

METHOD FOR THE MEASUREMENT OF THE SENSITIVITY OF VASCULAR BEDS TO ISCHEMIA

Jonathan S Maltz and Thomas F. Budinger

Department of Functional Imaging, Lawrence Berkeley National Laboratory, University of California,
Berkeley, CA 94720
jon@eecs.berkeley.edu

Abstract- We identify a novel performance parameter of arterial beds and propose a convenient, non-invasive method for its measurement. The parameter relates the amount of reactive hyperemic flow that ensues after blood flow occlusion to the duration of occlusion and thus may be viewed as a measure of the sensitivity of the downstream vascular bed to the level of ischemia. The measurement is performed by inducing three successive progressively longer periods of ischemia in a limb using a pneumatic cuff. During the periods between occlusions, reactive hyperemic flow is measured at a finger using laser Doppler fluximetry (LDF). Total hyperemic response is calculated for each occlusion by averaging the LDF time series over the interocclusion interval. The vascular sensitivity parameter is calculated as the gradient of the line fit to the three mean response measurements. We evaluate the method in 18 subjects and find that orally administered nicotine significantly reduces the sensitivity to ischemia by 34%.

Keywords- ischemia, reactive hyperemia, nicotine, endothelial function, myogenic mechanisms.

1. INTRODUCTION

Intact vasomotive mechanisms are required if the metabolic requirements of tissue are to be matched to oxygen supply [1]. In conditions such as myocardial ischemia, Alzheimer's disease and ischemic vascular dementia, these mechanisms are deficient [2]. Hypoperfusion due to vascular insufficiency is a likely contributor to the genesis of cardiovascular disease and some types of dementia. The ability to conveniently characterize the integrity of acute ischemic response is a desirable clinical goal.

In this paper, we propose a technique to measure the "gain" of the feedback mechanism which modulates vascular tone and caliber under ischemic stimulus. The method requires no sensor contact with the subject and provides a measure that is independent of baseline blood flow. It is also normalized with respect to limb composition, which is a major determinant of the magnitude of absolute reactive hyperemic response [3].

Peak hyperemic flow and its integral (often referred to as "debt repaid") are considered to be measures of functional vasodilatory reserve of a vascular bed. These quantities are thought to be more

This work was supported in part by the National Heart, Lung, and Blood Institute of the U.S. Department of Health and Human Services under grants HL-07367, R01-HL50663 and P01-HL25840, in part by the National Institute on Aging grant R01-AG-05890, and in part by the Director, Office of Science, Office of Biological and Environmental Research, Medical Sciences Division of the U.S. Department of Energy under contract DE-AC03-76SF00098.

greatly affected by the tone of resistance vessels (arterioles and capillaries) than that of conduit vessels (arteries) [4].

Reactive hyperemic flow is normally measured using venous occlusion plethysmography (VOP). This method allows the flow-versus time characteristic after arterial occlusion to be quantified. Values of peak hyperemic flow and total hyperemic flow are usually considered important parameters. These quantities can be difficult to interpret, since they are strongly dependent on the tissue composition within the limb to which VOP is applied [3].

Our method is based on performing successive blood flow occlusions of increasing length, for example 15, 30 and 60 seconds. Between occlusions, cutaneous perfusion is measured at the middle finger for a period of 30 seconds. The reactive hyperemic response is averaged over the 30 seconds. This provides three data points. We have observed an approximately linear relationship between average response and occlusion duration in most subjects. The slope of the line fit to these points is thus proposed as a measure of sensitivity to ischemia.

In this paper we describe the technique in detail and present preliminary results of human subject studies in which the effect of oral nicotine on the sensitivity parameter is evaluated.

2. MEASUREMENT TECHNIQUE

Figure 1 illustrates the application of the measurement technique to the human forearm. A pneumatic cuff is placed around the upper arm of a seated subject. The arm rests on a flat surface at chest height, as shown in the upper left of Figure 1. A baseline measurement of cutaneous perfusion on the dorsal side of the middle phalange of the middle finger is made using a laser Doppler imager (Moor LDI, Moor Instruments, Devon, UK). The cuff is inflated for O_1 seconds, and then released. Measurement ensues for M_1 seconds. The cuff is then inflated for O_2 seconds, released and a perfusion measurement is made for M_2 seconds. Finally, the cuff is inflated for O_3 seconds, and then released. A final perfusion measurement is made for M_3 seconds.

Perfusion measurements obtained after occlusion O_n during measurement interval M_n are averaged over this interval. A straight line is fitted using an unweighted least squares method to the three resulting mean perfusion values. The gradient m of this line serves as the dependent variable in the statistical analysis of the data.

3. EVALUATION IN HUMAN SUBJECTS

Our preliminary studies involved performing the measurement described above before and 30 minutes after the administration of

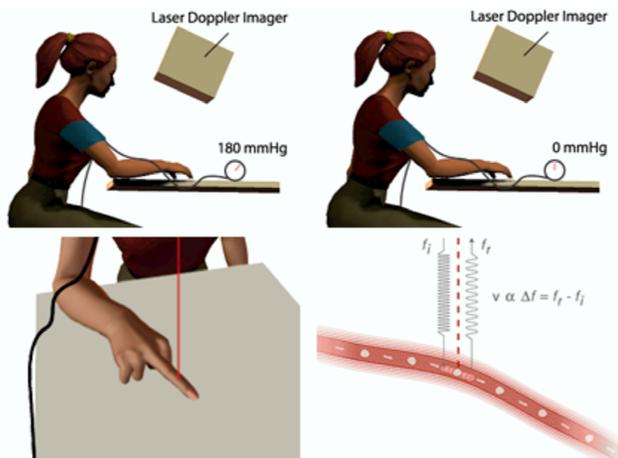


Fig. 1. Procedure for measuring vascular ischemic sensitivity using laser Doppler imaging. A cuff around the upper arm is inflated to occlude blood flow. It is then released. While reactive hyperemia ensues, a measurement of cutaneous perfusion is made. The frequency of the reflected laser light is shifted in proportion to the speed of the red blood cells in the cutaneous circulation.

4mg of sublingual nicotine in the form of chewing gum. Time intervals were set at $O_1 = 15s$, $O_2 = 30s$, $O_3 = 60s$ and $M_1 = M_2 = M_3 = 30s$.

Each subject was instructed to chew the gum for about 90 seconds, then kept it in the mouth for a period of about 5 minutes until the mild tingle subsided. Chewing was repeated for about 10 seconds, then the gum was returned to the side of the mouth for a further 5 minutes. This intermittent chewing continued for a total of 30 minutes.

Hypertensive subjects and users of tobacco products were excluded from the study. Subjects were asked to fast for 12 hours before the study and to avoid taking medication known to alter vascular response.

A total of 23 studies were performed on 18 subjects (14 male, 4 female). In 18 of the studies, a dose of 4mg nicotine was administered. In the other 5 studies (3 female, 2 female), the same protocol was followed except that no gum was chewed during the 30 minute rest period between tests.

Mean subject age was 27.3 ± 1.0 years for the group that received the 4mg nicotine and 27.1 ± 2.0 years for the 0mg group.

The experimental protocol was approved by the university institutional review board and experiments were conducted in compliance with institutional guidelines. Subjects gave informed consent to participate in all experiments.

4. RESULTS

Where applicable, all values are given as the mean \pm the standard error of the mean (SEM).

Figure 2 illustrates a typical set of raw data. The area under each curve yields one of the three data points to which a line is fitted.

A typical response before and after nicotine appears in Figure 3.

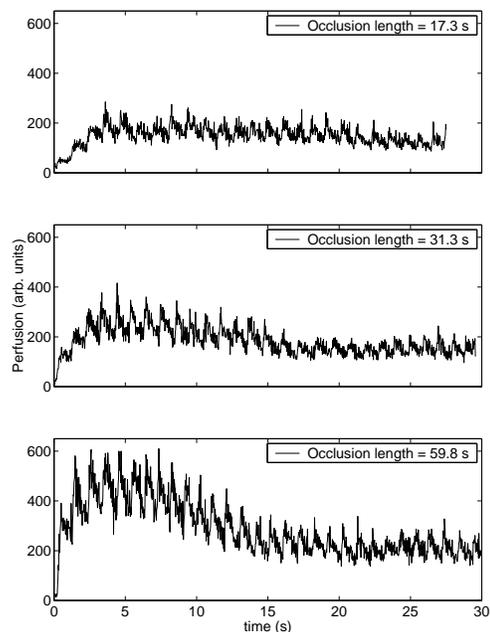


Fig. 2. Typical raw perfusion data traces output by the laser Doppler imager during three successive reactive hyperemic periods.

Figure 4 shows the mean gradients and mean gradients \pm their SEMs for all subjects before and after 0mg (N=5) and 4mg (N=18) nicotine. In this figure the lines are arbitrarily plotted to intercept at the 15 second time point. Table 1 compares values of baseline perfusion and line gradients for both study groups.

While baseline perfusion falls by approximately 30% in the second test regardless of whether nicotine is administered or not, this change was not statistically significant. Likewise, the large increase in gradient when 0mg of nicotine was administered is not significant. However, the 4mg nicotine dose significantly decreased the gradient by 34% ($p < 0.05$).

The mean perfusion response for the 60s occlusion was significantly decreased (by 32%) when 4mg of nicotine was administered but not when no gum was chewed. Statistical significance was not achieved for the effect of nicotine on the responses to shorter occlusions.

5. CONCLUSION

Our preliminary results indicate that the proposed method is able to detect impairment of incremental response to ischemic severity by an agent that is known to act primarily as a vasoconstrictor and inducer of endothelial dysfunction.

Many more studies are required in order to determine repeatability and sensitivity with respect to measurement location.

A limitation of this study is that while nicotine is predominantly a sympathetic vasoconstrictor, it may also directly stimulate cholinergic receptors on endothelial cells and lead to vasodilation. Sympathetic vasoconstriction [5] is likely to impair the myogenic dilation response that is thought to contribute the most to reactive hyperemic flow. The balance of the dilation that leads to re-

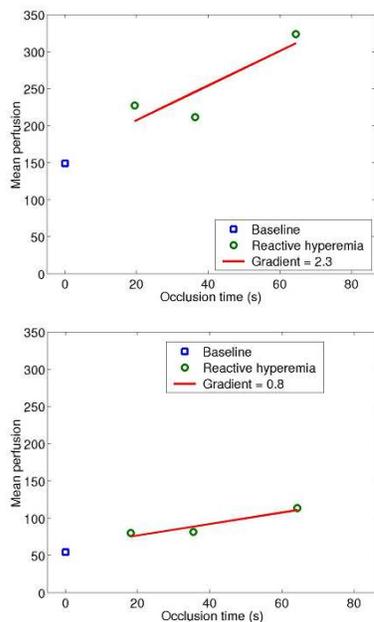


Fig. 3. The upper figure shows a typical response before nicotine. The lower figure illustrates a reduced sensitivity to ischemia after 4mg of nicotine when compared to the upper figure.

active hyperemia is probably endothelium dependent, with nitric oxide, prostaglandins, endothelium derived hyperpolarizing factor (EDRF), adenosine and accumulation of anions serving as endothelial vasodilatory stimuli. The effects of nicotine on the endothelium are controversial. Fewings et al. observed an increase in forearm blood flow after intra-arterial injections of nicotine that was sustained for 15-45 minutes [5]. Hand blood flow was initially decreased after the injection but then exceeded baseline levels for up to 30 minutes [6]. Acute administration of nicotine has been shown to impair the dilation of arterioles due to acetylcholine, via an oxidative mechanism, in the hamster cheek pouch [7, 8]. A more recent study observed that the effects of nicotine the production of NO via endothelial NO synthase (eNOS) are dependent on interactions with NADPH and oxygen radicals [9]. Thus, depending on conditions, mode of administration, and dose, nicotine may act to constrict or dilate vasculature. However, in the majority of reports, acute nicotine administration is reported to constrict arterioles. Our results are thus consistent with most other studies.

In our study, although uncommon, we have witnessed cases where nicotine increased our sensitivity measure. Evaluation of the method using a drug with a more specific pharmacological action (such as the NO synthase inhibitor L-NAME) would thus be preferable.

The selection of the occlusion interval lengths is presently not optimized. The fact that the perfusion response following the 60s occlusion is itself significantly reduced by nicotine while the reductions in the responses to the shorter intervals are smaller and less significant suggests that longer occlusion times might allow us to obtain a more valuable sensitivity measure.

It is also clear from Table 1 that the variance of the slope “after

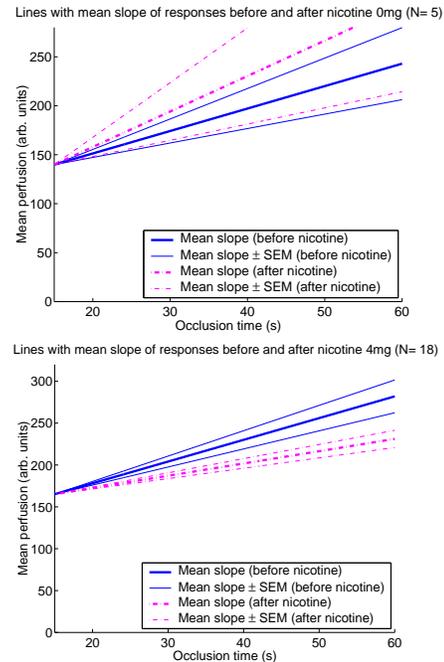


Fig. 4. The upper figure shows the mean slopes \pm their SEMs before and after 0mg nicotine. There is no significant difference between the mean slopes for the 5 subjects. The lower figure represents the corresponding changes observed after 4mg nicotine in 18 subjects. A large (34%) significant decrease in mean slope is evident ($p < 0.05$).

0mg nicotine” is much higher than that “before 0mg nicotine”. This may indicate some interaction between the two successive sets of occlusions. It may be necessary to increase the interval between the two tests to more than 30 minutes or to conduct the second test on the contralateral limb.

In summary, we present a method that can practically be applied to quantitate the responsiveness of the peripheral vascular system to mild ischemia. This method has the potential to measure changes in the reactivity of subjects under conditions such as smoking, hypertension, atherosclerosis, diabetes and sick cell disease. It may also be employed to determine the effects of various vasoactive drugs (e.g. statins and inhibitors of angiotensin-converting enzyme, cholinesterases and phosphodiesterases) on ischemic sensitivity.

6. REFERENCES

- [1] T M Griffith, “EDRF and the control of flow in arteries,” in *Flow-dependent regulation of vascular function*, pp. 178–213. American Physiological Society, 1995.
- [2] J. C. de la Torre, “Impaired cerebrovascular perfusion. Summary of evidence in support of its causality in Alzheimer’s disease,” *Ann N Y Acad Sci*, vol. 924, pp. 136–152, 2000.
- [3] T. C. Wascher, R. Bammer, R. Stollberger, B. Bahadori, S. Wallner, and H. Toplak, “Forearm composition contributes

to differences in reactive hyperaemia between healthy men and women," *Eur J Clin Invest*, vol. 28, no. 3, pp. 243–248, Mar 1998.

- [4] W. M. Chilian, C. L. Eastham, and M. L. Marcus, "Microvascular distribution of coronary vascular resistance in beating left ventricle," *Am J Physiol*, vol. 251, no. 4 Pt 2, pp. H779–H788, Oct 1986.
- [5] J. D. Fewings, M. J. Rand, G. C. Scroop, and R. F. Whelan, "The action of nicotine on the blood vessels of the hand and forearm in man," *Br J Pharmacol*, vol. 26, no. 3, pp. 567–79, Mar. 1966.
- [6] J. D. Fewings, M. L. Roberts, A. V. Stepanas, and R. F. Whelan, "The effects of decreased muscle blood flow on post-exercise hyperaemia in the human forearm," *Aust J Exp Biol Med Sci*, vol. 43, no. 4, pp. 547–52, Aug. 1965.
- [7] W. G. Mayhan and K. P. Patel, "Effect of nicotine on endothelium-dependent arteriolar dilatation in vivo," *Am J Physiol*, vol. 272, no. 5 Pt 2, pp. H2337–42, May 1997.
- [8] W. G. Mayhan, G. M. Sharpe, and P. Anding, "Agonist-induced release of nitric oxide during acute exposure to nicotine," *Life Sci*, vol. 65, no. 17, pp. 1829–37, 1999.
- [9] B. H. Tonnessen, S. R. Severson, R. D. Hurt, and V. M. Miller, "Modulation of nitric-oxide synthase by nicotine," *J Pharmacol Exp Ther*, vol. 295, no. 2, pp. 601–6, Nov. 2000.

Quantity	Before nicotine	After nicotine 0mg	% change	<i>p</i> value
Baseline perfusion	133.3 ± 34.7	92.8 ± 20.6	–30.36	0.34
Gradient of line	2.3 ± 0.8	3.6 ± 2.0	57.95	0.55
Mean perfusion (15s occl.)	159.5 ± 41.0	120.2 ± 22.3	–24.64	0.42
Mean perfusion (30s occl.)	194.0 ± 58.4	133.0 ± 25.4	–31.45	0.37
Mean perfusion (60s occl.)	262.1 ± 72.2	271.8 ± 88.0	3.70	0.93
Quantity	Before nicotine	After nicotine 4mg	% change	<i>p</i> value
Baseline perfusion	160.0 ± 29.8	106.4 ± 15.2	–33.50	0.12
Gradient of line	2.6 ± 0.4	1.5 ± 0.2	–43.51	0.03 [†]
Mean perfusion (15s occl.)	187.3 ± 26.2	143.1 ± 15.3	–23.57	0.15
Mean perfusion (30s occl.)	213.7 ± 29.8	150.1 ± 18.3	–29.76	0.08
Mean perfusion (60s occl.)	302.7 ± 36.0	206.3 ± 21.4	–31.82	0.03 [†]

Table 1. The upper table contains measurement parameters for experiments on 5 subjects given 0mg nicotine. The lower table lists the corresponding measurements for 18 subjects given 4mg nicotine. Statistical significance at the 95% confidence level is denoted by [†].