NEUROVASCULAR RESPONSES TO SEQUENTIAL DEEP INSPIRATIONS ASSESSED VIA LASER-DOPPLER PERFUSION CHANGES IN DORSAL FINGER SKIN

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BACKGROUND

A vasomotor reflex that is triggered by a rapid and deep inspiration causes an arteriolar vasoconstriction and induces a transient decrease in skin blood flow. Reports of this phenomena appeared over 50 years ago[1-2], but many details of the sympathetic neural pathways involved are not yet known. Although the reflex causes vasoconstriction, an initial small blood flow increase may precede it and often a flow increase follows (figures 3-4).

This inspiratory gasp vascular response (IGVR) has most often been measured on plantar aspects of toes and fingers with laser-Doppler[3-4] or photoplethysmography[5] and has been used to study aspects of neurovascular function in many conditions including diabetes[6], Raynauds Phenomenon[7], erythromelagia[8] and leprosy[9].

RATIONALE

An important issue related to the use and interpretation of IGVR findings is its variability within the same subject and among subjects. Factors that may affect the magnitude of the vasoconstictive component include skin temperature, age, gender and vital capacity[4,10] . This variability may represent an intrinsic limit to the utility of the IGVR tests. However, systematic studies of the magnitude of variability and its implications for experimental design & interpretation of findings are sparse. Such variability affects the ability to detect possible differences when comparing normal subjects to patient groups and also affects the ability to detect changes that might be induced by rapidly acting therapeutic interventions in patients with suspected neurovascular deficts.

OBJECTIVES

The present study was undertaken to characterize key features of normal IGVR variability and to explore sampling strategies that might minimize the impact of this variability Specifically, it was our initial objectives to:

(1) Estimate the extent to which the number of sequential IGVR samples used to estimate mean responses for individual subjects effects variability among subjects
(2) Estimate the extent to which IGVR sample size effects the ability to detect acute changes in IGVR responses that would be associated with acute interventions in individual subjects

METHODS

SUBJECTS: Twenty-eight volunteers (14 male) were studied. Subjects had no history of cardiovascular or respiratory abnormality, hypertension or diabetes.

PROCEDURES: Subjects sat in a height adjustable chair with hands placed palm down on a support surface. A laser-Doppler probe was placed on the right index finger dorsum (figure 1A). A small thermocouple was placed under the probe and the finger wrapped with elastic self-adhering bandaging material (figure 1B). The hand was covered with a towel and skin temperature monitored. Testing began when a steady state was reached (15-20 minutes). During this interval subjects were instructed as to the required breathing maneuver and were given chances to practice. The instruction was to take a deep and rapid inspiration starting at the end of a normal quiet expiration and hold it for 10 sec (figure 2).

PROTOCOL: The test protocol consisted of a series of 21 sequential inspiratory gasps (IG) taken at two minute intervals. Average finger skin blood perfusion (SBF) during two-minute intervals preceding each successive gasp were used as the reference perfusion for its following inspiratory gasp vascular response (IGVR). IGVR was calculated using the minimum SBF during the gasp (SBF_{min}) and the average SBF (SBF_0) as shown in figure 3.

Skin and room temperatures were continuously monitored and recorded every two minutes corresponding to each of the inspiratory gasps. After the test, blood pressure was measured and an occlusion (200 mmHg) of the brachial artery for three minutes was used to determine the laser-Doppler biological zero. This value was routinely subtracted from all raw SBF data prior to analyses.

ANALYSES

The dependence of overall variability among subjects on the number of sequential IG samples used to estimate each subjects mean IGVR was determined as shown in figure 4.

To estimate the extent to which IG sample-set size affects the ability to detect acute (within subject) changes in IG responses, sample-set sizes of 1, 2, 3, 4, 5 and 6 sequential IGVR were used as illustrated in figure 5.

Inspiratory Gasp Response

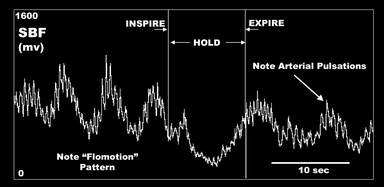
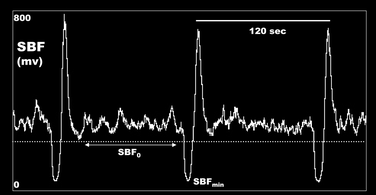


Figure 2

Calculation of IGVR



$$IGVR = \frac{SBF_0 - SBF_{min}}{SBF_0} \times 100$$

Figure 3

Example of Estimating Overall SD using Triplicate Sample-Sets

This sampling strategy results in 19 triplicate samples for each of the 28 subjects. Each sample-set provides a separate estimate of the mean IGVR for that subject. To estimate an overall SD, the SD of the estimated mean of each sample-set is averaged across subjects and the average of these 19 is used as an estimate of the overall SD of the mean IGVR for the full group. The same approach was used for samples-set sizes of 1, 2, 4, 5, 6 and 10 sequential IG samples.

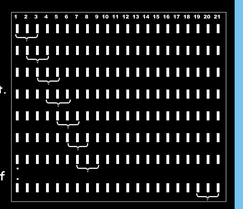


Figure 4

Sampling Stragegy to Estimate SD of Separated Sample-Sets

Effects of IG sample-set size on the ability to detect acute changes were estimated with set sizes of 1-6 IGVR. For each set, SD of differences between 1st & 2nd sets were determined. As the goal was to estimate detectible levels of change between "baseline" & an "intervention", 1st sets simulated baselines & sets starting at 10 & 20 minutes afterwards simulated intervention responses. The diagram shows a 20" separation example. For triplicate samples, responses 1-3 are the 1st set & responses 8-10 are the response set an so on up to a sample-set size of six. Correlations between 1st & 2nd sets were also determined. Results were used to estimate a minimum number of subjects needed to detect an IGVR change of either 10 or 20% of the overall mean for = 0.05 and a power of 90%.

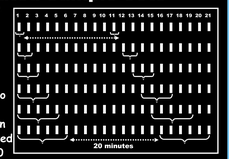


Figure 5

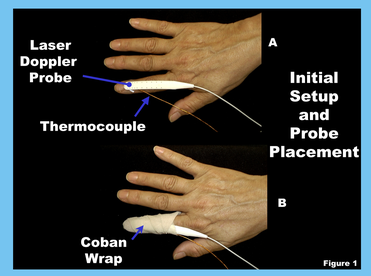


Figure 1

RESULTS

Summary of Subject Data

	Male (N=14)	Female (N=14)	Total Group (N=28)
Age (years)	32.9 7.9	33.3 13.2	33.1 10.7
Height (cm)*	177 6.3	167 5.8	172 8.0
Weight (Kg)*	82.4 11.0	62.2 9.0	72.3 14.3
Pressures (mmHg)			
Systolic	128 20	117 13	123 17
Diastolic*	89 13	79 8	85 12

Values are mean sd. *parameter values significantly greater for males (p<0.01).

Figure 6

Overall Sequential Parameter Values

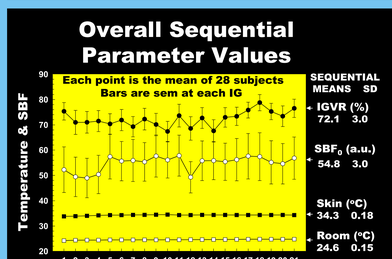


Figure 7

Estimated Minimum Number of Subjects to Detect a 20% IGVR Difference Between Groups

IG's in each sample set	Number of sequential sample sets	Estimated overall mean	Variance Across Subjects SD	CV	sd	Minimum Subjects per group to detect 20% difference*
1	21	72.2 3.0	21.1	29.4	4.4	46
2	20	72.1 2.4	19.2	26.7	3.2	38
3	19	72.0 2.3	18.7	26.0	3.0	36
4	18	71.9 2.2	18.3	25.5	2.7	34
5	17	71.8 2.0	18.1	25.3	2.5	34
6	16	71.7 1.8	18.0	25.2	2.2	33
10	12	71.4 1.1	17.6	24.7	1.4	32
21	1	72.2	16.9	23.4		29

*Tabulated values of N are for an of 0.05 and power of 90%

Figure 8

Estimated Minimum Number of Subjects for Detecting 10% and 20% changes in IGVR

IG Set Size	SD and Correlation of paired-differences: 10 minutes SD	R	20 minutes SD	R	Minimum Required Subjects*: 10 % change 10 min N	20 min N	20% change 10 min N	20 min N
1	17.1	0.661	19.1	0.615	60	74	15	19
2	14.2	0.761	15.8	0.670	41	51	11	13
3	11.4	0.830	14.5	0.722	26	43	7	11
4	10.3	0.859	13.2	0.753	22	36	6	9
5	10.6	0.844	11.7	0.799	22	28	6	7
6	10.7	0.842	11.8	0.795	22	28	6	7

*Tabulated values of N are for an of 0.05 and power of 90%

Figure 9

Paired-Difference SD

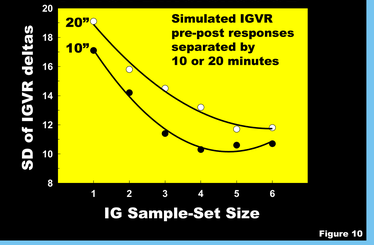


Figure 10

Paired Correlations

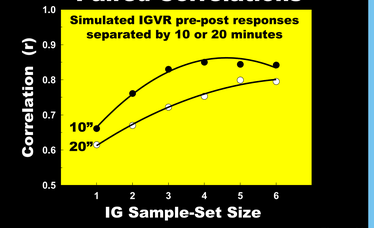


Figure 11

Minimum N to Detect 10% & 20% Changes

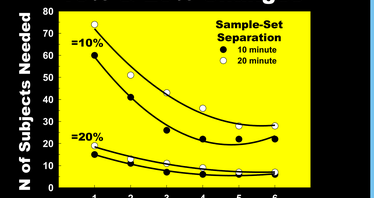


Figure 12

CONCLUSIONS

1. In this group of 28 healthy subjects the overall mean perfusion decrease induced by 21 sequential inspiratory gasps and measured at the finger dorsum was 72% of each responses immediately preceding two-minute baseline blood perfusion.

2. This magnitude of IGVR is comparable to values reported for those obtained at finger palmer surface that is rich in arterial-venous anastamoses (AVAs). This suggests that the magnitude of the IGVR is not fully dependent on AVA presence.

3. Variability of the IGVR, both within and across subjects, depended on the number of sequential sample-sets included in the estimation of the mean IGVR and on the time separation between sample-sets for within subject measures.

4. The ability to statistically detect differences in IGVR between normal subjects and patients with suspected neurovascular deficits thus depends on the number of IGVR samples used to characterize the mean response and on the number of subjects N.

5. Similarly, the ability to detect acute changes in IGVR, potentially associated with effects of rapidly acting therapeutic interventions that modify IGVR, depends on the IG sample-set size, the correlation between time separated sample-sets and on N.

6. The analyses provide a framework for, and specific estimates of, the minimum number of subjects needed to detect specified IGVR differences between groups or changes in IGVR after such interventions.

7. Application of these findings to results reported in the literature suggests that some previously drawn conclusions lack suitable statistical underpinnings.

REFERENCES

1. Bolton B et al. Vasoconstriction following deep inspiration. J Physiol 1936;86:83-94
2. Gilliatt RW. Vaso-constriction in the finger after deep inspiration. J Physiol 1948;107:76-88
3. Khan F et al. Quantification of sympathetic vascular responses in skin by laser Doppler flowmetry. Int J Microcirc 1991;10:145-153
4. du Buf-Vereijken PWG et al., Skin vasomotor reflexes during inspiratory gasp: Standardization by spirometric control does not improve reproducibility. Int J Microcirc 1997;17:86-92
5. Barron SA et al., DC photoplethysmography in the evaluation of sympathetic vaosomotor responses. Clin Physiol 1993;13:561-572
6. Wilson SB et al., Detection of microvascular impairment in type I diabetics by laser Doppler flowmetry. Clin Physiol 1992;12:195-208
7. Wollersheim H et al., Laser Doppler evaluation of skin vasomotor reflexes during sympathetic stimulation in normals and in patients with primary Raynaud's phenomenon. Int J Microcirc 1991;10:33-42
8. Littleford RC et al., Impaired skin vasomotor reflexes in patients with erythromelalgia. Clin Sci 1999;96:507-512
9. Abbot NC et al.,Vasomotor reflexes in the fingertip skin of patients with type 1 diabetes and leprosy. Clin Autonom Res 1993;3:189-193
10. Lau YT et al., Cutaneous vasoconstrictor response induced by inspiratory gasp in relation to sex and age. Clin Sci 1995;89:233-237