, such as acetazolamide and halothane [33]. Monitoring of changes in CBF is therefore important to maintain adequate cerebral perfusion and prevent secondary or iatrogenic tissue injury [2]. Consequently, there is a need for real-time non-invasive, continuous monitoring of CBF during surgery, in intensive care units and clinical research.

One limitation to this study is the fact that correlation was tested at only two time points, post acetazolamide injection. This limitation is accounted for the lack of a comparator, which can track CBF changes over time non-invasively. Thus, ^{133}Xe-SPECT, which mandate a washout period of the tracer, provides only snapshots for the relative change in CBF at certain intervals. This factor also limits the ability to show gradual changes in CBF over time.

We conclude that UT-NIRS detects a localized increase in CFI following acetazolamide in healthy subjects, which at 15 min correlate to regional and global CBF as measured by ^{133}Xe-SPECT. CFI is sensitive to acute changes following acetazolamide. This study suggests that UT-NIRS is a promising methodology for real-time non-invasive assessment of local cortical hemodynamics, but further studies are needed to further validate the possible application of the this technology in research and clinical settings.

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Conflict of interest Dr. Schytz, Dr. Guo, Dr. Jensen and Dr. Ashina report no disclosures in any relation to this study. Dr. Kamar is vice president of medical affairs at Ornim Medical. Dr. Gress is a member of Ornim Medical Inc.'s scientific advisory board and holds stock options of the company. Dr. Nini is a medical advisor of Ornim.

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