Reproducibility of cerebral blood volume measurements by near infrared spectroscopy in 16 healthy elderly subjects

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Abstract

Near infrared spectroscopy (NIRS) is a non-invasive method to monitor cerebral haemodynamics. Used either alone or in combination with other non-invasive methods such as transcranial Doppler sonography, this technique is well suited for use in cerebrovascular research in ageing. Reproducibility of NIRS, however, has only been determined in neonates and adults. We applied controlled desaturation (the O₂-method) to measure the cerebral blood volume (CBV) with NIRS in 16 healthy subjects aged 65 to 88. This method uses deoxygenated haemoglobin (the concentration of which is manipulated by desaturation) as an intravascular tracer for NIRS. We determined repeatability (between tests interval: 2 min), short-term reproducibility (intervals of 20 and 40 min) and long-term reproducibility (interval > 2 weeks). We found a coefficient of variation (CV) of 12.5% for repeatability and a CV of 11.7% for short-term reproducibility. The CV for long-term reproducibility was 15%. We conclude that NIRS can reproducibly measure CBV in subjects aged 65 and older, using the O₂-method. In this group of healthy subjects, this method was well tolerated.

Introduction

With advancing age, global cerebral blood flow declines.¹ Moreover, there is an increasing prevalence of factors such as orthostatic hypotension and postprandial hypotension that could further impair perfusion of the brain.²⁻⁴ In clinical practice, these findings can lead to dizziness, loss of consciousness or syncope and cerebral ischaemia.³ Research in this field requires continuous haemodynamic monitoring of both systemic blood pressure and cerebral perfusion. Magnetic resonance imaging (MRI) and positron emission tomography (PET) are two important techniques to investigate cerebral haemodynamics, but these methods are limited to monitoring in the supine position, and may have limited availability. Near infrared spectroscopy (NIRS) offers non-invasive monitoring of cerebral haemodynamics with high temporal resolution at low cost.⁵ It can easily be adapted to allow continuous monitoring during a 90-min postprandial test, or during head-up tilt or active standing. NIRS can measure beat-to-beat variations in the concentrations of oxygenated (O_2Hb) and deoxygenated (HHb) haemoglobin. We have previously shown that these measurements are reproducible in healthy elderly subjects.⁶ In addition, we have demonstrated that NIRS-recorded changes in O_2Hb and HHb , brought about by finger tapping, correlated well with changes in BOLD-signal recorded by fMRI.⁷

Techniques have been developed in neonatal research that enable NIRS to measure the more intuitive haemodynamic parameters, cerebral blood flow (CBF) and cerebral blood volume (CBV).⁸⁻¹² In contrast with the situation in neonates, in adults only CBV can be measured reproducibly.^{9,10} CBV is affected by changes in cerebrovascular dilatation, arterial-to-venous-transit time and CBF.¹³ To measure CBV, O_2Hb and HHb are used as an intravascular tracer, or 'dye'. A gradual decrease in the fraction of inspired oxygen results in a slow desaturation of haemoglobin, hence its name 'the O_2 -method'.¹¹ The reproducibility of this method has been examined in neonates and adults, and its internal validity has been tested in an animal model, but no data are available for subjects above the age of 65.^{10,11,14} Because ageing is associated with reduced cerebral blood flow, brain atrophy and changes in respiratory physiology, this method may be less reliable in older subjects. We designed this study to test the reproducibility of CBV measurements in subjects between 65 and 90 years old.

Methods

Study design

CBV was measured in two cortical regions: the frontal and frontoparietal cortex. It has previously been demonstrated that the frontal cortex is susceptible to deoxygenation in orthostatic hypotension.³ The repeatability, short-term and long-term reproducibility were tested. Repeatability was assessed in three sets of repeated CBV measurements (see figure 1). Each set consisted of two identical desaturation periods with a normoxic interval of 1 min. Short-term reproducibility was assessed by inserting a 20 min interval between the first and the second set. During this interval, the subjects remained in a supine position. Between the second and the third set, we used a longer interval of 40 min. After 20 min, we performed an intervention that is likely to be used in future studies assessing CBV with NIRS. This intervention consisted of a 60° head-up tilt test for a period of 5 min. The purpose was to test the possible effect of a tilt-test procedure on optode position and fixation. Movement of the optodes due to tilting could have a negative effect on the reproducibility of the CBV measurements following this tilt test. We waited 20 min before measuring CBV, to cancel out the effect that tilting itself has on CBV. Finally, long-term reproducibility was tested by repeating a CBV test after an interval of two or more weeks. All measurements were performed and analysed by the same researcher (JC).

This protocol was approved by the Ethics Committee of our institution.

Figure 1. Graphical overview of the experiment.

The bottom half shows the SaO₂-curve, where the desaturation episodes can easily be identified. The upper half of the graph shows the parameters measured by NIRS. Oxygenated haemoglobin decreases during desaturation (peaks facing downwards); deoxygenated haemoglobin rises during desaturation (upward peaks); and total haemoglobin (which represents the sum of the previous parameters) should remain constant during desaturation (a straight line).

Subjects

We recruited 16 healthy volunteers responding to a newspaper announcement, 12 men, 4 women, mean age 73 years, range 68–87. All subjects were examined by a geriatrician. They were leading an active and independent life, had no history of cardiovascular or cerebrovascular disease, had a normal cognitive screening (mini mental state examination score >24 and normal clock drawing test), and used no medication. They had normal ECGs, and normal duplex examination of the carotid and vertebral arteries. All subjects gave their written informed consent.

CBV measurements

We used a continuous wave NIRS instrument (Oxymon, Artinis Medical Systems, The Netherlands). A detailed description of the materials and methods used to measure CBV has been published previously.¹⁰ The distance between the transmitter and the receivers was 5.5 cm to assure deep enough penetration of the infrared light into the brain to exclude substantial contamination from the extra-cerebral circulation. The probes were held in place by a custom-made elastic headband and were applied to the skin with light pressure. We applied a fixed differential path length factor of 6.6 (corresponding to age 50) in all subjects.¹⁵ This factor corrects for the distance that the light travels through the cerebral tissue. No data are available on the actual variation of the differential path length factor with age above the age of 50. For the purpose of this reproducibility study, however, this is of little significance.

All experiments were performed in the morning. Beat-to-beat arterial pressure was recorded using a finger plethysmograph (Finapres, Ohmeda 2300, USA). Arterial saturation (SaO₂) was recorded with a pulse oximeter (N200 Nellcor Puritan Bennett, USA) with a reflectance sensor attached to the forehead, and end tidal CO₂ (etCO₂) was monitored using a capnograph (N1000, Nellcor Puritan Bennett, USA). All analogue signals were synchronized and stored together with the NIRS data on a PC for off-line analysis.

During desaturation, the subjects were asked to breathe through a face mask, and hypoxia was induced using a stepwise computer controlled reduction of the inspired oxygen fraction (FiO₂) by changing an O₂/N₂ gas mixture (Bronckhorst Hitec, Ruurlo, The Netherlands). We aimed for a gradual decrease in SaO₂ of 10% below baseline within 3 to 5 min, after which FiO₂ was immediately increased to 33%. As a result of this, SaO₂ returned to normal values within 10 to 30 s.

CBV analysis

CBV analysis was performed off-line using a software application written in Matlab (Oxysoft, Artinis Medical Systems, The Netherlands). The start and end of each desaturation episode were visually identified from the graphic representation of the changes in [O₂Hb], [HHb] and SaO₂ (see figure 2). The theoretical explanation of CBV calculation from these data has been described in detail before.¹⁰⁻¹² In short, the concentrations of [O₂Hb] and [HHb] measured by NIRS vary with the concentration of cerebral haemoglobin [cHb] and its absorptive property, which depends on its oxygenation state (SaO₂). Because total cerebral haemoglobin remains constant during desaturation, [O₂Hb] and [HHb] will change equally but in opposite direction, and their average is used in the equation as the differential haemoglobin signal ($Hb_{diff} = ([O_2Hb] - [HHb])/2$). [cHb] is then derived from the relationship of the change in Hbdiff and SaO₂. This relationship can be calculated using the slope method, which applies linear regression ($Hb_{diff} = slope * SaO_2 + constant$) to determine the slope, which equals [cHb]. CBV is derived from [cHb] by multiplying with a constant k that incorporates brain density, cerebral-to-large-vessel haematocrit ratio and unit conversion, and this result is divided by the subjects haemoglobin [Hb] concentration:

$$CBV(\text{mL } 100 \text{ g}^{-1}) = k * [cHb]/[Hb]$$

Statistical analysis

All CBV tests were processed regardless of their quality, except for those instances where CBV could not be calculated due to a disturbance in either the NIRS signal or the pulse oximetry signal, mostly due to motion artefacts or disconnection of the probes leading to signal loss. These instances are reported as missing values or uninterpretable tests.

For repeatability, we compared the two measurements in each of the three sets. For reproducibility, we compared the average values for the three sets. We calculated coefficients of variation (CV) and two-tailed Pearson correlation coefficients. Differences were tested with a paired sample t -test.

The overall CBV for each subject was calculated as the mean of the three CBV sets. Because of the small size of the group and the unevenly spread ages, we did not investigate the correlation between age or gender and CBV.

We determined mean values for blood pressure and etCO₂ measurements during 3 min of baseline recording and during 1 min at the end of the first desaturation episode, and compared these values using a paired-sample t -test.

Figure 2. Example of a CBV measurement.

The lower part of the graph shows the saturation curve, identifying the period of desaturation. The upper part shows the effect of desaturation on the parameters measured by NIRS, oxygenated and deoxygenated haemoglobin. The dotted lines mark the manually chosen points for the calculation of CBV using the slope method (see the text for details).

Results

Group values (mean $\pm$ SD) for cHb were $28.3 \pm 9 \mu\text{mol L}^{-1}$. The results of the CBV measurements are summarized in table 1. The CBV for the whole group was $0.98 \pm 0.38 \text{ mL } 100 \text{ g}^{-1}$. There was a small and non-significant difference between the CBV in the first and last set ($-0.1 \text{ mL } 100 \text{ g}^{-1}$, $p = 0.2$). Mean etCO_2 was $34.7 \pm 3.6 \text{ mmHg}$ during baseline and $34.8 \pm 4.2 \text{ mmHg}$ during desaturation. The average mean arterial pressure (MAP) was $75.7 \pm 9.5 \text{ mmHg}$ during baseline and $78.9 \pm 9.9 \text{ mmHg}$ during desaturation ($p = 0.034$).

Table 1. Summary of individual data for age, gender and CBV values. This table provides age and CBV values for all subjects, as well as mean values for the whole group. Three sets of two CBV measurements

were performed with intervals of 20 min, 40 min and more than 1 week. Each CBV value represents the average of the two CBV measurements in that particular set. Missing values are uninterpretable CBV measurements.

Subject	Age		CBV (mL 100 g ⁻¹)			
	Gender	(years)	Baseline	20 min	40 min	≥1 week
1	Female	70	0.52	0.56	0.51	0.52
2	Female	68	1.28	0.96	1.11	0.47
3	Female	70	0.64	0.86	0.81	0.61
4	Female	73	0.77	0.80	0.77	0.89
5	Male	69	1.20	1.06	1.05	1.17
6	Male	70	Missing	0.63	0.54	1.04
7	Male	68	1.06	1.25	1.39	1.23
8	Male	75	0.84	0.77	0.80	1.00
9	Male	69	1.17	1.29	1.11	1.00
10	Male	82	1.21	1.08	0.91	1.03
11	Male	74	0.87	0.64	0.50	Missing
12	Male	70	1.30	1.32	1.10	1.47
13	Male	73	1.76	1.27	1.56	1.33
14	Male	75	1.16	1.46	1.42	1.35
15	Male	75	0.65	0.68	0.58	1.17
16	Male	87	0.84	0.68	0.59	0.88
Mean		73	1.02	0.96	0.92	1.01
SD		5.2	0.33	0.29	0.34	0.30
Min		68	0.52	0.56	0.50	0.47
Max		87	1.76	1.46	1.56	1.47

The results for repeatability are demonstrated in table 2. We found no difference in repeatability between the three sets, or between the two measurement sites, and therefore all measurements were pooled. The CV for these pooled data was 12.5% (95% CI: 9.3–15.8%). A Bland–Altman plot is presented in figure 3.

Table 2. Coefficients of variation (CV) for repeated CBV tests, expressed as mean values with 95% confidence interval. The first pair of CBV tests (CBV-set 1) was recorded at the baseline, the second (CBV-set 2) was performed after 20 min of supine rest, and the third (CBV-set 3) after 40 min of supine rest, interrupted midway by a tilt test for 5 min. Missing values are reported as the percentage of the total number of CBV-sets (48) that were collected.

Optode position	Missing values	CBV-set 1 CV (%) (95% CI)	CBV-set 2 CV (%) (95% CI)	CBV-set 3 CV (%) (95% CI)
Frontal	8	11.8 (5.9–17.6)	11.7 (7.4–16.1)	11.0 (5.2–16.8)
Parietal	15	10.9 (6.3–15.5)	14.3 (5–23.6)	17.3 (8.7–25.9)

Figure 3. Repeatability of CBV tests.

Analysis as proposed by Bland and Altman of repeated CBV tests calculated using the slope method (see the text for details). In 16 subjects, six sets of two repeated (interval: 1 min) CBV measurements were performed. The average of these two measurements is plotted against their difference

The mean CV for the third set of repeated CBV tests, which was obtained 20 min after a tilt table intervention, was not significantly different from that of previous CBV sets. Pearson's correlation coefficient for the repeated tests was $R = 0.81$, $p < 0.001$. 11 out of 96 sets (11%) could not be analysed because either or both CBV tests were uninterpretable.

For short-term reproducibility, we found a good correlation between the three CBV values ($R > 0.8$, $p = 0.01$), and the CV was 11.7% (95% CI: 8.3–15%). For the CBV values obtained on different days (with an interval of two or more weeks), correlation was acceptable ($R=0.5$, $p=0.05$) and the CV was 15% (95% CI 5–25%). A Bland–Altman plot for long-term reproducibility is presented in figure 4.

The quality of a measurement using the slope method can be expressed by the linear correlation coefficient (r^2) of the relationship between the changes in Hb_{diff} and SaO_2 . Mean r^2 for our measurements was 0.92 ± 0.05 . We found a correlation between the goodness of fit (a high value for r^2) and the CV ($R = 0.3$, $p = 0.05$). All CBV values with $r^2 > 0.95$ had a CV of 10% or less.

Figure 4. Long-term reproducibility of CBV measurements.

Analysis as proposed by Bland and Altman. In 16 subjects, CBV was determined twice with an interval of more than 2 weeks. The average of the two CBV values is plotted against their difference.

Discussion

The main finding of our study is that CBV measurements with NIRS can be performed with the same reliability in old subjects as they can in other age groups. Repeatability in our study resulted in a CV of 12.5%, which is almost equivalent to the CV of 11.7% that was found for this method in adults and the CV of 11.5% that was found in neonates.^{10,11} Of note, the study of Van de Ven *et al* was performed in our institution using identical equipment and methodology. These investigators have discussed the differences with reproducibility results from studies using other methodology.¹⁰

An important new outcome of our study is that, even with a prolonged interval of several weeks, measurements of CBV with NIRS in this age group of healthy elderly subjects were reproducible with a CV of 15%, and showed an acceptable correlation. Over such a period, individual CBV is subject to considerable physiological change. The option to perform repeated measurements of CBV over time with a non-invasive method like NIRS offers an interesting perspective for cerebrovascular research.

The reproducibility of CBV measurements with NIRS in our study compares favourably to other methods. For example, PET measures CBV with a CV of 20%¹⁶, and MRI measures CBV with a CV of 14%¹⁷.

The fact that we did not validate our NIRS measurements with a second method to determine CBV is a limitation in the design of this study. However, even though there are several methods available to measure CBV, none of these can actually serve as a gold standard. Haemodynamic measurements with NIRS in animals, neonates and adults have been compared with MRI, immunolabelling, jugular venous occlusion plethysmography and PET.¹⁸⁻²¹ The correlation between the two methods studied varied from very poor to excellent. Only one of these studies, comparing NIRS with MRI, actually used the desaturation method to determine CBV. In this study, the group mean value for CBV obtained with NIRS was similar to that found with MRI, but intra-individual correlation between the two methods was poor.²¹ We judged that validation with a second neuroimaging tool that cannot serve as a gold standard would yield little extra information for the purpose of our reproducibility study.

A potential problem with the desaturation method is that it could influence CBV. Prolonged hypoxia, especially around or below a SaO₂ of 80%, can induce vasodilatation and increase CBF and CBV. On the other hand, desaturation could produce compensatory hyperventilation, which causes vasoconstriction and reduces CBF. In our study, we found no evidence for hyperventilation during desaturation, as etCO₂ remained stable. Blood pressure rose slightly during desaturation, which is consistent with previous investigations. Because we performed paired CBV measurements with a short interval, any substantial effect of the desaturation procedure itself on CBV would result in the second CBV measurement being systematically higher or lower than the first. We did not find such an effect in our study. In addition, we constantly monitored the total haemoglobin concentration measured by NIRS, which did not change during our desaturation procedures.

We did not investigate the possible effects of altered brain geometry with ageing on the outcome of CBV measurements. For instance, brain atrophy with ageing would increase the depth of the cerebral tissue relative to the probes. This could unfavourably affect the relative contribution of extracerebral tissue to the NIRS signal. In order to investigate this, a method comparing different source-detector distances would be needed. Kohri *et al.*²² for example demonstrated that with source-detector distances of 3 cm, the cerebral tissue contributed to 55% of the signal, versus 69% when using a 4 cm distance. We did not investigate these

aspects in this study for several reasons. First, we wished to compare results directly with the previous study by Van de Ven *et al* in younger subjects, using an identical technique. Second, the source-detector distance used with the Oxymon-device is 5.5 cm, which is larger than is generally available in other NIRS devices, and represents the optimal distance for this device to minimize noise from extra-cerebral tissues. Third, a previous study in our laboratory has measured the depth between NIRS optodes and cerebral cortical tissue using MRI in a small group of young (35 ± 9 years) and ageing (73 ± 3 years) subjects, and found no evidence for an increase with ageing.⁷

Possibly related to this matter is the finding of low CBV values in our group. Even though estimation of individual values for CBV was not the primary focus of this study, this finding merits further discussion. For comparison, mean CBV determined by PET was 3.8 ± 0.7 mL 100 g^{-1} in a group with a mean age of 51.8 (± 15.1) years.¹⁶ The study of Van de Ven *et al*, using NIRS, found a mean CBV value of 3.66 ± 0.82 mL 100 g^{-1} in a group of adults with a mean age of 31 ± 10 years. Future studies are needed to further investigate the low CBV values we found in our age group. Despite similar brain geometry in young and ageing subjects, a reduction with ageing in the deoxyhaemoglobin response to cortical activation was apparent both with NIRS and fMRI in a previous study.⁷ One of many possible explanations for this finding is a reduced cortical haemoglobin content with ageing. A future study using different source-detector distances and measuring brain geometrics with MRI may serve to confirm in a larger group that there is no evidence for reduced brain tissue sample due to brain atrophy. In addition, a study investigating a larger, intermediate age group may demonstrate if a true reduction of CBV measured by NIRS age occurs with advancing age.

Conclusion

The O₂-method to measure changes in CBV with NIRS can be applied in subjects aged 65 years and older. Despite many possible sources of variation in older subjects, including reduced global cerebral perfusion, brain atrophy and altered respiratory physiology, we found that reproducibility in our study was comparable to investigations in younger subjects. We conclude that NIRS is a safe and reliable tool to monitor changes in CBV in cerebrovascular research in the ageing population.

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