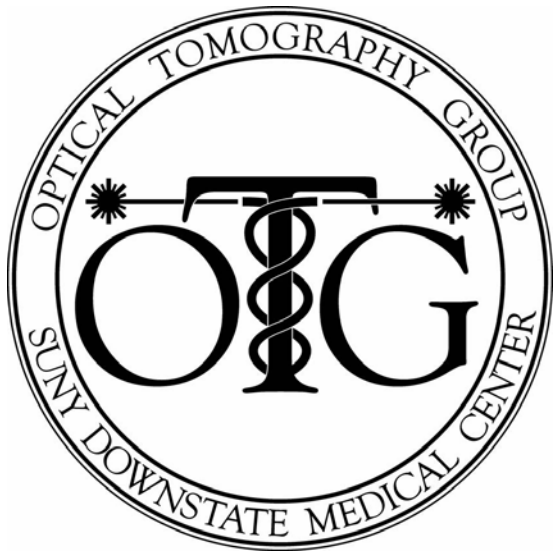
Evaluation of peripheral forearm perfusion in diabetics after an ischemic challenge with dynamic optical tomography

Nelson A. Franco[1], Michael S. Katz[1], Harry L. Graber[2], Christoph H. Schmitz[2], MaryAnn Banerji[3], Adam J. Braithwaite[5], Yaling Pei[5] , Michael Zenilman[1], and Randall L. Barbour[2,5]

Departments of Surgery[1],Pathology[2] and Medicine[3], SUNY Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, New York 11203
Department of Vascular Surgery[4], Staten Island University Hospital, 475 Seaview Avenue, Staten Island, New York 10305
NIRx Medical Technologies,LLC[5], 15 Glen Cove, Long Island, NY



INTRODUCTION

Diabetes mellitus (DM) is a chronic systemic disease with a tremendous medical burden on the world population today. Much of the morbidity and mortality of DM has been linked with peripheral vascular disease (PVD) and its pervasive manifestations. It has been shown that patients with DM with peripheral vascular disease have more severe arterial disease and poorer outcomes than normal controls.[1] Dysfunctional autoregulation of the vascular system in DM develops prematurely and aggressively resulting in debilitating claudication, tissue loss, and ultimately the need for surgical revascularization or amputation. Taken together, the vascular manifestations of DM account for the majority of the morbidity of the disease.

Dynamic optical tomography (DYNOT), a novel non-invasive modality for the functional imaging of the spatiotemporal dynamics of the vascular system, detects the microvascular circulation and the corresponding tissue oxygenation state without exposure to ionizing radiation or nephrotoxic dyes. The principle of DYNOT relies upon the fundamental concept that light in the near-infrared spectrum can propagate through tissue and is differentially absorbed by oxyhemoglobin and deoxyhemoglobin.[2] DYNOT employs the use of a coordinated array of NIR source-detector pairs oriented circumferentially around a limb to study the relative levels of oxyhemoglobin (HbO2), deoxyhemoglobin (Hbred), and subsequently total hemoglobin (Hbtot). Our previously published body of work has validated the principles and physiological rationale for DYNOT.[3] [4] [5]

We had previously demonstrated a correlation between the rate of accumulation of total hemoglobin and an individual's HbA1c level using a venous occlusion provocation model. We demonstrated that the less disciplined a diabetic's glycemic control (higher HbA1c), the lower the rate of total hemoglobin accumulation measured in that patient (See Figure 1a).[7]

We now sought to apply an anerobic-anerobic provocation to the evaluate the vascular response. Specifically, we used a brief, graded periods of arterial ischemia and the subsequent reperfusion as our metric to evaluate differences in the vascular bed response between normal patients and patients with DM2. (Figure 3a & b)

It has been well-documented by previous studies that the peripheral vasodilatory autoregulation systems such as reactive hyperemia is impaired in patients with DM2 [6] [8][10][11]. We found that we could measure statistically significant differences in the maximal amplitude of the detector response between diabetic and euglycemic groups (See Figure 1b).

We show that following an ischemic provocation, it is in the initial reperfusion phase and the peripheral tissue's recovery following the metabolic insult, that useful information about tissue and vasomotion disturbances can be determined.

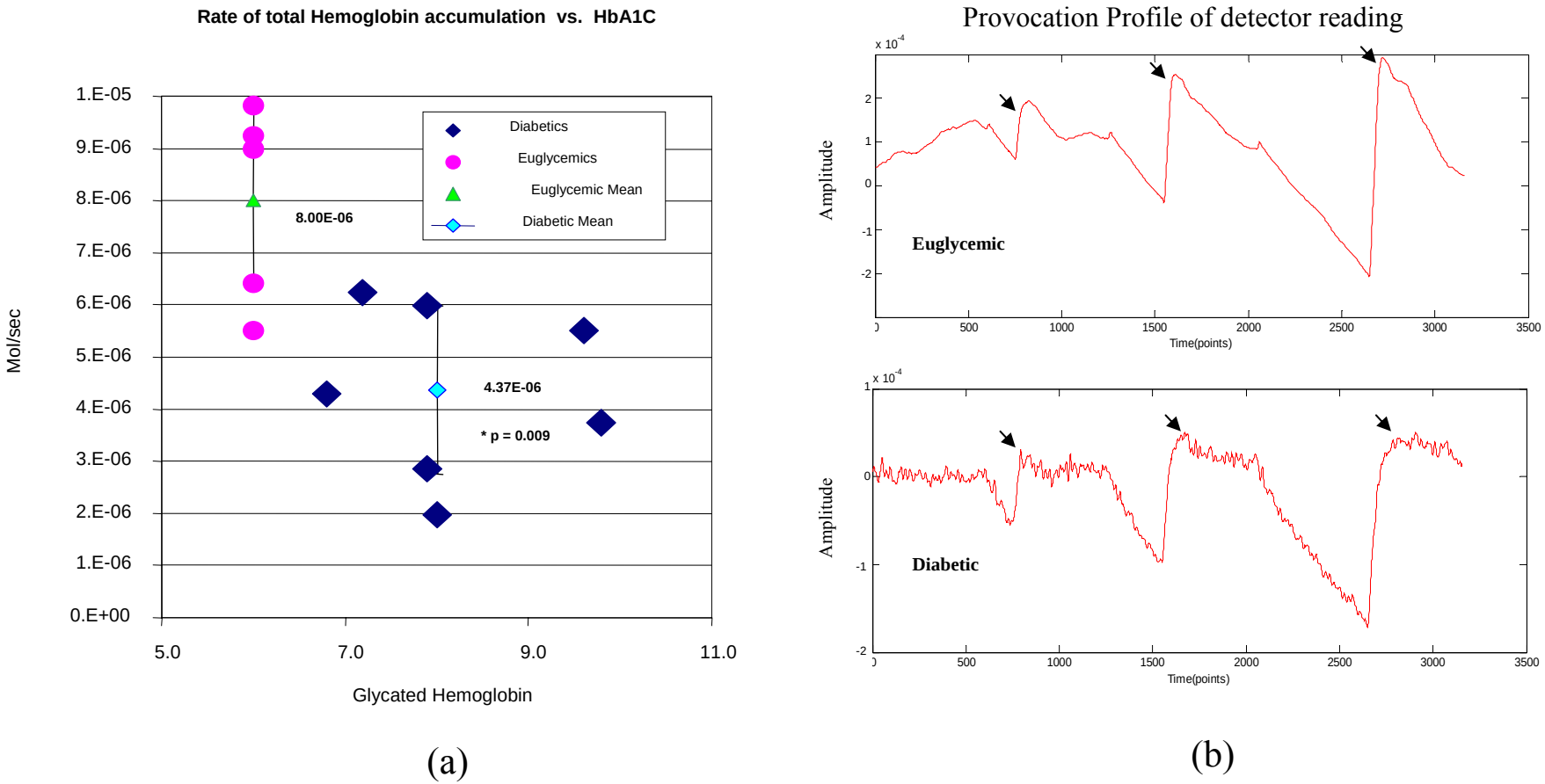


Figure 1: (a) Relation between total hemoglobin inflow rate and HbA1C level. There was a statistically significant decrease in total hemoglobin accumulation as HbA1C levels increased. (b) The amplitude of the detector signals (arrowheads) were significantly lower in the diabetic than in the euglycemic patient groups.

METHODS

Nineteen volunteers were measured; 20% were female, 40% were ethnic minorities. Volunteer ages ranged from 30 to 62. Seven of the volunteers were clinically diagnosed, poorly to moderately-controlled diabetics receiving only oral therapy ($HbA1_C$ <9%). Patients diagnosed as diabetics had a duration of diagnosis greater than 5 years. The groups were aged matched. One patient, in addition to already having a diagnosis of DM, was also a clinically diagnosed vasculopath with documented peripheral vascular disease.

The volunteer was then placed in a seated recumbent position and remained at rest during the measurements. The volunteer was allowed to acclimate to his/her position in the chair and the environment for ten minutes before any experimental provocations were initiated. Observation of vital signs permitted an objective indication that no significant change in physical activity occurred during the measurement period. Measurements were obtained from a dual-wavelength (760nm and 830nm) optical tomographic iris measurement head (Figure 2) positioned around the left prone forearm.

Experimental provocations consisted of inflation of a blood pressure cuff positioned around the left arm proximal to the imaging head to a pressure of 180 mmHg to produce arterial occlusion and induce a mild period of tissue ischemia. This provocation was performed 3 times. The provocation lasted 60, 120, and 240 seconds with 400 seconds between each provocation. Figure 3c illustrates the detector response profile to this graded arterial occlusion provocation.

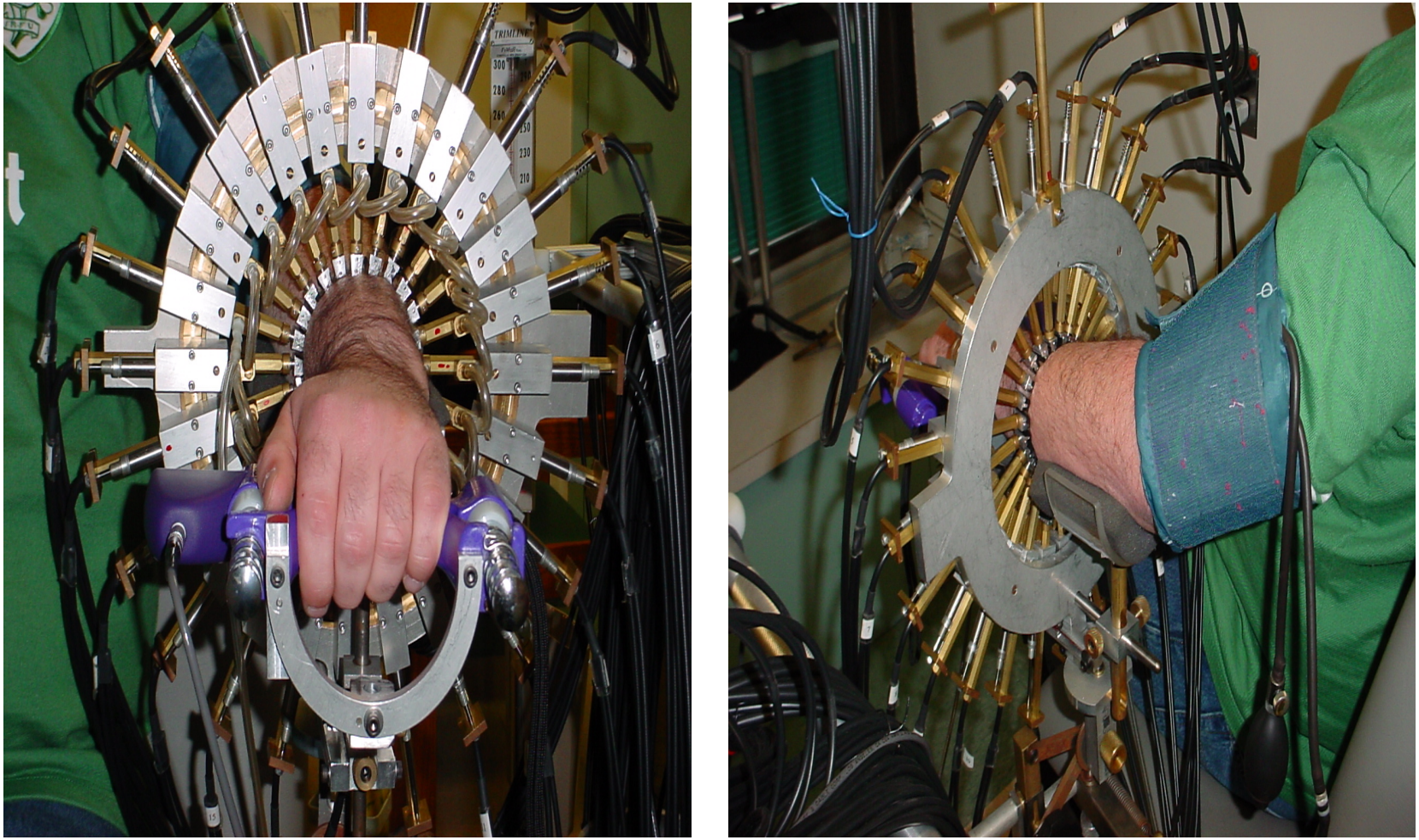
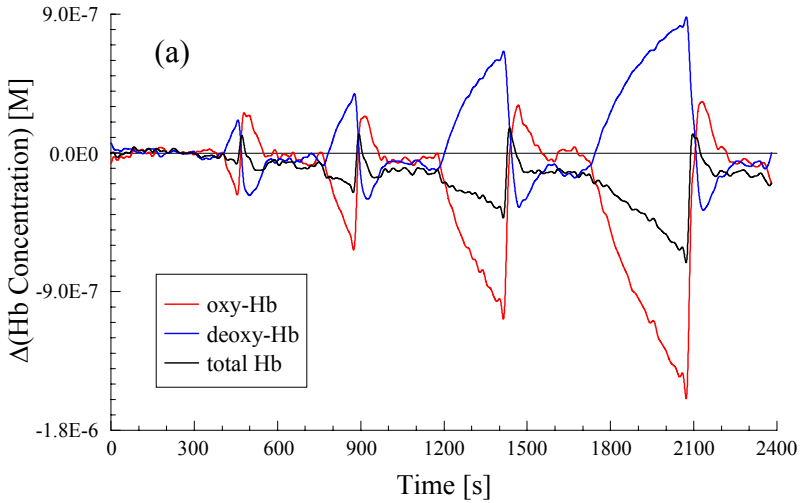


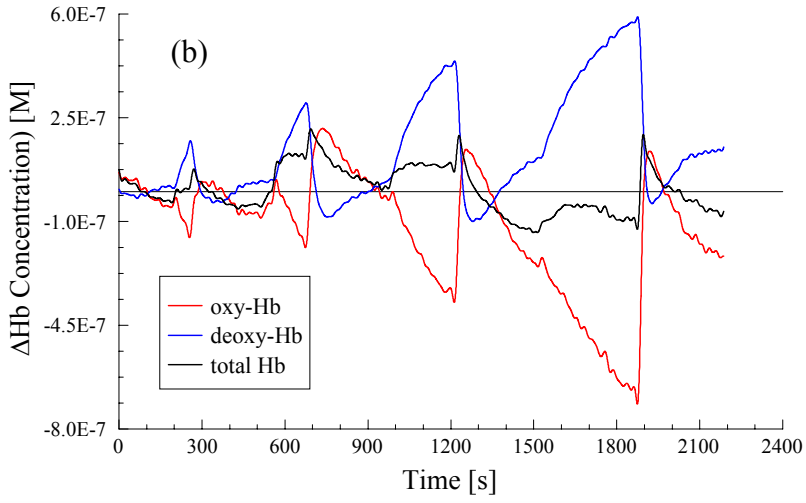
Figure 2. Typical placement of the Iris Imaging Head on the forearm and the orientation of the volunteer's forearm during the measurements.

Hemoglobin Fluctuations, Spatially Integrated Across Reconstructed Image

Euglycemic



Type-2 Diabetic



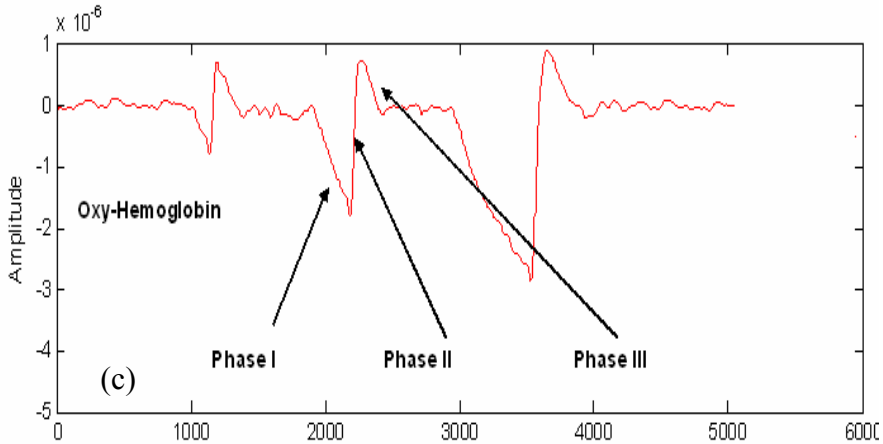


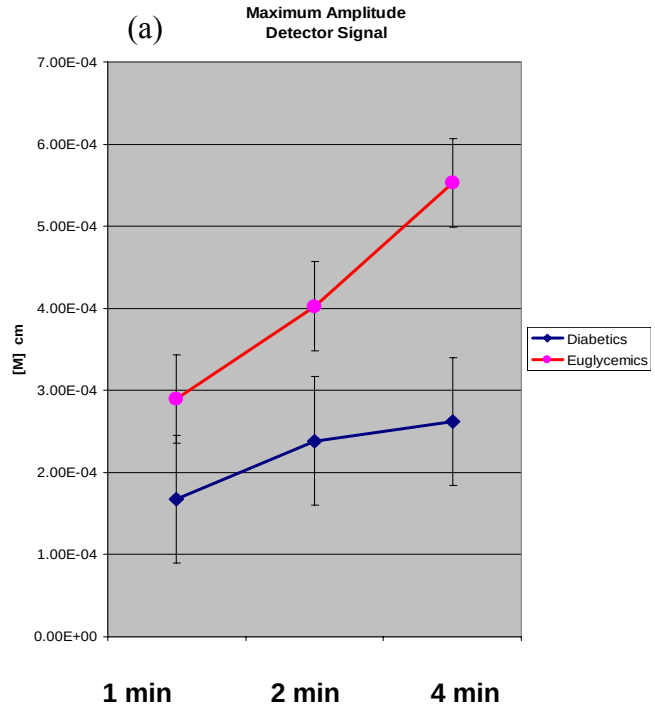
Figure 3. (a) and (b) show the divergent detector response profiles for oxy- and deoxy-hemoglobin in a euglycemic patient compared to a diabetic patient. (c) Phase 1 is defined as the actual ischemic event from point of cuff inflation to cuff release. Phase 2 of the response profile is the period of reactive hyperemia. Phase 3 is the return of the detector signal to baseline.

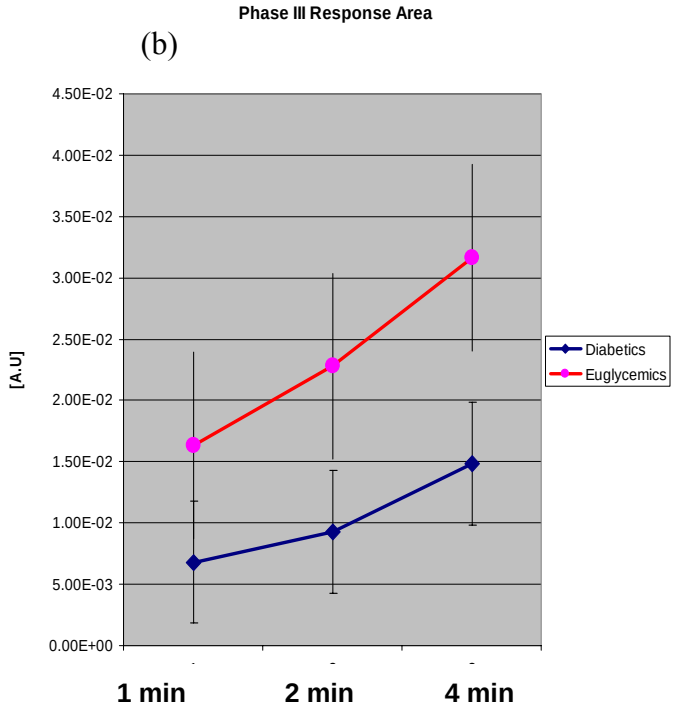
DATA ANALYSIS and RESULTS

Changes in Hb concentration (Figure 3) were computed from the reconstructed two-wavelength absorption coefficients at each FEM mesh node, by solving a simple algebraic system of two equations in two unknowns. For the detector readings time series, relative concentration changes for the Hb states were estimated via a modified Lambert-Beer law. 1) A general linear model (GLM) algorithm was applied to the detector data or the image time series to find the best fit of each time series to a model function created by spatially averaging over all source-detector pairs or, in the case of reconstructed images, over all pixels in each image. 2) A rate analysis strategy was employed to quantify changes in vascular compliance by evaluating the maximal transient response of the optical signal to the occlusion maneuver.

We observed a statistically significant difference for the decrease in response strength between the two groups ($n_{euglycemic} = 6$, $n_{diabetic} = 4$). We also quantified the change in area under the response signal curves of Phase III as a measure of the reactive hyperemic response magnitude. Figure 4 shows the results of this analysis. The statistical methods used for the small group comparisons were made by means of the Mann – Witney test.

Image recovery was achieved using the Normalized Difference Method [8]. As previously shown, this algorithm is markedly insensitive to expected uncertainties in boundary conditions, which are unavoidable in experimental methods. A truncated singular value decomposition procedure is used to solve the linear perturbation equation. The absorption coefficient images were subsequently post-processed to produce image time series for oxygenated hemoglobin (Hb) concentration changes.





	Euglycemic (n=12)	Diabetic (n=7)	p-value
E1	2.90E-04 ±5.41E-05	1.67E-04 ±7.82E-05	.0190
E2	4.02E-04 ±1.00E-05	2.38E-04 ±8.92E-05	.0059
E3	5.53E-04 ±2.17E-05	2.62E-04 ±1.33E-05	.0059

	Euglycemic (n=12)	Diabetic (n=7)	p-value
E1	1.63E-02 ± 7.70E-03	6.81E-03 ± 2.75E-03	0.0019
E2	2.28E-02 ± 7.59E-03	9.25E-03 ± 3.25E-03	0.0102
E3	3.16E-02 ± 9.88E-03	1.48E-02 ± 1.15E-02	0.0084

Figure 4. The maximum amplitude of Phase 2 detector readings (a) and the integrated area of the Phase 3 detector response (b) show a significant decrease in the detector response profile of the diabetic versus the healthy volunteer. This finding demonstrates impairment of autoregulation after an ischemic challenge.

Phase 1		Healthy (n=6)		Diabetic (n=4)	
	n	std	n	std	p-value
1 min	184.33	8.98E+01	182.75	6.99E+00	0.4540
2 min	262.17	4.33E+01	254.00	5.54E+01	0.4575
4 min	240.33	8.01E+01	308.50	6.62E+01	0.1204

	GLM coeff mean	std	Coeff mean	std	p-value
1 min	1.85E-06	1.23E-06	2.22E-06	1.15E-06	0.3749
2 min	4.74E-06	2.52E-06	4.80E-06	2.37E-06	0.4575
4 min	6.96E-06	4.60E-06	1.01E-05	7.27E-06	0.1956

Table 1. (a) Comparison of the total number of pixels (n) of GLM Coefficients > threshold value of .25E-6 [M] •cm between Healthy and Diabetic groups. (b) Comparison of the mean GLM coefficients > threshold value between the two groups. Groups were aged matched. Volunteers younger than 30 were excluded from comparison

Phase 2		Healthy (n=6)		Diabetic (n=4)	
	n	std	n	std	p-value
1 min	239.00	3.62E+01	195.75	4.35E+01	0.0829
2 min	253.17	2.24E+01	198.50	4.90E+01	0.0549
4 min	234.67	5.80E+01	223.00	3.54E+01	0.2191

	GLM coeff mean	std	Coeff mean	std	p-value
1 min	1.54E-06	3.67E-07	1.32E-06	5.61E-07	0.2377
2 min	1.97E-06	4.93E-07	2.44E-06	1.37E-06	0.3745
4 min	2.57E-06	8.62E-07	2.77E-06	1.41E-06	0.4512

Table 2. (a) Comparison of the total number of pixels (n) of GLM Coefficients > threshold value of .25E-6 [M] •cm between Healthy and Diabetic groups. (b) Comparison of the mean GLM coefficients > threshold value between the two groups.

Phase 3

	Healthy (n=6)		Diabetic (n=4)		
	n	std	n	std	p-value
1 min	259.17	4.24E+01	157.75	4.23E+01	0.0829
2 min	290.83	4.04E+01	200.50	6.56E+01	0.0549
4 min	246.67	6.37E+01	236.50	8.65E+01	0.2191

	GLM coeff mean	std	Coeff mean	std	p-value
1 min	3.24E-06	1.88E-06	1.15407E-06	3.08E-07	0.0549
2 min	3.00E-06	1.43E-06	2.48015E-06	1.27E-06	0.3325
4 min	4.44E-06	1.32E-06	1.3995E-06	2.75E-07	0.0140

Table 3. (a) Comparison of the total number of pixels (n) of GLM Coefficients > threshold value of .25E-6 [M] •cm between Healthy and Diabetic groups. (b) Comparison of the mean GLM coefficients > threshold value between the two groups.

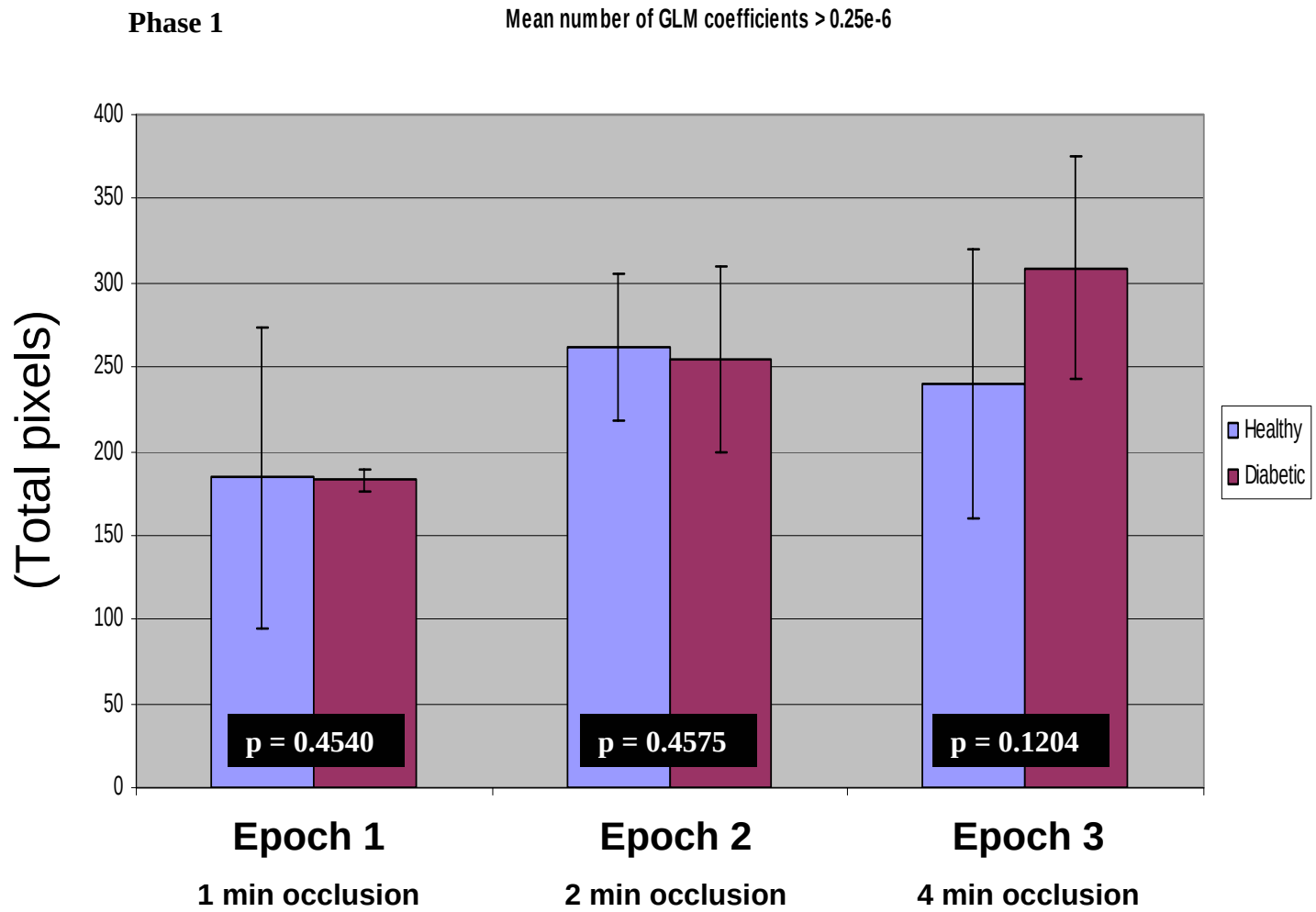


Figure 5. No statistical significance was found in the number of GLM coefficients greater than the threshold value of 0.25E-6 [M] •cm when diabetic and healthy groups were compared against each other.

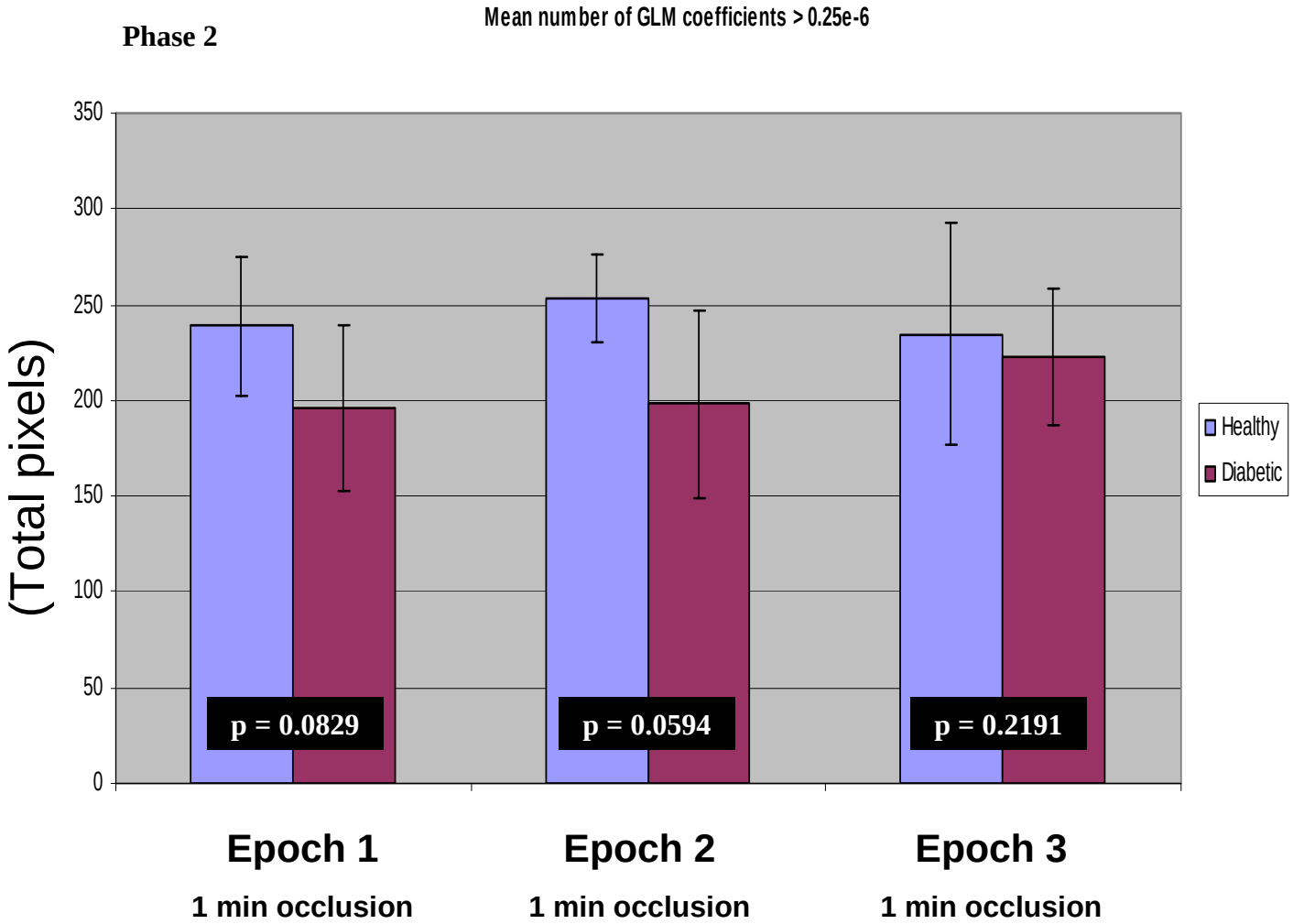


Figure 6. There was no statistical difference noted between the the diabetic and healthy groups within each Epoch provocation.

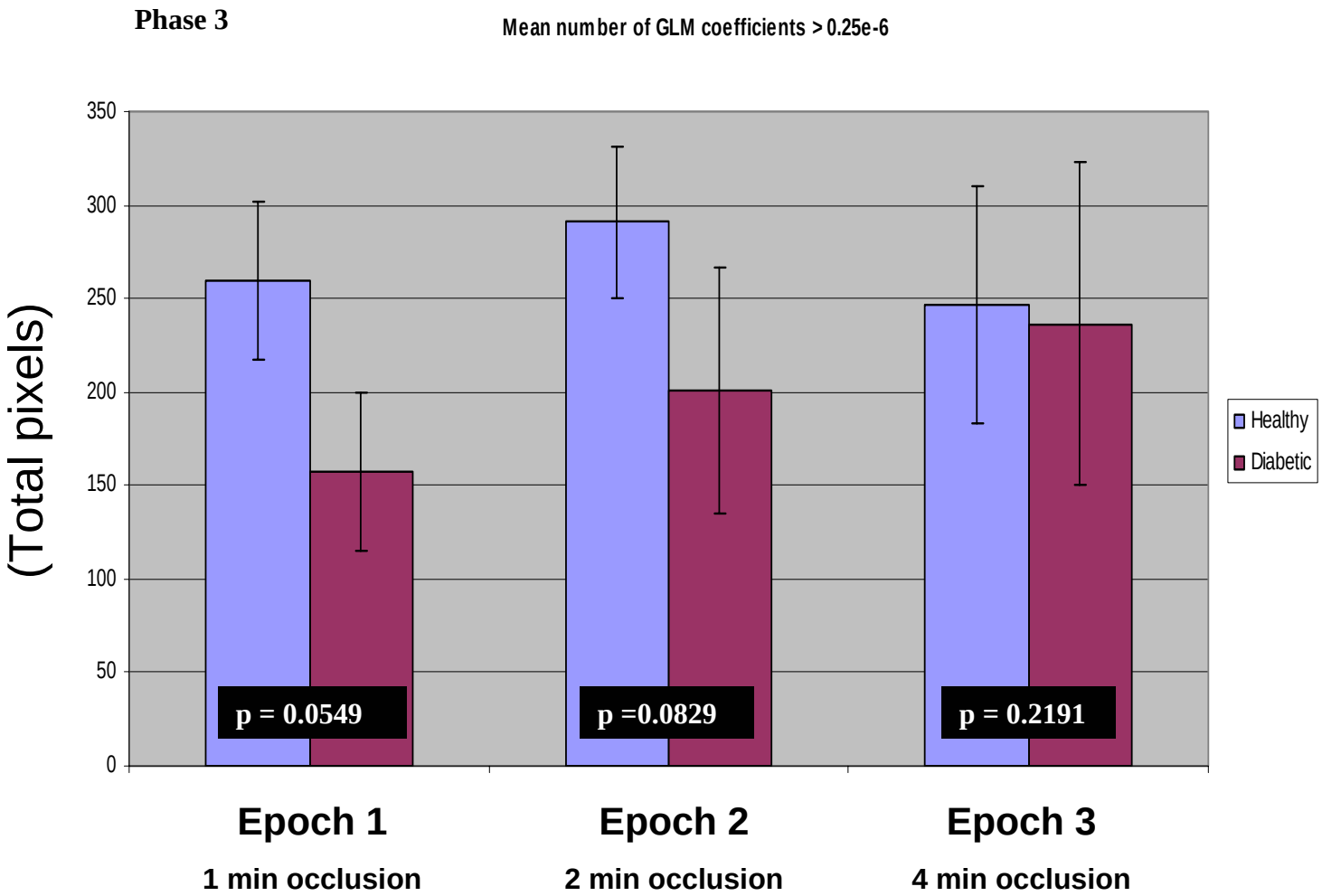


Figure 7. No clear statistical differences were noted between the diabetic and the healthy group in phase 3 of the provocation.

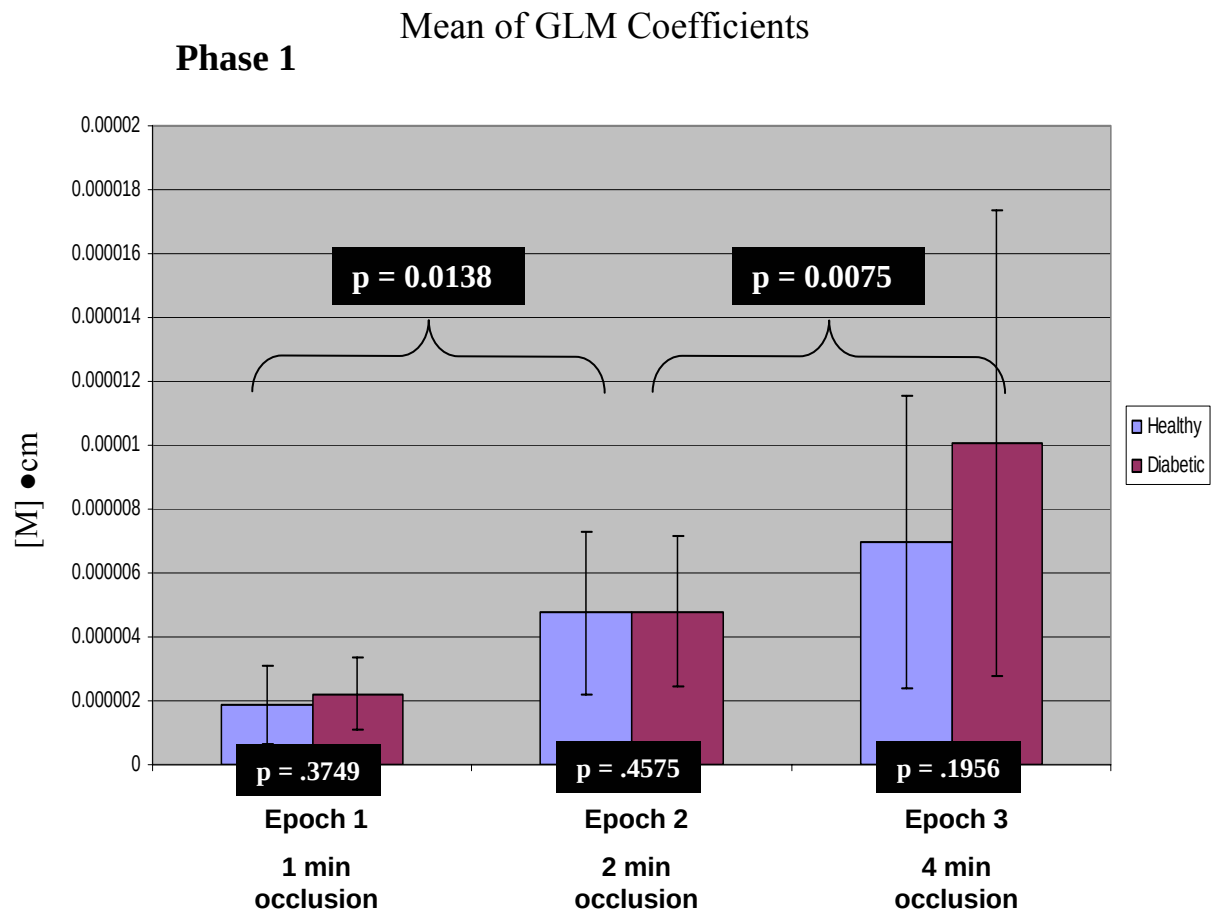


Figure 8. The a statistically significant increasing value of the mean of the GLM coefficients reflects the magnitude of the metabolic insult caused by the arterial ischemia provocation. As seen in Figure 3c, the duration of the arterial ischemia provocation results in a greater decrease of oxyhemoglobin volume in the forearm.

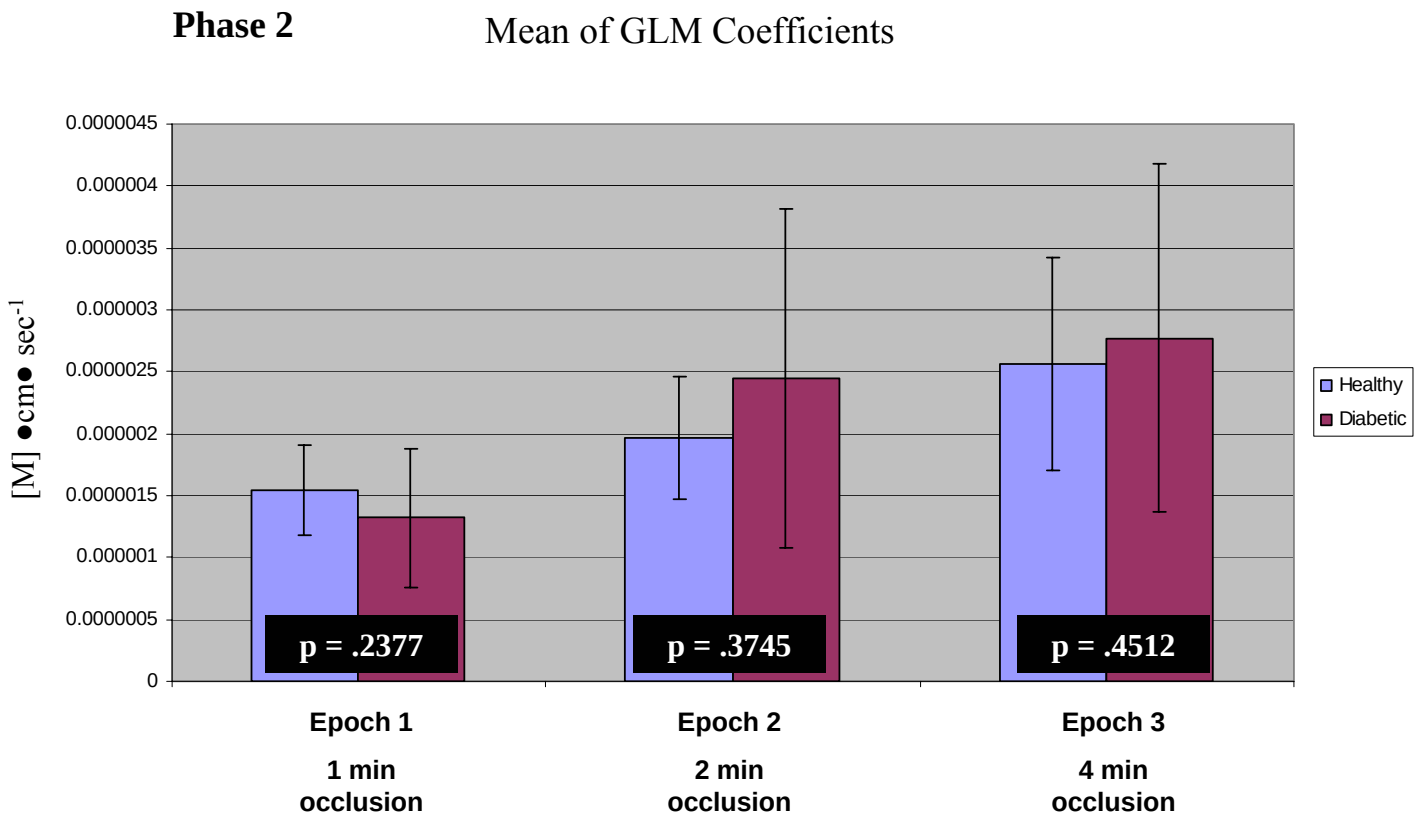


Figure 9. The a statistically significant increasing value of the mean of the GLM coefficients is seen from E1 to E2. As seen in Figure 3c, the duration of the arterial ischemia provocation results in a greater decrease of oxyhemoglobin volume in the forearm.

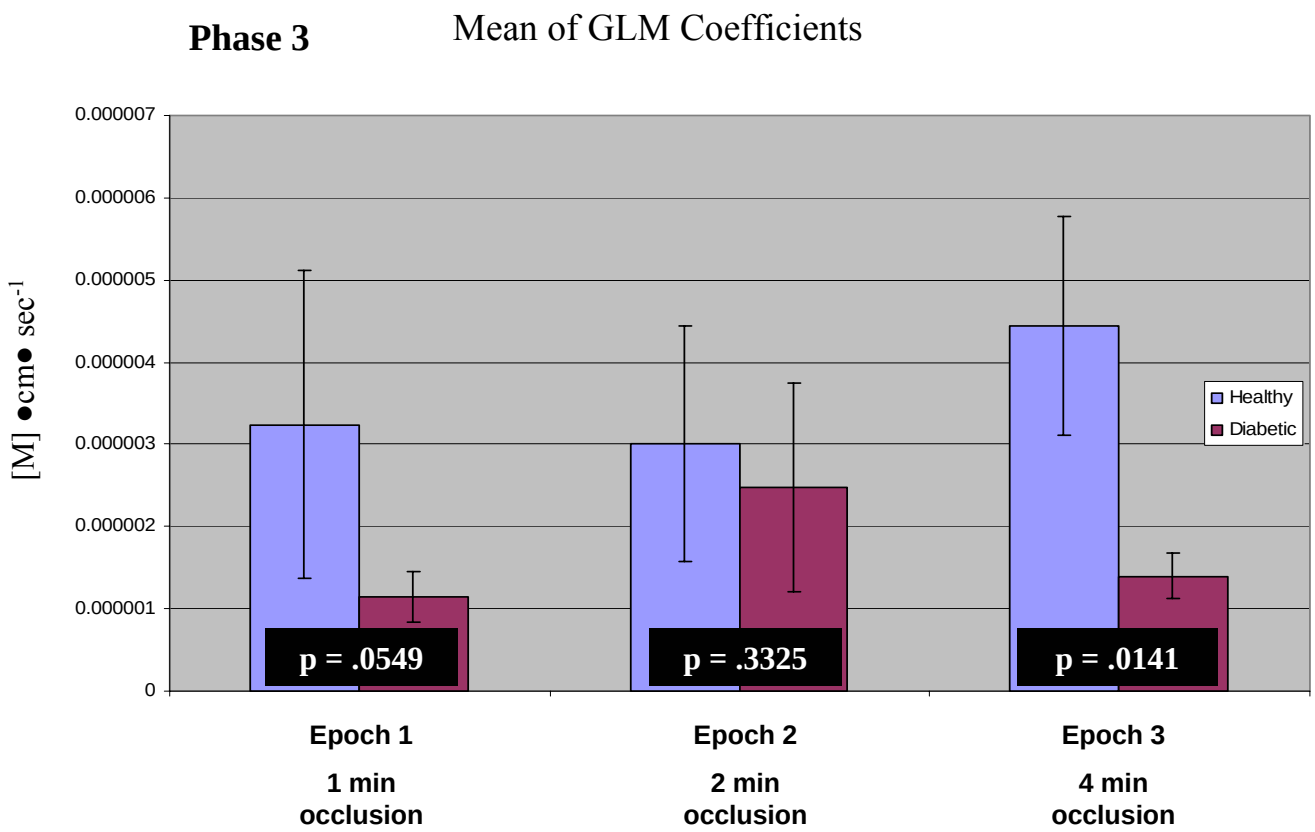


Figure 10. There is a significant statistical difference in the response in the Phase 3 mean value of the GLM coefficients. While the healthy patients continue to mount a greater reactive hyperemia in response to the length of the ischemic provocation, the diabetic patient fails to respond in the same manner

Phase 2

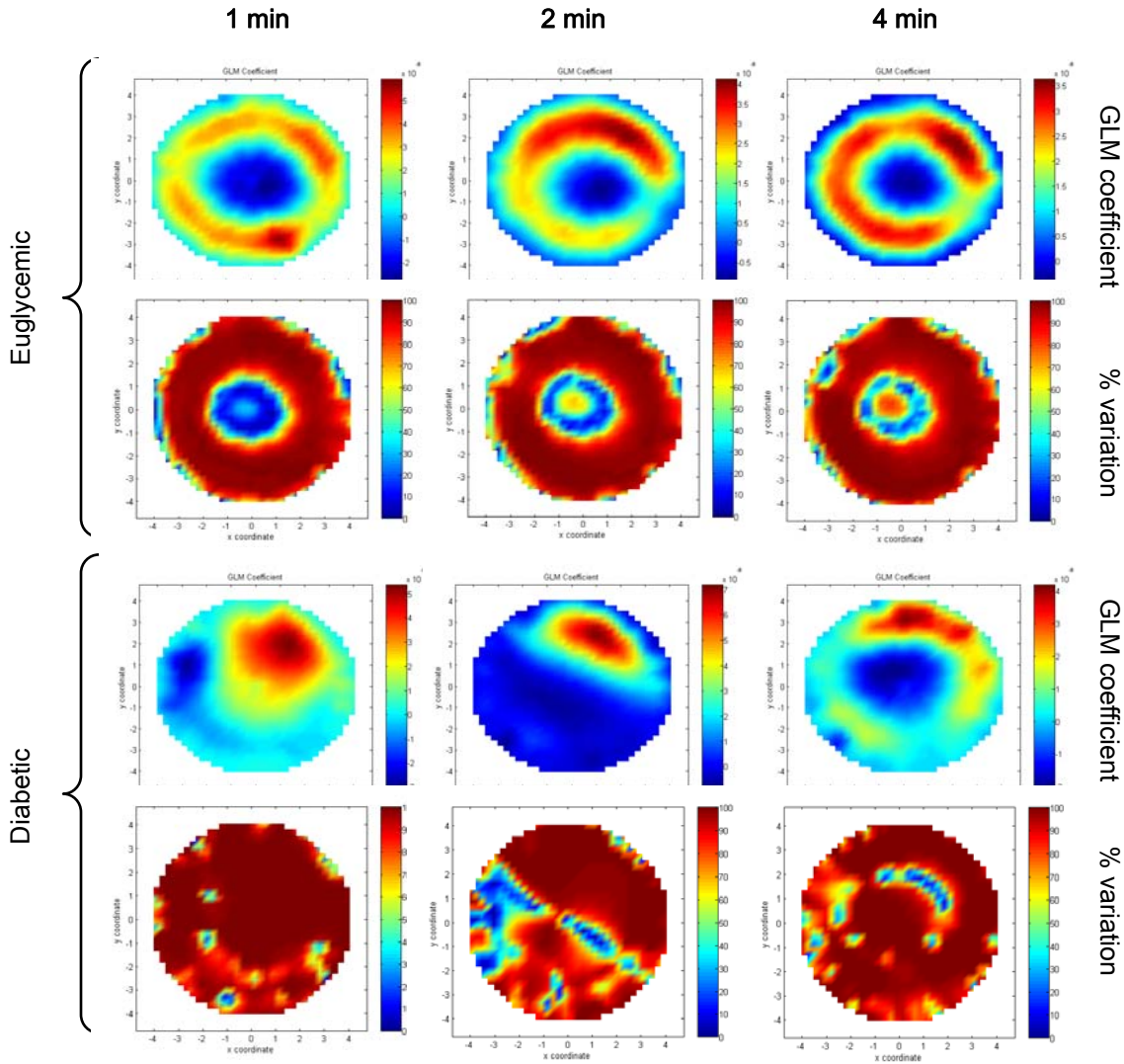


Figure 11. Spatial maps of the GLM coefficients and percentage-of-variance-accounted-for-by-the-model-function (PVA) values, for one healthy and one diabetic subject. The model function used for these computations was Phase II (reactive hyperemia)

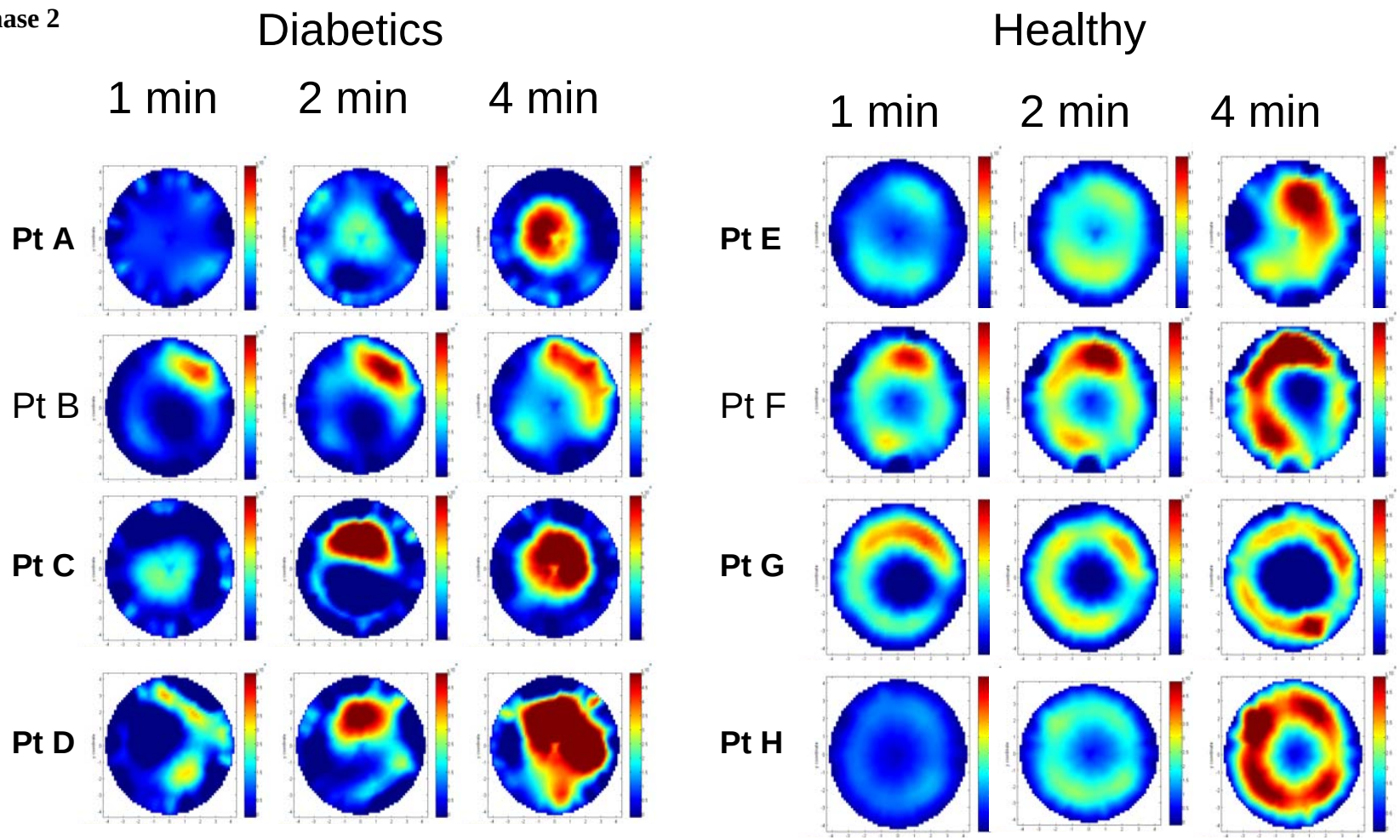


Figure 12. GLM Coefficients of Phase 2 Reactive Hyperemia comparing Epochs 1 through 3 for selected patients from diabetic and euglycemic groups. There is a difference between the spatial distributions of the model functions in the GLM parameter maps. These distributions roughly correspond to the muscle bellies of the extensor and flexor muscle compartments of the forearm.

During reactive hyperemia, oxygen becomes replenished and vasodilator metabolites are washed out of the tissue causing the resistance vessels to regain their normal vascular tone and thereby return flow to normal levels. The longer the period of occlusion, the greater the metabolic stimulus for vasodilation leading to increases in peak reactive hyperemia and duration of hyperemia.[9]

As demonstrated by our analysis of the peak volume change during the reactive hyperemia, the euglycemic group responded with 50% to 60% greater volume change than the diabetic group. When the area of the of the Phase III response, there is also a 40% to 50% greater response on behalf of the euglycemic volunteer as compared with the diabetic patient. This finding correlates well with the finding of vasodilatation related to the hyperemic response reported in the vascular literature.

The spatial maps of the GLM coefficients of the Phase II model function show that in the euglycemic patient, the model function is distributed throughout the arm. Those areas correspond to the muscle bellies of the extensor and flexor muscles of the forearm. Forrest et al., found this same type of distribution in muscle blood flow during reperfusion.[10] However, in the diabetic volunteer, we see a derangement in the typical pattern of reactive hyperemia. Akbari et al., reported that his group found impairment of both macro and microcirculation during induced hyperglycemia. Endothelial derived relaxing factor (EDRF) was cited as a major factor that causes smooth muscle relaxation and thus, vasodilation. DM2 and its derangement of glycemic control plays a role in reducing EDRF and attenuating the vasodilation needed for a normal hyperemic response.[11]

CONCLUSION

- Our measurements show our ability to detect a statistically significant decrease in a diabetic volunteer's ability to mount a hyperemic response after an ischemic trial.

- DYNOT time series measurements of the forearm using graduated periods of arterial ischemia as a provocation maneuver have proven sensitive to detecting differences in the reactive hyperemia response that enable us to discriminate between euglycemic and diabetic populations.

- DYNOT time series measurements of the reactive hyperemia phase following a brief period of arterial ischemia can provide a simple and useful method of evaluating vascular dysfunction in the clinical setting.

REFERENCES

[1] Jude, E.B. et al. Peripheral Arterial Disease in Diabetic and Nondiabetic Patients. *Diabetes Care* 2001; Vol. 24 No 5, August.

[2] Mancini, D.M. et al. Validation of near-infrared spectroscopy in humans. *Journal of Applied Physiology* 1994; 77(6): 2740- 2747.

[3] Barbour, R.L. et al. Optical tomographic imaging of dynamic features of dense-scattering media. *Journal of the Optical Society of America* 2001; 18: 3018- 3036.

[4] Schmitz, C.H. et al. Instrumentation and calibration protocol for imaging dynamic features in dense-scattering media by optical tomography. *Applied Optics* 2000; 39: 6466-6486.

[5] Schmitz, C.H. Instrumentation for real-time dynamic optical tomography. *Photon Migration, Optical Coherence Tomography, and Microscopy* 2001; (Proceedings of SPIE, VOl. 4431), pp. 282-291.

[6] Stansberry, K.B. el al. Impaired peripheral vasomotion in diabetes. *Diabetes Care* 1996; 19: 715-721.

[7] Franco, N.A, et al Dynamic optical tomographic imaging of the human forearm for quantification of the vascular bed response in diabetics and non-diabetics, *Biomedical Topical Meetings on CD-ROM* (The Optical Society of America, Washington, DC, 2004), presentation #WF37.

[8] Tooke, J.E. Microvascular function in human diabetes: a physiological perspective. *Diabetes* 1995; 44: 721-726.

[9] Y. Pei, H. L. Graber, and R. L. Barbour, "Influence of systematic errors in reference states on image quality and on stability of derived information for DC optical imaging," *Appl. Opt* 2001; 40 5755–5769.

[10] Inpoue T, Tomoike H, Hisano K, Nakamura M. Endothelium determines flow dependent dilatation of the epicardial coronary in dogs. *J Am Coll Cardiol* 1988;11:187-91.

[11] Forrest, I, Lindsey, T., Romanschin, A., et al, The rate and distribution of muscle blood flow after prolonged ischemia, *J Vasc Surg* 1989; 10:83-8.

[12] Anderson TJ, Uehata A, Gerhard MD, Meredith IT, Knab S, Delegrange D, et al. Close relation of endothelial function in the human coronary and peripheral circulation. *J Am Coll Cardiol* 1995;26 1235-41.

ACKNOWLEDGEMENT

This research was support in part by the National Institutes of Health (NIH) under Grants R21-HL67387, R21-DK63692 and R41-CA96102.