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The influence of demographic factors on phase-modulated spectroscopy in adults

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SUMMARY

The increased complexity of phase-modulated spectroscopy (PMS) compared with incoherent light techniques of near-infrared spectroscopy is justified if measurement of path length is necessary. In order to assess the variability of optical transmission in the head of adults, 96 subjects of varying age, gender and skin pigmentation were studied using an experimental three-wavelength time-shared PMS device. Optical path length was measured at each wavelength, and saturation and haemoglobin-free path length were calculated. Path length varied linearly with the separation of the optical probes, but gender, age or skin pigmentation were not associated with differences in path length. Haemoglobin saturation averaged 68% and varied with age in a non-uniform fashion. Haemoglobin-free path length differed between genders, being 8% longer in women than in men. Measurements could not be performed at 5 cm due to optical attenuation in 36% of subjects. These subjects were more often young, but they were not otherwise distinguished by gender or pigmentation differences. Subjects in whom measurements could be obtained at 5 cm had longer haemoglobin-free path lengths than did subjects in whom optical attenuation prevented these measurements. These data confirm the occurrence of significant differences in optical transmission in adults and support the use of PMS techniques that measure path length.

1. INTRODUCTION

Phase-modulated spectroscopy (PMS) has the potential to provide quantitative assessment of cerebral oxygenation using equipment that is technologically simpler than time-resolved (TRS) techniques, and that corrects for the interpersonal variability in factors such as haemoglobin concentration and tissue scattering; factors that cannot be distinguished by techniques that do not measure path length. Most studies utilizing PMS in adult humans have involved limited numbers of subjects, thus preventing analysis of demographic factors that may influence the measurement process and potentially underestimating interpersonal variability. This study was undertaken to assess those factors. Specifically, we planned to measure optical path length in the head using phase-modulated technology in a population of adult subjects of varying age, gender, and skin pigmentation, and to use the measurements to determine whether demographic factors influence the measurement of path length and derived values of haemoglobin saturation and haemoglobin-free path length.

2. MATERIALS AND METHODS

Data were obtained from 96 subjects who gave informed consent. Most subjects were patients awaiting surgery, although a few were recruited in other ways. Subjects were not medicated prior to study. Path length was measured using a three-wavelength phase-modulated spectroscopy system (NIM Inc., Philadelphia, PA). Light of 754, 785 and 816 nm wavelengths was modulated at 200 MHZ and conducted to the subject using a fibre optic bundle. Scattered light was returned to the photomultiplier by another fibre optic bundle. After amplification, the signals for each wavelength were demodulated using time-shared circuitry so that amplification and bandwidth characteristics were identical for all wavelengths. Analog voltages were sampled by computer and stored for subsequent analysis. Calibration was performed using an adjustable optical bench, with polarizing filters to adjust the optical intensity.

The fibre optic bundles were attached to the subject using a foam-backed, perforated, rigid holder so that the spacing between probes was constant among subjects. Probes were placed as high on the forehead as practical and measurements were made with subjects sitting or semi-recumbent. Measurements were performed at two separations. If adequate light intensity could be obtained, measurements were obtained at 5 cm separation and 4 cm separation. If light intensity at 5 cm was inadequate, then measurements were obtained at 4 and 3 cm of probe separation. Haemoglobin saturation was calculated using the relationship between phase shift and absorption described by Sevick *et al*. (1991). Haemoglobin-free path length is related to the scattering and background absorption properties of the tissues and is calculated using Levy's equation (Levy *et al*. 1995).

Statistical analysis was performed using analysis of variance, linear regression or χ^2 techniques as appropriate, with $p < 0.05$ considered statistically significant.

3. RESULTS

Eight subjects were recruited into each demographic group, defined by the criteria listed in table 1. Measurements at two separations were obtained in 90 of the 96 subjects, and data were obtained at one separation for the remaining six subjects.

The distribution of subjects in whom measurements could not be made at 5 cm was not uniform among demographic groups, with two-thirds of the young subjects requiring shorter separations, but only 11% of the subjects over age 65 (table 2), $p < 0.001$. No such differences were observed for pigmentation or gender. Measurements could not be obtained at 5 cm in 40% of men and 33% of women, and the distribution of these cases by skin pigmentation was 31% light-skinned and 42% dark-skinned.

As would be expected, path length at 785 nm varied with separation between optical probes. The differences, as shown in figure 1, are all highly significant ($p < 0.00005$). To correct for this effect of path length, data obtained at 4 cm of separation were analysed for demographic differences. If all data points are analysed (including one outlier that produces highly suspect results), no significant differences are seen. If this single case is eliminated, changes in saturation are seen to be related to age, and gender differences are associated with differences in haemoglobin-free path length (table 3).

Finally, path length at 785 nm, haemoglobin-free path length, and saturation were compared at 4 cm separation between the groups of subjects in whom

Table 1. *Demographic criteria*

age	gender	pigmentation
< 30	male	light
30–65	female	dark[a]
> 65		

[a] Predominantly African–American with a small number of Indian and Hispanic.

Table 2. *Measurement at 5 cm*

	yes	no
young	8	24
middle-aged	24	8
old	30	2

$\chi^2 = 32.5$, $p < 0.005$.

Table 3. *Demographic effects at 4 cm probe separation*

	L_{785}[a] /cm	saturation /fraction	L_0[b] /cm
age			
young	51.8	0.69	68.2
middle-aged	52.3	0.65[c]	69.0
old	51.9	0.68	74.0
gender			
female	52.3	0.68	74.3
male	51.5	0.68	68.9[d]
pigment			
light	51.4	0.68	69.5
dark	52.4	0.68	71.4

[a] L_{785} is the path length of 785 nm light
[b] L_0 is the haemoglobin-free path length
[c] Differs from young, $p < 0.01$
[d] Differs from female, $p < 0.05$

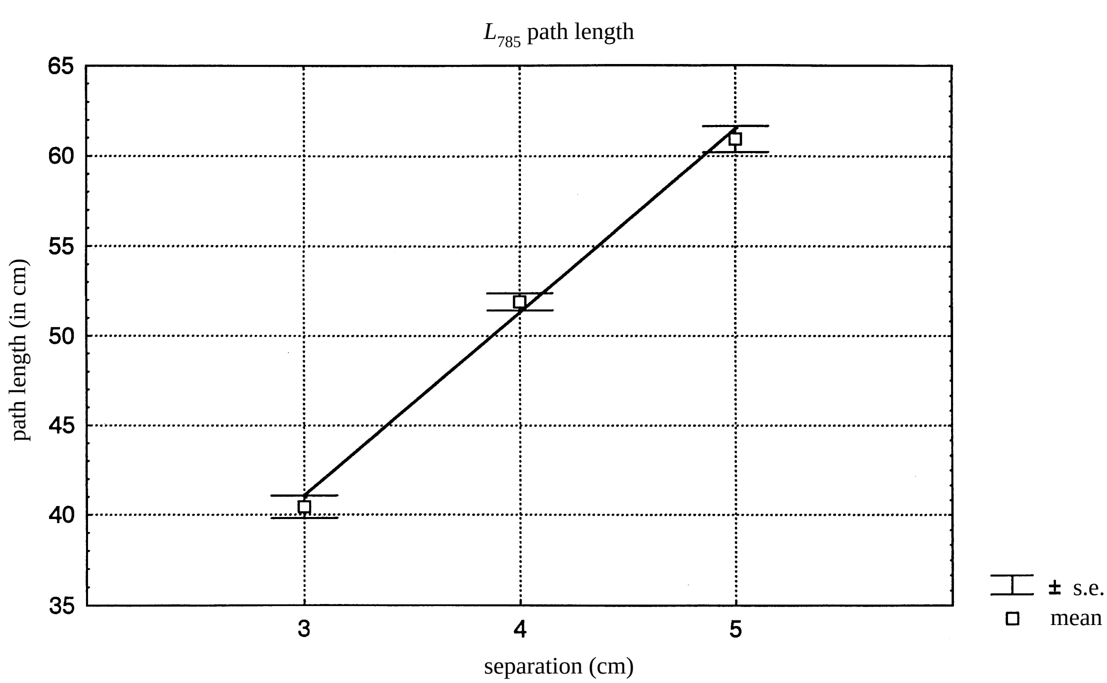

Figure 1. The relationship between the measured path length of 785 nm light and the separation of the optical probes is shown. The relationship is highly linear, but the standard errors are small because of the large number of measurements at each separation (30, 92, and 58 at 3, 4, and 5 cm respectively).

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Table 4. *Results grouped by maximum probe separation*

	A	B	
785 nm path length/cm	52.3	51.7	NS
saturation/fraction	0.69	0.67	NS
haemoglobin-free path length/cm	64.9	73.8	$p < 0.005$

A, subjects in whom measurements at 5 cm probe separation could not be obtained.
B, subjects in whom measurements at 5 cm probe separation could be obtained.
NS, not significant.

measurements could be obtained at 5 cm and those in whom they could not (table 4). Haemoglobin saturation and the 785 nm path length did not differ, but haemoglobin-free path length was longer in those in whom a measurement at 5 cm was obtainable.

4. DISCUSSION

The major advantage of coherent light techniques (PMS and TRS) for near-infrared spectroscopy is the ability to measure path length and thus distinguish between concentration and path length effects. If the path lengths were easily predicted from demographic or other data, measurement would be unnecessary and simpler techniques using incoherent light would suffice. In several ways, these results support the need to measure path length. The measured path lengths at 785 nm are highly linear with respect to separation. This correlation has high statistical significance but relatively poor predictive value, as suggested by the r^2 of 0.68. Looked at another way, in spite of the small standard error of the means, the standard deviations were sufficiently large that 30% of the measured values at each data point differed from the mean by 10% of the mean value. Such large variability precludes the use of the data in figure 1 for predicting path length.

Additional evidence of differences in the parameters of near-infrared light transmission among groups of individuals was demonstrated. A group of subjects was identified in whom the increased optical density prevented measurement at 5 cm separation. This group had shorter haemoglobin-free path lengths than did the group in whom 5 cm measurements were possible, suggesting less scattering and more absorption. Either haemoglobin or non-haemoglobin absorption could explain this phenomenon, although increased haemoglobin absorption would require a second difference to explain the observed differences in haemoglobin-free path length. The source of this additional absorption is purely speculative, but could include increased absorption in overlying muscles (myoglobin absorption), absorption in the bony matrix of the skull, or increased absorption in cerebral tissues due to higher concentrations of oxidative enzymes such as cytochrome aa_3. Perhaps the most interesting hypothesis is that the individuals with greater absorptions have fever scattering micro-organelles and, correspondingly, more intracellular water. Such intracellular structures are known to produce scattering (Beauvoit *et al.* 1995) and a reduction in scattering would produce shorter haemoglobin-free path lengths. The replacement of these scattering structures by water would increase the 'background' absorption (Liu *et al.* 1995), thus further reducing the haemoglobin-free path length. By combining these two effects, relatively small changes in intracellular composition might produce measurable differences in haemoglobin-free path length. Such differences might also explain the observation that the haemoglobin-free path length in women was about 8% longer than the haemoglobin-free path length in men (74.3 vs 68.9 cm). Previous studies (Van der Zee *et al.* 1992) did not have a sample size sufficient to identify these differences related to gender.

The method used to estimate haemoglobin saturation is essentially a ratio of sums and differences of path lengths, and can be extremely sensitive to measured errors if the denominator is small. In spite of this, saturation estimates were extremely reproducible within groups, so that clinically unimportant differences were identified as statistically significant. The source of this variation among groups is not clear, but factors such as concurrent disease, which was not controlled in subject selection, may have played a role in these observations.

In summary, these data demonstrate that there are significant differences in optical transmission characteristics of near-infrared light in adults, differences that cannot be predicted based on demographic factors. These differences must be measured in order to make meaningful calculations of haemoglobin saturation. Phase modulation techniques allow the measurement of path length and thus appear preferable to techniques that use average or assumed values for optical transmission.

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