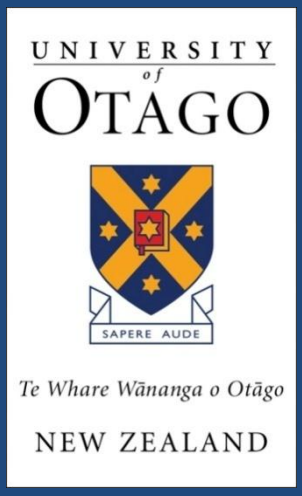

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BLOOD PRESSURE AND CEREBRAL BLOOD FLOW CONTROL STUDY

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SPONSORS THE SURGICAL RESEARCH TRUST

LOCATION Department of Surgery and Anaesthesia
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BLOOD PRESSURE AND CEREBRAL BLOOD FLOW CONTROL STUDY

Supervised by: Dr. Shieak Tzeng

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Cerebrovascular carbon dioxide reactivity is a measure of how CO2 alters blood flow in the blood vessels which supply the brain with oxygen and nutrients. Impaired CO2 reactivity is known to be associated with an increased risk of stroke, TIA 'mini-strokes' and brain hemorrhage. CO2 reactivity has the potential to be used for assessing how healthy a subject's brain vasculature is in patients suffering from hypertension, heart failure, carotid artery stenosis and other blood vessel diseases. The ability to accurately measure CO2 reactivity in at risk patients is vital, as it would possibly allow early detection, better treatment and maybe even prevention of cerebrovascular diseases. We sought to find out whether two currently used methods of measuring CO2 reactivity, the classical 'Rebreathing' method and a newer technique involving exposure to CO2 enriched air and controlled hyperventilation, provided us with comparable results. At this time we cannot say which method is better, as both have advantages and disadvantages associated with them. The rebreathing method does not require a pressurized gas-canister full of known concentration CO2 but takes longer to perform, can cause the subject to feel claustrophobic and is more difficult to consistently complete. The in-house method is faster and provides a wider range of readings but was found to place more stress on the subject because it requires a period of hyperventilation, causing subjects to feel nauseous. However, while we found that each method provided consistent results in themselves, we found no association in the results given between methods.

ABSTRACT

Cerebrovascular carbon dioxide (CO_2) reactivity measures have the potential to be used in the diagnosis and subsequent treatment of a variety of cerebrovascular diseases, such as carotid artery stenosis, hypertension and heart failure. Impaired CO_2 reactivity is known to be associated with increased risk of stroke, TIA and subarachnoid haemorrhage. It is not currently known whether various methods of measuring CO_2 reactivity provide accurate, comparable and repeatable results.

We measured blood flow velocity in the middle cerebral artery (MCA_v) at varying exposures of CO_2 (PCO_2) in 15 subjects using 3 different methods, the classical rebreathing method and 2 variations of our own variable exposure method using transcranial Doppler ultrasonography (TCD). We found that all 3 methods provide repeatable results, and the variations of our variable exposure method provided comparable values of CO_2 reactivity. However, we found that the rebreathing method does not provide comparable results with either form of the variable exposure method; therefore the variable exposure method does not provide a viable substitute for the classical method. It is still unknown whether either the classical technique or our method actually provide correct measures for CO_2 reactivity, so we cannot say whether either method is better than the other, although in terms of subject comfort it seems the rebreathing method is more bearable, albeit longer and more difficult to set up.

INTRODUCTION

Measurements of cerebrovascular CO_2 reactivity have become widely recognised as a surrogate of cerebrovascular reserve, and therefore have the potential to be used in the clinical assessment of cerebral vascular function in patients suffering from a variety of vascular diseases, such as: hypertension, heart failure and carotid artery stenosis. An impairment of CO_2 reactivity is known to be associated with an increased incidence of ischaemic stroke, transient ischaemic attack (TIA) and subarachnoid haemorrhage. Given that treatment decisions for such afflictions can be influenced by our assessment of cerebral vascular function, so it is imperative that methods of measuring CO_2 reactivity correspond to each other and are technically reproducible.

We sought to determine whether CO_2 reactivity estimated from enhanced percentage CO_2 inhalation and voluntary hyperventilation produces comparable results to those derived from the classical rebreathing technique, and we wanted to know whether both methods gave repeatable results. The rebreathing method exposes the subject to slowly increasing amounts of CO_2 while the variable exposure method exposes the subject to both hypercapnia and hypocapnia, which gives a wider range of data but also left us with the possibility that the order of exposure has an effect on results received. Therefore an additional aim of our experiment was to determine whether an order-effect was present.

MATERIALS AND METHODS

Our experiment involved three methods, each of which were tested on fifteen healthy, non-smoking participants who were not on any respiratory or cardiovascular medications. To determine reproducibility each method was tested three times successively with five minute resting periods between repetitions on each subject. After completion of the third repetition for a method the subject was given a ten minute resting period before moving on to the next method, and the order of testing was randomised. Protocols A and B were always paired, if a subject was to be subjected to three repetitions of Protocol A first then the next method would always be the three repetitions of Protocol B, and vice versa.

Participants were screened to ensure they had no respiratory, cardiovascular or cerebrovascular disease, and that they had no family history of such diseases. Participants were informed of the details of each experimental procedure and signed a consent form, and were asked to abstain from caffeinated or alcoholic beverages and exercise for a minimum of 12 hours prior to experimentation. Subjects were also asked to avoid eating in the two hours beforehand.

The participant's heart rate (HR) and beat-to-beat blood pressure were measured continuously throughout the experiment using a 3 lead electrocardiogram and finger cuff photoplethysmography, respectively. End tidal CO_2 ($P_{ET}CO_2$) and O_2 ($P_{ET}O_2$) levels were measured using a nasal sampling line and a gas analyser. In order to measure cranial blood flow velocity (MCAv) two transcranial Doppler (TCD) ultrasonography probes were affixed to the subject's head using an adjustable headband. The headband had to be on tightly to provide good readings and to prevent it from moving out of place, several participants found this to be uncomfortable so we loosened it between recordings when requested. One probe was placed on each transtemporal acoustic 'window', an area above the zygomatic arch where bone tissue is relatively thin. Blood flow velocities in the sphenoidal segment (M1) of the middle cerebral artery (MCA) were measured. If there were any doubts as to whether the correct vessels had been a simple test was used, we compressed the ipsilateral carotid artery to observe whether the arterial blood flow was diminished, which could easily be recognised as a drop and subsequent rise in mean blood flow velocity on the TCD display. National Instruments 'LabView 11' was used to process the data.

Protocol A: Hypercapnia-Hypocapnia

Participants respired in time with a metronome set for a steady 12 breaths per minute throughout the entire protocol. Many participants would subconsciously begin to breathe deeper than normal when breathing in rhythm, so they were given time to normalise their breathing depth before starting the protocol. After a few (~6) normal depth paced breaths the subject was exposed to a CO_2 enriched air mixture (5%CO_2 initially, 8%CO_2 in the second bottle use in that later half of the experiments, 21%O_2, balanced NO_2) from a pressurised air-tank via an anaesthetic delivery gas mask. The subject was asked to continue to breathe with a normal depth while exposure to CO_2 was progressively increased by increasing gas flow through the mask. A PCO_2 increase of ~5 torr was enough to gain a noticeable change in MCAv, but an increase of 7-12 torr was not uncommon. After 90s the gas flow was progressively decreased and the mask slowly removed to allow for a slow, steady decrease in $P_{ET}CO_2$ back to baseline levels. Once $P_{ET}CO_2$ had been returned to normal the subject was asked to breathe progressively deeper and deeper, lowering $P_{ET}CO_2$ down to a nadir via controlled hyperventilation. Once again a decrease of ~5 torr from baseline was normally all that was necessary to gain a noticeable change in MCAv. Once an acceptably low $P_{ET}CO_2$ was reached the subject was asked to continue breathing at that depth until 90s was completed.

Protocol B: Hypocapnia-Hypercapnia

Protocol B was, in essence, the reverse of protocol A. Once again participants respired in time with the metronome constantly, and after they had become accustomed to breathing in rhythm a few (~6) normal depth breaths were recorded. The subject then began the hypocapnic section of the protocol first, and they were asked to breathe progressively deeper and deeper. Once an acceptable decrease in $P_{ET}CO_2$ was achieved the subject was asked to start breathing progressively shallower in order to slowly return to baseline $P_{ET}CO_2$ levels. The subject was then exposed to a progressive increase in CO_2 via the gas mask, and once 90s was over gas flow was halted completely.

Protocol C: Rebreathing

For this protocol the subject wore a mask that could be attached to two empty bags, one of which was always attached and the other opening was left free until the beginning of the experiment. The mask was held onto the subject's head over their nose and mouth using elastic straps, and the mask was designed so that, ideally, there was a vacuum seal over the mouth. The nasal sampling tube was removed from the nose and connected to the mask, and supplemental oxygen was able to be supplied should the subject's $P_{ET}O_2$ fall below acceptable levels (<100 torr). A metronome for 12 breaths per minute was set up but the subject did not have to breathe in rhythm with it initially. The subject was asked to hyperventilate for 15 seconds at their own natural rate to reduce $P_{ET}CO_2$ and give a wider range of values. At the end of the 15 seconds hyperventilation the second gas bag was attached to the mask, sealing it and forcing the subject to rebreathe their own expired air in time with the metronome. This continued for five minutes, or if the participant began to feel uncomfortable and unable to continue or wanted to stop. At the end of the five minutes the second bag was removed and the participant was allowed to breathe normally for a five minute recovery period.

RESULTS

Table 1: Baseline parameters before experiments

	Protocol A	Protocol B	Protocol C	P-value
Heart rate (beats.min^{-1})	70.5 ± 6.7	71.9 ± 7.2	73.9 ± 9.3	0.12
$MCAv_{mean}$ (cm.s^{-1})	56.7 ± 12.8	56.2 ± 12.3	60.6 ± 12.4	0.06
MAP_{mean} (mmHg)	77.6 ± 9.7	75.1 ± 11.1	75.3 ± 10.2	0.57
$P_{ET}CO_2$ (mmHg)	34.5 ± 4.4	34.2 ± 4.5	34.1 ± 3.9	0.77

Values are mean ± SD.

Abbreviations: $MCAv_{mean}$ - middle cerebral artery velocity; MAP_{mean}, mean arterial blood pressure; $P_{ET}CO_2$, partial pressure of end-tidal CO_2

Table 2: CO2 reactivity values

	Protocol A	Protocol B	Protocol C	P-value
Integrated curves	1.99 ± 0.50	1.84 ±0.46	1.99 ± 0.65	0.40

Table 3: Intraclass correlation results for the repeatability of a method and the repeatability between methods

	Protocol A	Protocol B	Protocol C	Coefficient repeatability methods
Coefficient repeatability method repetitions	0.82	0.82	0.37	0.77

Comparisons of methods

In order to compare the different methods between each other, the CO_2 reactivity value was calculated for each participant for each repetition of the different protocols. In this case, the whole curve (named integrated curve) of each repetition was taking into account, except for the rebreathing method for which the hyperventilation section was systematically cut. Once again, the mean per participant and per method was done.

However, the curve of the rebreathing method is completely different from the variable exposure method curves. The former consists only of an increase in CO_2, but the latter cover a wide range of CO_2 values.

Repeatability of methods

To verify the repeatability of all three methods, the averaged CO_2 reactivity values for each repetition of the protocol were used.

Table 1 shows the averaged baseline values for heart rate, $MCAv_{mean}$, MAP_{mean} and $P_{ET}CO_2$ for each protocol. None of these parameters change significantly ($p>0.05$) between protocols. Averaged CO_2 reactivity values generated from the integrated curves are similar in all three tests ($p>0.05$), as shown in Table 1. Moreover, the high value of the intraclass correlation coefficient (0.77) presented in Table 3 means that the variability within measures for one test is close to the variability existing between values for different methods, which implies good reproducibility between methods.

Comparisons between tests bring us more precise information about the links existing between them. The R^2 value of the regression between Protocols A and B is 0.75 (figure 1), with a p-value less than 0.05, meaning that the results of both methods are closely related, which is confirmed by the limits of agreement test. The integrated curve between Protocols A and B (figure 2) shows an almost horizontal curve with, a 95% CI around 0.5. Therefore, the differences between Protocol A and B test values seem to be

fixed and unchanging with variations of CO_2. Neither of the comparisons between Protocols A and B with Protocol C (figure 1) presents a satisfactory linear regression ($p>0.05$). However, the limit of agreement allows us to say that the differences in the results between methods are fixed for all values of CO_2. On the other hand, 95% CI are around 1, higher than those for the comparison between Protocols A and B (figure 2).

Table 3 shows that the intraclass correlation coefficient is high (0.82) for both A and B, and consequently they seem to be reliable and reproducible. However, the intraclass correlation coefficient is lower (0.37) for the rebreathing test.

The strong associations between protocols A and B indicate that an order effect is not present.

Figure 1: Linear regressions between methods.

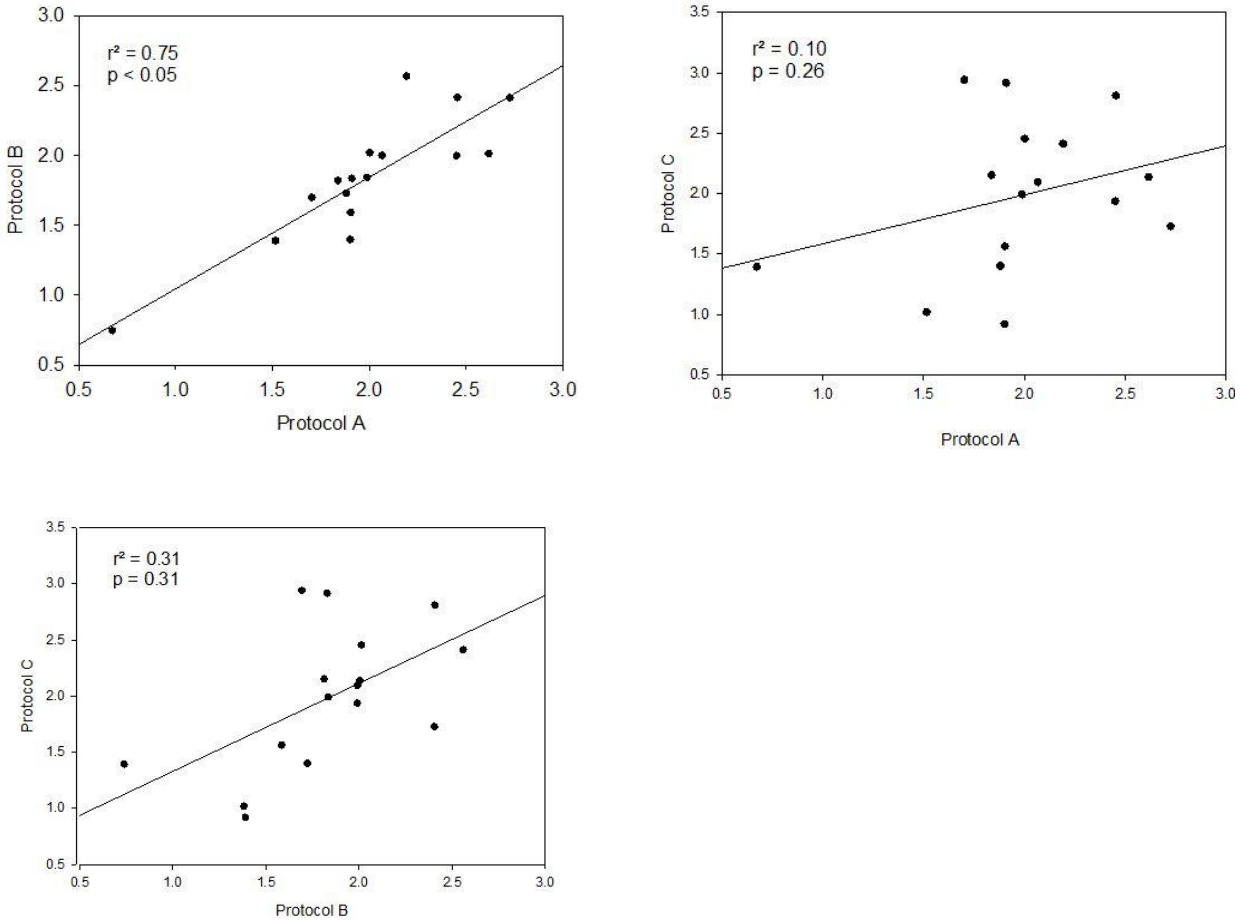
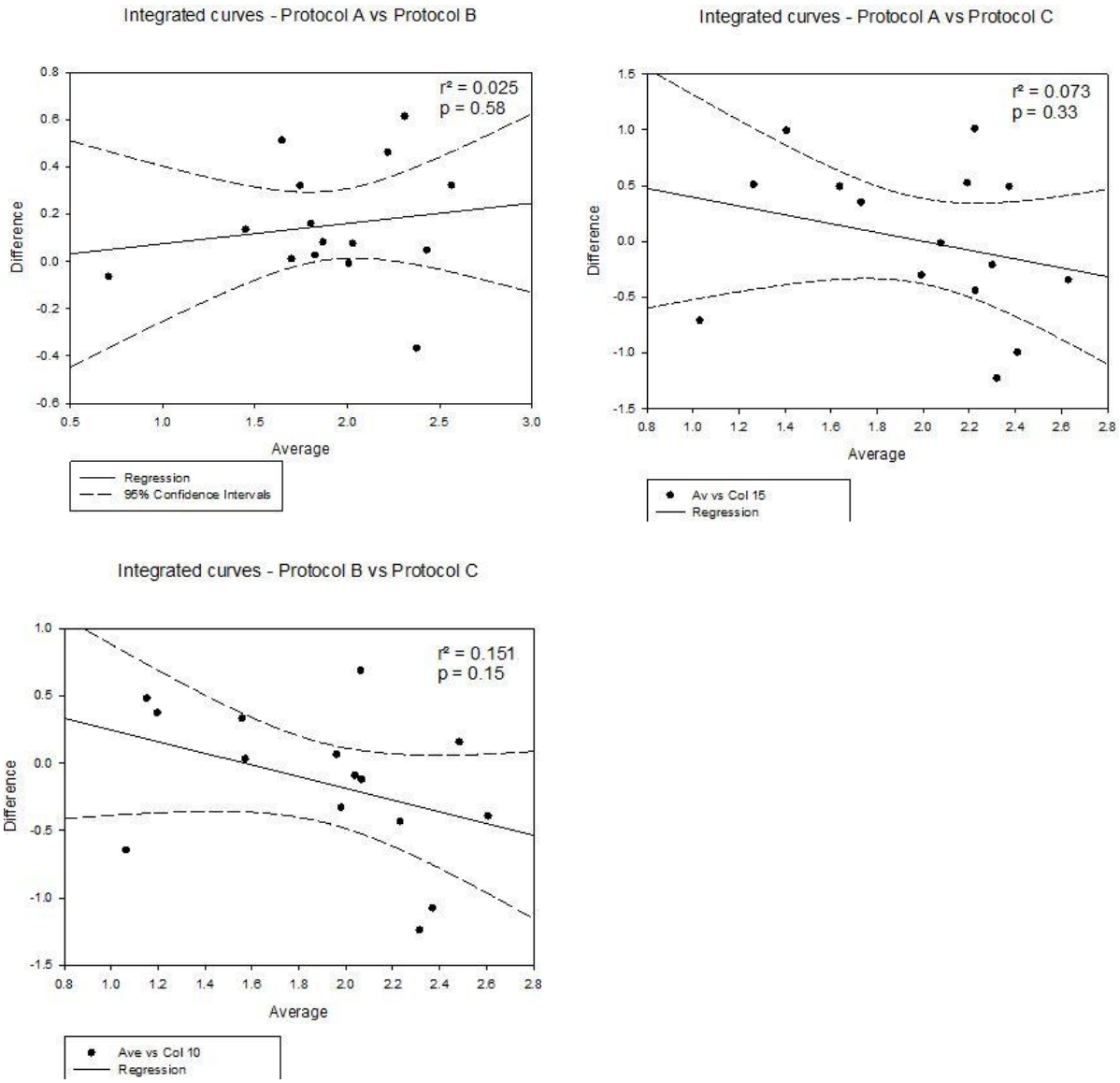


Figure 2: Integrated curves between methods



DISCUSSION

With our findings we were able to confirm that all three techniques provide reproducible results for measuring carbon dioxide reactivity in the middle cerebral artery. However, we found no correlation between our enhanced CO_2 inhalation and voluntary hyperventilation technique and the classical rebreathing technique, implying that the two do not give comparable results. They are therefore not suitable surrogate measures for one another.

Our third aim was to determine whether there was an order effect present in the two versions of the variable exposure method. We found that both variations of the technique gave very similar results with strong limits of agreement, and that there was

no order-effect overall. Therefore the variable exposure method can be performed in either order and provide the same results but, as stated before, it cannot be used as a substitute for the rebreathing method.

As both methods are measuring the same thing we cannot at this point determine which method is best, and both have advantages and disadvantages. The rebreathing technique can be performed without needing a pressurised gas canister with a known CO_2 concentration, but it takes longer, can make the subject feel claustrophobic because of the restrictive gas-mask and usually requires a supplemental supply of O_2 to prevent hypoxia, and in several cases we had to restart halfway through because the O_2 supply threw off the $P_{ET}CO_2$ readings. The variable exposure method is quicker to perform and easier to set up, and it also provides a greater range of $P_{ET}CO_2$ against MCAv measurements due to it comprising of both a hypercapnic and hypocapnic section, as opposed to the entirely hypercapnic rebreathing technique. We did discover one major disadvantage with our method; the majority of our volunteers found it less comfortable than the rebreathing method. Complainants found that the variable exposure method's periods of extended hyperventilation left them feeling slightly nauseous after repeated trials, and one came close to fainting and had to lie down for several minutes to recover.

This can be attributed to the physiological effects that alterations in CO_2 have on vascular tone. CO_2 is a potent vasodilating metabolite, and 'CO_2 reactivity' is the term given to the quantification of this effect. Decreased amounts of CO_2 will result in vasoconstriction, decreasing O_2 perfusion to the brain, heart, liver and other tissues, which caused the nausea experienced by several subjects, and can also cause exhaustion and eventually syncope if it is allowed to continue. CO_2 also forms an important part of the blood buffer system, any CO_2 decrease causes a resultant increase in H^+, therefore hyperventilation causes respiratory acidosis, while vasodilation caused by hypercapnia causes respiratory alkalosis. Acidosis can compound the O_2 starvation effect of CO_2 restricted vessels as due to the Bohr effect, causing acidified haemoglobin (Hb) to have a decreased O_2 binding affinity, reducing the amount of O_2 picked up by Hb in the lungs. Alkalosis can also result in lowered O_2 offloading in tissues due to the same effect, decreased H^+ results in a higher binding efficiency for O_2 in Hb, resulting in less O_2 perfusion at tissues with a higher CO_2 concentration.

CONCLUSIONS

Because there are differences in the two techniques that we used it was important to know whether they gave comparable results for CO_2 reactivity. We now know that the variable exposure method is not comparable with the classical CO_2 rebreathing technique, and at this time we do not know which method provides us with the best results. CO_2 reactivity has the potential to be used as an assessment criteria of cerebral

vascular function, and consequently in the diagnosis and treatment of a variety of cerebrovascular diseases, such as ischaemic stroke, TIA 'mini-strokes' and subarachnoid haemorrhage.

However, more investigation is needed before we can begin to use CO_2 reactivity measures clinically.

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Conflicts of Interest, The authors have no conflicts to declare.

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