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Quantifying The Effects Of Nicotine And Nitroglycerin On The Microvasculature

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Abstract

Nicotine can stimulate both sympathetic and parasympathetic ganglionic cells depending on which ganglionic cells of the ANS it is able to access. This study involved the transdermal delivery of low doses of nicotine and nitroglycerin to the forehead and finger microvasculature so as to test the hypothesis that, although both drugs will cause vasodilation at the forehead, the nature of the dilation will differ as a consequence of their different sites of action within the neuro-microvascular pathways. With IRB approval, the local effects of transdermal nitroglycerin and nicotine were investigated on the forehead and finger of 10 healthy volunteers resting supine in a temperature-regulated room (22±1°C). Each micropatch was placed on the forehead and finger beneath a laser Doppler flowmetry (LDF) probe which measured the relative blood flow. The changes in the height (AC) and the baseline (DC) components of the laser Doppler signal were assessed as well as the power of the signal at different frequencies. Both drugs caused significant vasodilation at the forehead vasculature. The increase in the AC component was larger for nicotine than for nitroglycerin. Similarly the DC component showed a larger increase for nicotine than for nitroglycerin. There was no significant increase in either component at the finger. The FFT analysis showed marked oscillatory activity at the nicotine application site for virtually every frequency that we studied up to about 0.3. Both nicotine and nitroglycerin caused vasodilation of the forehead microvasculature while not at the finger. Nicotine may turn out to be the ideal transdermal preparation for testing a disorder such as diabetic neuropathy and the impact of diabetes on endothelium-dependent vasodilation. Since intact postganglionic parasympathetic fibers are required for nicotine to exert its vasodilatory effect on the microvasculature, application of a nicotine micropatch not only would test the vessel but also the post-ganglionic pathway and, hence, potentially identify the autonomic neuropathic component.

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INTRODUCTION

NICOTINE

Nicotine is a drug consumed by millions of people worldwide. It is the main compound responsible for addiction to tobacco. Nicotine replacement therapy in the form of gum, transdermal patches, inhalers and nasal sprays is also widely used and is a strategy employed for smoking cessation. It is a very intriguing drug from a physiologic standpoint. The majority of data related to nicotine is focused on the systemic effects of a relatively large dose of nicotine administered intravenously or locally and on its role as a smoking cessation tool.

The effects of transdermal nicotine on the local microvasculature are not well studied. However, as technology has improved, tools such as laser doppler flowmetry (LDF) have emerged for measuring local blood flow. In this study, we investigated the effects of transdermal nicotine on the skin microvasculature at different sites. We then compared this response to nitroglycerin (a known vasodilator) in order to assess whether there were differences in the response given the different mechanisms of action for each drug.

It is well established that nicotine can stimulate sympathetic ganglionic cells in low and moderate concentrations and thereby causes vasoconstriction and increased heart rate among other effects [1]. It can also paralyze these cells at high doses. Less appreciated is the fact that nicotine can also stimulate parasympathetic ganglionic cells and also block them at high doses. Hence, the effects of nicotine at a given site is

dependent on which ganglionic cells it is able to gain access to and which branch of the autonomic nervous system is dominant in that region.

As previously mentioned, nicotine (NIC) is a neurotransmitter that can activate both sympathetic and parasympathetic pathways. It is released by pre-ganglionic neurons of both systems resulting in the release of norepinephrine (by postganglionic sympathetic fibers) and acetylcholine (by postganglionic parasympathetic fibers).

Acetylcholine (ACh) is a known vasodilator. It is an endothelium dependent vasodilator [2] which means that it requires intact endothelial cells to produce vasodilation. This is because ACh, acting on ACh receptors on these cells, stimulates release of the substances that cause vasodilation of the smooth muscle cells. Using invasive measurements, it has been shown that the vasodilatory effect of acetylcholine is impaired in patients with coronary artery disease [3]. Since ACh requires intact endothelial cells in to function, ACh can be used to assess endothelial function.

A previous study by Schonberger et al [4] has used LDF to compare the response of a prepared ACh transdermal patch with commercially available nitroglycerin transdemal patch. They found an increase in blood flow in response to the application of these patches. They were limited in that there is no commercially available acetylcholine patch and hence had to produce their own. However, this was the first study of its kind using LDF in conjunction with a translucent drug patch to measure changes in blood flow. Since no ACh patch is available, transdermal nicotine is a good alternative in that it stimulates ACh release via the mechanisms described above. Moreover, a commercially available transdermal nicotine patch is already available.

Using these patches with different mechanisms of action may provide insight into the mechanism of blood flow regulation in tissues rich in cholinergic innervations (e.g. centrally located regions) but not in tissues where such innervation is sparse or lacking (e.g., more peripheral regions).

Systemic nicotine affects the cardiovascular system and typically leads to direct and indirect increases in heart rate, blood pressure and also causes vasoconstriction. This is due to its sympathomimetic effects as it stimulates the release of catecholamines. These increases in heart rate and blood pressure increase myocardial work and oxygen demand. Moreover, coronary vasoconstriction can lead to a decrease in blood flow to the heart. These effects of nicotine can lead to significant morbidity and mortality especially in individuals with underlying coronary artery disease. Given its mechanism of action, application of transdermal nicotine to the forehead activates parasympathetic pathways and thereby causes vasodilation of the local microvasculature. This may provide insight into the mechanism of blood flow regulation in tissues rich in cholinergic innervations (e.g., centrally located regions) but not in tissues where such innervation is sparse or lacking (e.g., more peripheral regions).

When administered systemically, nicotine can gain access to pre-/post- ganglionic junctions of both divisions of the autonomic nervous system. However, if given in a small dose via the transdermal route, it should have no effect on the sympathetic junctions since the synapse occurs in the paraverterbral sympathetic chain. Alternatively, it may have an effect on parasympathetic junctions: in tissues which are innervated by the parasympathetic nervous system, the pre/post synapse occurs near the end organ (e.g., within the vessel walls).

Transdermal nicotine patches deliver on average between 15-22mg of nicotine per day depending on the type of patch used and whether it is used during sleep. [1] The nicotine is slowly absorbed and blood plasma levels rise gradually over the course of 6-10 hours, then stay at steady state for 7-8 hrs eventually declining during the last 6 hours. The amount absorbed into the blood stream varies by individual and is determined by several factors including nicotine clearance, cutaneous blood flow, skin temperature etc. The half life of nicotine is about 2-3 hours and it is metabolized by the liver, and to a lesser extent in the lung and brain. Studies have shown the 21mg transdermal nicotine is safe [5]. In this study, transdermal nicotine patches were left in place for 30 minutes minimizing systemic effects substantially. In addition, the patches used were markedly smaller than the original patch and only measured about 2cm X1cm.

NITROGLYCERIN

Nitroglycerin is a drug used to relieve the symptoms of angina. It has generally been assumed that nitroglycerin relieves symptoms via the release of nitric oxide (NO), which relaxes vascular smooth muscle cells. This occurs independently of acetylcholine and other means of releasing NO (since the NO is supplied by the nitroglycerin).

In light of the different mechanisms of action of nicotine and nitroglycerin, we set out to measure and quantify the response to local transdermal doses of nicotine and nitroglycerin in different body regions. This will be accomplished by measuring the blood flow directly using laser Doppler flowmetry. We will further be able to measure

the autonomic response by measuring the frequencies of the response. This is due to the fact that the sympathetic and parasympathetic systems oscillate at different frequencies.

LASER DOPPLER FLOWMETRY

Laser doppler flowmetry (LDF) is a non invasive method that has many practical applications which include the measurement of microvascular blood flow. This allows us to monitor blood flow changes to the tissue and thus can be a useful tool to assess the effects of drugs on the tissue. While LDF does not provide absolute values, it gives us a means of measuring relative changes. Hence, once a baseline flow is established, relative increases or decreases in flow can be determined and quantified. Measuring skin perfusion has many clinical applications as many diseases can affect the skin such as diabetic microangiopathy, Raynaud's phenomenon, peripheral vascular disease leading to various complications such as ulcers, neuropathies etc. In addition, it has clinical applications in plastic surgery especially in flap surveillance, burns and pharmacology.

Hence, as a clinical tool, LDF allows the monitoring of skin blood perfusion in real time, continuously and non invasively. The theory behind LDF, as its name implies, is based on the Doppler effect which was described by Johan Christian Doppler . LDF are designed using a fiber optic probe which is about 0.25mm between the transmitting and

receiving fibers allowing for a sampling depth of approximately 1mm for human skin. Coherent light directed towards the tissue causes photons to be scattered by both moving and static objects. In the case of a moving object, the doppler effect occurs causing a change in photon frequency. The reemitted light is then directed towards a photodetector in which the combination of the shifted and non shifted light frequencies give rise to a stochastic photocurrent. The properties of this photocurrent is related to the properties of the blood cells present in the illuminated volume as they move at their different velocities. Light can be viewed as a particle with some wave characteristics. The doppler effect results in the scattering of light due to its interactions with the red blood cells.

These red blood cells are moving at very low velocities (mm/s) and hence give rise to extremely small frequency shifts, much smaller than that of the incident light. Several mathematical equations and models are thus used to give an estimate of the blood flow. Several models have been proposed using complex mathematical equations. In other for these models to work, certain assumptions need to be made. These assumptions may not be fully true in biologic tissue but give us a good framework in which to make these calculations. A few of these assumptions are outlined by Humeau et al [1] and are as follows :

1. Light suffers multiple scattering in the static tissue matrix, hence becoming diffusely scattered.
2. The fraction of photons undergoing multiple doppler shifts is negligible.
3. The blood cell velocities are randomly distributed in direction within the scattering volume, meaning that the network of microvessels, in effect, is

random on a length scale defined by the mean distance between RBC scattering events.

4. The statistics of the blood cell velocity field is uniform throughout the scattering volume.

In summary, LDF measurements have evolved from the intial reports by Riva et al in 1972, who reported that microvasculature blood flow measurements could be measured with the Doppler effect. Several studies have been carried out to improve the technique and measurements and the theory has now been established. LDF will only continue to grow as a tool for measuring tissue perfusion, a very important indicator of tissue health.

STATEMENT OF PURPOSE

To measure the response of the forehead and finger microvasculature to transdermal nicotine and transdermal nitroglycerin using laser doppler flowmetry on healthy volunteers. Since these drugs work by different mechanisms, this can be used as a novel non invasive way to assess the function of the microvasculature as we can compare the changes to disease states.

METHODS

The study was carried out on 10 healthy non-smoking volunteers between the ages of 20 and 26 who were instructed to refrain from caffeine for at least twenty four hours. After informed consent was obtained, the subjects lay on a bed in a semi-recumbent position in a temperature controlled room (22±1C) and were monitored using an ECG and non-invasive brachial artery blood pressure cuff. A baseline blood pressure and pulse were obtained at the beginning of the experiment in other to monitor any systemic changes.

A 6-mm diameter circle was obtained from the two patches: a clear nitroglycerin patch (Minitran, 3M Pharmaceuticals, Northridge, CA) and transdermal nicotine (Nicoderm CQ, GlaxoSmithKline). Each subject received both the nicotine and nitroglycerin patch in separate sessions. The order was randomly determined.

Each micropatch (i.e the nicotine or nitroglycerin patch) was placed on the forehead beneath a laser Doppler flowmetry (LDF) probe (PF5010, Perimed), a second LDF probe was placed on the finger, while the third LDF probe served as the control for the forehead. There was no finger control since there were only three probes available. LDF readings were recorded continuously at 200 Hz for 20 minutes, with a data acquisition system (PowerLab, ADInstruments) and analyzed with commercially available software (Chart, ADInstruments). This procedure was repeated for both sets of patches.

The changes in the height (AC) and the baseline (DC) components of the laser Doppler signal were assessed for each drug and compared with placebo using a paired t test.

Microvascular oscillatory patterns were delineated with spectral analysis for each LDF tracing using a 32K FFT size and a Hamming window. Three different frequency ranges (0.05-0.11, 0.12-0.19 and 0.2-0.3 were selected and analyzed for each site. The basis for these three bands is addressed below. The low frequency bands (0.05- 0.11Hz) represent oscillations in the microvasculature due to sympathetic activity. These sympathetic-induced oscillations are limited to low frequencies (<9 cycles/s) [6]. The oscillations occurring at higher frequency bands (> 9 cycles/s) represent parasympathetic mediated activity. The cholinergic etiology of these HF oscillations was confirmed by their elimination by systemic administration of atropine. [7]. The higher frequency band (0.12-0.3 Hz) was divided into an intermediate frequency band (0.12-0.19Hz) and a high frequency band (0.2-0.3Hz). This is in order to distinguish between oscillations transmitted to microvasculature due to respiration (which typically has a frequency of ~0.2Hz) or a cholinergic mediated process occurring at the level of the microvasculature.[6]

The presence of systemic effects was monitored at the control site, as well as by measurements of heart rate and blood pressure.

RESULTS

TIME DOMAIN RESULTS

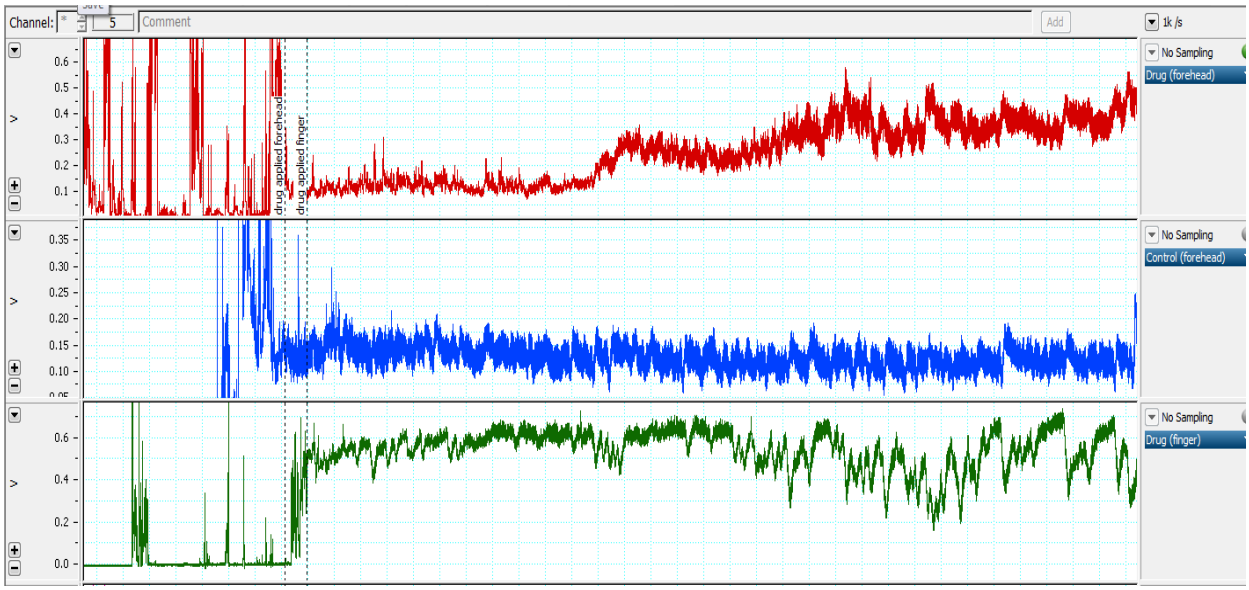

Figure 1a- Nicotine Laser Doppler Tracings showing forehead, placebo and finger as drug from patch is absorbed over time.

Figure 1a shows a tracing from a single subject. Channel 1 (uppermost channel) shows the LDF tracing for nicotine at the forehead. Channel 2 (middle channel) shows the placebo at the forehead. Channel 3 (bottom channel) shows the tracing for nicotine at the finger site. The forehead tracing shows an upward change as time progresses. The placebo tracing remains unchanged while the finger tracing shows large fluctuations but does not seem to increase.

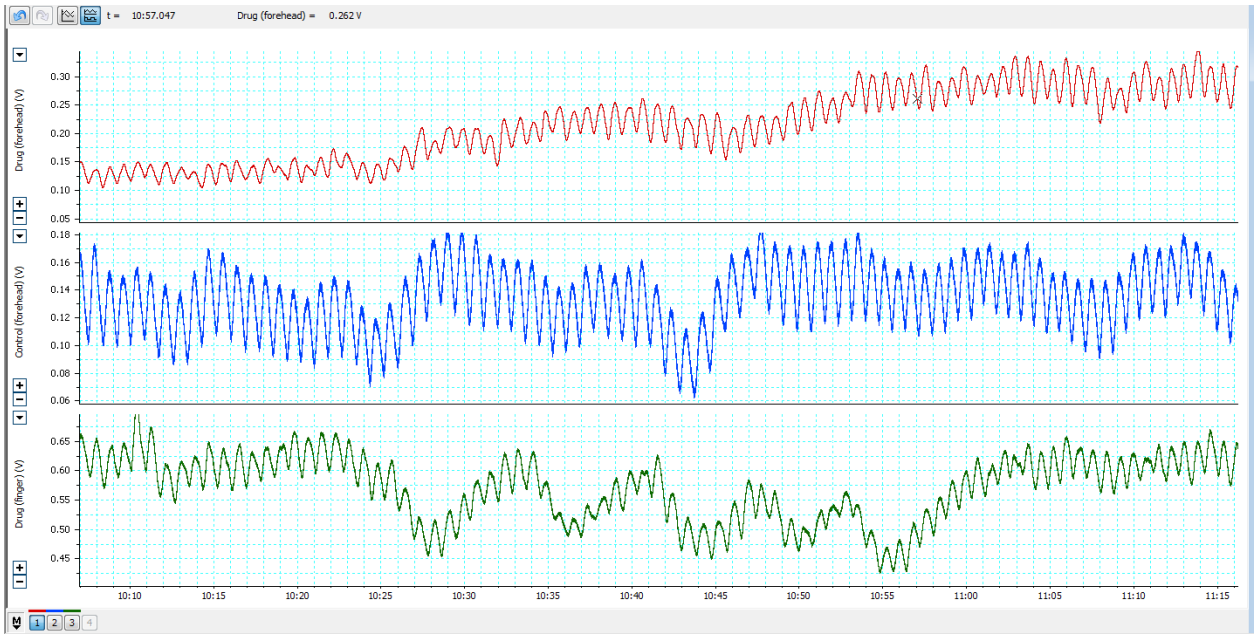

Figure 1b- Nicotine Laser Doppler Tracings magnified

Figure 1b above shows a magnified portion of the Figure 1a. This illustrates that the tracing is composed of individual oscillating waveforms. The forehead tracing shows an increase in the LDF signal accompanied by an increase in the height of the individual waveforms. The placebo tracing shows no increase in LDF signal value and the individual waveforms remain unchanged. The finger tracing shows a minimal increase in LDF signal along with large fluctuations in the signal.

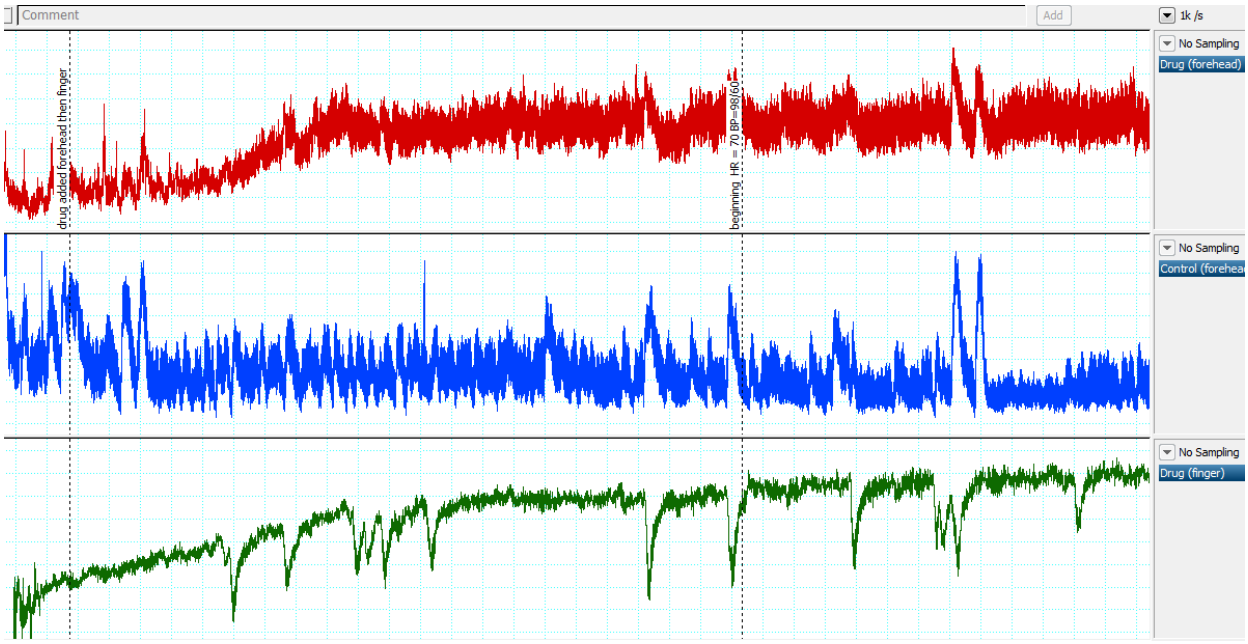

Figure 2a- Nitroglycerin Laser Doppler Tracings of forehead, placebo and finger in response to drug from patch being absorbed over time.

Figure 2a shows a tracing from a single subject. Channel 1 (uppermost channel) shows the LDF tracing for nitroglycerin at the forehead. Channel 2 (middle channel) shows the placebo at the forehead. Channel 3 (bottom channel) shows the tracing for nitroglycerin at the finger site. The forehead tracing shows an upward change as time progresses. The placebo tracing remains unchanged while the finger tracing shows an increase.

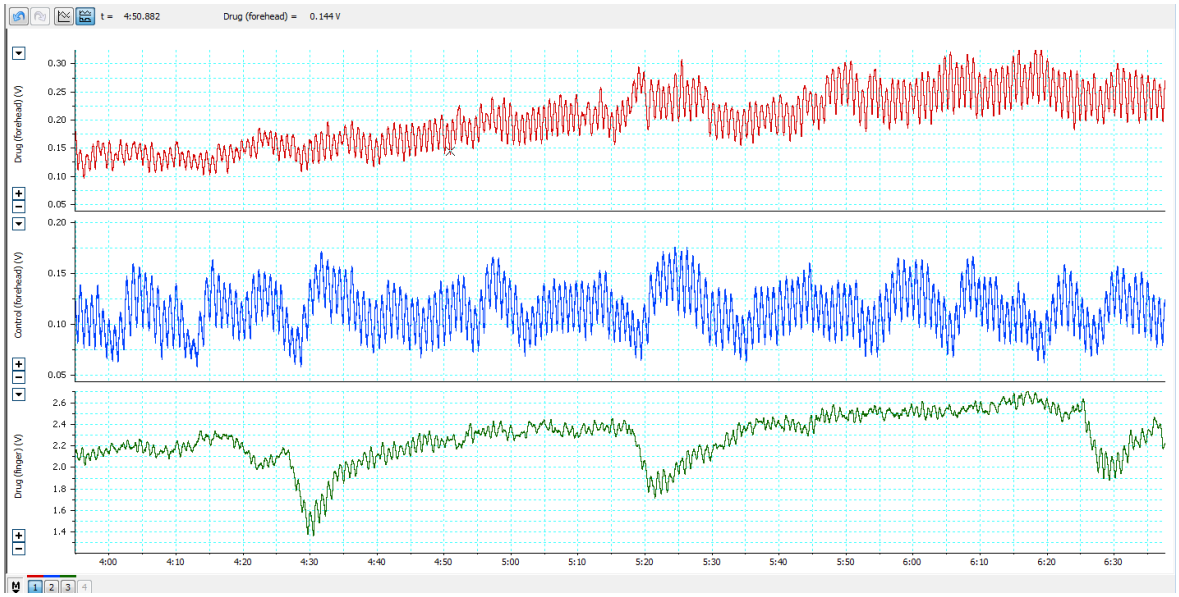

Figure 2b- Nitroglycerin Laser Doppler Tracings Magnified

Figure 2b above shows a magnified portion of the Figure 2a. This illustrates that the tracing is also composed of oscillations which change over time as nitroglycerin is absorbed. The forehead tracing shows an increase in the LDF signal accompanied by an increase in the individual waveforms. The placebo tracing shows no change in LDF value and the individual waveforms remain unchanged. The finger tracing shows a very minimal increase in the LDF signal.

TABLE 1- FOREHEAD NICOTINE BASELINE VALUES				
SUBJECT	FOREHEAD NICOTINE PLACEBO BaselinePRE	FOREHEAD NICOTINE PLACEBO BASELINE POST	FOREHEAD NICOTINE BASELINE PRE	FOREHEAD NICOTINE BASELINE POST
1	0.2048	0.157	0.1625	0.4727
2	0.1108	0.09523	0.06363	0.3985
3	0.1106	0.1007	0.07804	0.4955
4	0.05285	0.079915	0.1124	0.369
5	0.5156	0.6549	0.062565	0.1807
6	0.13355	0.1103	0.04961	0.1921
7	0.067565	0.067315	0.07411	0.1115
8	0.01739	0.02531	0.02633	0.03677
9	0.048315	0.04924	0.04824	0.13385
10	0.0375	0.037735	0.0692	0.361
MEAN	0.129897	0.1377645	0.0746625	0.275162
SD	0.146363266	0.185752101	0.038167714	0.162741626
median	0.0890825	0.0875725	0.066415	0.27655
min	0.01739	0.02531	0.02633	0.03677
max	0.5156	0.6549	0.1625	0.4955

*BaselinePRE – defined as the trough of the waveform before drug administered

* BaselinePOST- trough value after drug administered.

TABLE 2- T TEST PROCEDURE NICOTINE FOREHEAD BASELINE					
Difference = FOREHEAD NICOTINE BASELINERATIO – FOREHEAD NICOTINE BASELINE RATIO					
N	MEAN	STD DEV	STD ERR	MIN	MAX
10	2.5835	1.9069	0.6030	-.0589	5.4388
		DF	T value	Pr> |t|	
		9	4.28	0.002	

Table 1 above compares the changes in Forehead Baseline values pre and post drug administration for nicotine compared to placebo. Baseline is defined as the trough of the waveform. At the forehead, transdermal nicotine patch resulted in a significant increase in the baseline value with an increase in the mean from 0.07 to 0.27 while the mean remained unchanged for placebo. The t test in table 2 showed these results to be statistically significant with a p value of 0.002.

TABLE 3- NICOTINE FOREHEAD HEIGHT VALUES				
SUBJECT	FOREHEAD PLACEBO HEIGHT PRE	FOREHEAD PLACEBO HEIGHT POST	FOREHEAD NICOTINE HEIGHT PRE	FOREHEAD NICOTINE HEIGHT POST
1	0.01495	0.021715	0.03086	0.1535
2	0.05835	0.05038	0.02308	0.1686
3	0.057995	0.05079	0.028125	0.14465
4	0.013885	0.02759	0.02661	0.05571
5	0.1237	0.1601	0.050205	0.1053
6	0.06601	0.06124	0.022295	0.06311
7	0.018085	0.01561	0.0205	0.03399
8	0.015715	0.003125	0.015305	0.01565
9	0.01996	0.02012	0.02063	0.05092
10	0.007589	0.0048435	0.01952	0.099155
MEAN	0.0396239	0.04155135	0.025713	0.0890585
SD	0.036777011	0.046127155	0.009725564	0.053330247
median	0.0190225	0.0246525	0.0226875	0.0811325
min	0.007589	0.003125	0.015305	0.01565
max	0.1237	0.1601	0.050205	0.1686

TABLE 4- T TEST PROCEDURE NICOTINE FORHEHEAD HEIGHT					
Difference = FOREHEAD NICOTINE BASELINERATIO – FOREHEAD NICOTINE BASELINE RATIO					
N	MEAN	STD DEV	STD ERR	MIN	MAX
10	2.45	2.0974	0.6601	0.1065	6.44
		DF	T value	Pr> |t|	
		9	3.72	0.0048	

Table 3 above compares the changes in Forehead Height values pre and post drug administration for nicotine compared to placebo. Height is defined as the peak of the waveform. At the forehead, transdermal nicotine patch resulted in a significant increase in the Height value with an increase in the mean from 0.03 to 0.09 while placebo remained unchanged from 0.039 to 0.041. The t test in table 4 showed that these results to be statistically significant (See Table 2) with a p value of 0.0048.

At the forehead, both baseline and height approximately tripled in response to transdermal nicotine with baseline increasing approx 3.85 times compared to height increasing approx 3 times.

Table 5- Forehead Nitroglycerin Baseline Values				
SUBJECT	FOREHEAD NITRO PLACEBO BaselinePRE	FOREHEAD NITRO PLACEBO Baseline POST	FOREHEAD NITRO BaselinePRE	FOREHEAD NITRO BaselinePOST
1	0.1357	0.2367	0.1094	0.2987
2	0.048345	0.051735	0.083635	0.1529
3	0.08125	0.08458	0.09732	0.2102
4	0.09746	0.1045	0.09796	0.2799
5	0.12545	0.11535	0.041265	0.08307
6	0.1187	0.1636	0.06358	0.1134
7	0.10465	0.09281	0.05311	0.1029
8	0.1031	0.13	0.1216	0.2981
9	0.05557	0.07837	0.05513	0.15685
10	0.07906	0.073775	0.092545	0.1675
MEAN	0.0949285	0.113142	0.0815545	0.186352
SD	0.028838006	0.053681355	0.026823487	0.081464272
median	0.10028	0.098655	0.08809	0.162175
min	0.048345	0.051735	0.041265	0.08307
max	0.1357	0.2367	0.1216	0.2987

TABLE 6- T TEST PROCEDURE NITROGLYCERIN FORHEHEAD BASELINE					
Difference = FOREHEAD NITRO BASELINERATIO – FOREHEAD NITRO BASELINE RATIO					
N	MEAN	STD DEV	STD ERR	MIN	MAX
10	1.0700	0.3720	0.1176	0.4053	1.7851
		DF	T value	Pr> |t|	
		9	9.09	<0.0001	

Table 5 above compares the changes in Forehead Baseline values pre and post drug administration for nitroglycerin compared to placebo. Baseline is defined as the trough of the waveform. As shown above, transdermal nitro patch resulted in a significant increase in the baseline value with an increase in the mean from 0.081 to 0.18 while the mean remained unchanged for placebo. The t test in table 6 showed these results to be statistically significant with a p value < 0.0001.

TABLE 7- FOREHEAD NITROGLYCERIN HEIGHT VALUES				
SUBJECT	FOREHEAD NITRO PLACEBO HEIGHT PRE	FOREHEAD NITRO PLACEBO HEIGHT POST	FOREHEAD NITRO HEIGHT PRE	FOREHEAD NITRO HEIGHT POST
1	0.03206	0.03869	0.02466	0.05583
2	0.004375	0.005625	0.02092	0.04572
3	0.046715	0.047415	0.04184	0.08282
4	0.07562	0.07338	0.0298	0.051145
5	0.03993	0.038935	0.017125	0.02869
6	0.04471	0.03073	0.02597	0.04026
7	0.0274	0.0226	0.020515	0.02514
8	0.0075	0.004063	0.024745	0.06122
9	0.023555	0.01672	0.01655	0.0553
10	0.38325	0.44855	0.02585	0.046125
MEAN	0.0685115	0.0726708	0.0247975	0.049225
SD	0.112469849	0.13367253	0.007289561	0.016535355
median	0.035995	0.03471	0.0247025	0.048635
min	0.004375	0.004063	0.01655	0.02514
max	0.38325	0.44855	0.04184	0.08282

TABLE 8- T TEST PROCEDURE NITROGLYCERIN FORHEHEAD HEIGHT					
Difference = FOREHEAD NITRO HEIGHT RATIO – FOREHEAD NITRO HEIGHT RATIO					
N	MEAN	STD DEV	STD ERR	MIN	MAX
10	1.0809	0.6800	0.2150	0.4006	2.6316
		DF	T value	Pr> |t|	
		9	5.03	0.0007	

Table 7 above compares the changes in Height values pre and post drug administration for nitroglycerin compared to placebo. Height is defined as the peak of the waveform. As shown above, transdermal nicotine patch resulted in a significant increase in the Height value with an increase in the mean from 0.024 to 0.049. The t test in table 8 showed that these results to be statistically significant (See Table 2) with a p value of 0.0007.

TABLE 9- FINGER BASELINE NICOTINE VS NITROGLYCERIN				
SUBJECT	FINGER NICOTINE BASELINE PRE	FINGER NICOTINE BASELINE POST	FINGER NITRO BASELINE PRE	FINGER NITRO BASELINE POST
1	2.229	1.4035	0.3017	0.3323
2	0.4115	0.6481	0.1053	0.1975
3	0.5828	0.5911	1.8585	2.931
4	0.55305	0.7157	0.6933	1.4985
5	0.7278	0.7576	1.916	1.8095
6	1.375	1.302	0.225	0.3595
7	1.461	1.846	0.7785	1.391
8	0.01541	1.5025	0.11305	0.8521
9	0.4083	0.50215	0.9962	0.84865
10	0.3719	0.6518	1.353	1.7375
MEAN	0.813576	0.992045	0.834055	1.195755
SD	0.66869102	0.473910518	0.686688323	0.849247482
median	0.567925	0.73665	0.7359	1.12155
min	0.01541	0.50215	0.1053	0.1975
max	2.229	1.846	1.916	2.931

TABLE 10- T TEST FINGER NITROGLYCERIN VS NICOTINE BASELINE					
Difference = FINGER NICOTINE BASELINE RATIO – FINGER NITROGLYCERIN BASELINE RATIO					
N	MEAN	STD DEV	STD ERR	MIN	MAX
10	-0.18	0.5483	0.1734	-1.0642	0.7480
		DF	T value	Pr> |t|	
		9	-1.06	0.3181	

At the finger, neither transdermal nicotine or nitroglycerin produced a statistically significant increase in the mean baseline value (Table 9). However, for nitroglycerin, all but 2 subjects showed an increase in baseline values. The t test (table 10) showed that there was no difference between nicotine and nitroglycerin at the finger.

TABLE 11- FINGER HEIGHT NICOTINE VS NITROGLYCERIN				
SUBJECT	FINGER NICOTINE HEIGHT PRE	FINGER NICOTINE HEIGHT POST	FINGER NITRO HEIGHT PRE	FINGER NITRO HEIGHT POST
1	0.4114	0.4029	0.15	0.1076
2	0.08622	0.09903	0.05793	0.1014
3	0.05805	0.06543	0.08577	0.11755
4	0.09755	0.1032	0.086535	0.18525
5	0.035665	0.02949	0.1441	0.2847
6	0.3898	0.4143	0.06231	0.1082
7	0.4243	0.4425	0.2642	0.2734
8	0.015825	0.3066	0.018315	0.3276
9	0.07122	0.2133	0.2469	0.21495
10	0.06453	0.09367	0.38325	0.44855
MEAN	0.165456	0.217042	0.149931	0.21692
SD	0.169491441	0.160758375	0.114761702	0.116277291
median	0.07872	0.15825	0.1153175	0.2001
min	0.015825	0.02949	0.018315	0.1014
max	0.4243	0.4425	0.38325	0.44855

TABLE 12- T TEST FINGER NITROGLYCERIN VS NICOTINE HEIGHT					
Difference = FINGER NIC HEIGHT RATIO – FINGER NITRO HEIGHT RATIO					
N	MEAN	STD DEV	STD ERR	MIN	MAX
10	0.0413	1.0658	0.3370	-1.1489	2.1243
		DF	T value	Pr> |t|	
		9	0.12	0.9053	

At the finger, neither transdermal nicotine or nitroglycerin produced a statistically significant increase in the mean height value (Table 11). However, for nitroglycerin, all but 1 subject showed an increase in height values. The t test (table 12) showed that there was no difference between nicotine and nitroglycerin at the finger.

FREQUENCY DATA

TABLE 13- FOREHEAD NICOTINE FREQUENCY BANDS						
SUBJECT	FOREHEAD NICOTINE LOW FREQ BAND PRE	FOREHEAD NICOTINE LOW FREQ BAND POST	FOREHEAD NICOTINE INTERMEDIATE FREQ BAND PRE	FOREHEAD NICOTINE INTERMEDIATE FREQ BAND POST	FOREHEAD NICOTINE HIGH FREQ BAND PRE	FOREHEAD NICOTINE HIGH FREQ BAND POST
1	5.63166E-06	0.000110025	2.49636E-07	2.97973E-05	3.35998E-06	1.7396E-05
2	3.72965E-06	3.53911E-05	2.0305E-06	1.70647E-05	3.0235E-06	2.82662E-05
3	4.45215E-06	0.000174027	3.38021E-06	1.70575E-05	4.68164E-06	1.27978E-05
4	3.65387E-06	2.30778E-05	1.27075E-06	7.47765E-06	4.60988E-07	3.95348E-06
5	1.7489E-06	3.20392E-06	1.96322E-06	1.35523E-06	7.83363E-07	2.13073E-06
6	1.58617E-05	0.000104949	6.3265E-06	4.65265E-05	1.85889E-06	2.96587E-05
7	8.1573E-06	5.85464E-06	2.26862E-05	8.25939E-06	2.96968E-06	1.10726E-06
8	7.65652E-07	2.78587E-06	1.01985E-06	1.03477E-06	3.96838E-07	8.78938E-07
9	6.81623E-06	2.64045E-05	3.52142E-06	7.21656E-06	1.36121E-06	6.61361E-06
10	7.0996E-06	1.6749E-05	1.29325E-06	6.79217E-06	1.21396E-06	3.85717E-06
MEAN	5.79167E-06	5.02467E-05	4.37416E-06	1.42582E-05	2.01101E-06	1.0666E-05
SD	4.24275E-06	5.86637E-05	6.66292E-06	1.4248E-05	1.43341E-06	1.09958E-05
median	5.04191E-06	2.47412E-05	1.99686E-06	7.86852E-06	1.61005E-06	5.28355E-06
min	7.65652E-07	2.78587E-06	2.49636E-07	1.03477E-06	3.96838E-07	8.78938E-07
max	1.58617E-05	0.000174027	2.26862E-05	4.65265E-05	4.68164E-06	2.96587E-05

TABLE 14- FOREHEAD NITROGLYCERIN FREQUENCY BANDS						
SUBJECT	FOREHEAD NITRO LOW FREQ BAND PRE	FOREHEAD NITRO LOW FREQ BAND POST	FOREHEAD NITRO INTERMEDIATE FREQ BAND PRE	FOREHEAD NITRO INTERMEDIATE FREQ BAND POST	FOREHEAD NITRO HIGH FREQ BAND PRE	FOREHEAD NITRO HIGH FREQ BAND POST
1	1.47979E-05	2.55146E-05	1.87651E-06	3.02624E-05	1.45156E-06	3.78071E-06
2	4.33692E-06	8.86632E-06	5.15565E-06	1.70664E-05	6.46802E-06	9.27677E-06
3	3.98981E-06	1.58643E-05	4.49222E-06	5.09366E-06	3.16375E-06	4.62797E-06
4	1.04493E-05	1.74053E-05	2.01758E-05	4.04614E-05	2.84992E-06	4.89007E-06
5	1.199E-05	2.33307E-05	6.69577E-06	1.44572E-05	9.30115E-06	8.34185E-06
6	4.02893E-06	2.38241E-06	6.50801E-07	1.68911E-06	4.50839E-07	5.93199E-07
7	1.19847E-05	1.80827E-06	1.48299E-05	1.41082E-05	3.29327E-06	4.1182E-06
8	4.35106E-06	1.89279E-06	6.91813E-07	7.08439E-06	3.05284E-07	6.54937E-07
9	2.79192E-06	6.94711E-06	1.12438E-05	4.3055E-05	1.03984E-06	7.04108E-06
10	1.55688E-06	5.32697E-06	9.4687E-07	1.69044E-06	1.19139E-06	1.85762E-06
MEAN	7.02774E-06	1.09339E-05	6.67591E-06	1.74968E-05	2.9515E-06	4.51824E-06
SD	4.73495E-06	8.95804E-06	6.70528E-06	1.53711E-05	2.88895E-06	3.01648E-06
median	4.34399E-06	7.90671E-06	4.82393E-06	1.42827E-05	2.15074E-06	4.37309E-06
min	1.55688E-06	1.80827E-06	6.50801E-07	1.68911E-06	3.05284E-07	5.93199E-07
max	1.47979E-05	2.55146E-05	2.01758E-05	4.3055E-05	9.30115E-06	9.27677E-06

TABLE 15 - FINGER NICOTINE FREQUENCY BAND DATA						
SUBJECT	FINGER NICOTINE LOW FREQ BAND PRE	FINGER NICOTINE LOW FREQ BAND POST	FINGER NICOTINE INTERMEDIATE FREQ BAND PRE	FINGER NICOTINE INTERMEDIATE FREQ BAND POST	FINGER NICOTINE HIGH FREQ BAND PRE	FINGER NICOTINE HIGH FREQ BAND POST
1	9.75286E-06	0.000109955	2.05979E-06	2.9737E-05	5.27039E-06	1.7429E-05
2	4.94911E-05	3.74795E-05	3.16592E-06	1.9876E-05	2.95105E-06	1.9453E-05
3	0.000107402	0.000167871	1.56064E-05	2.29289E-05	4.69651E-06	4.7293E-06
4	0.0413554	7.52615E-06	0.017088506	8.77705E-06	0.005713136	1.5021E-06
5	7.3962E-06	7.52765E-06	1.0757E-06	1.77838E-06	9.84427E-07	1.6627E-06
6	6.17572E-05	0.000761967	6.16374E-05	9.37107E-05	5.08541E-06	1.3548E-05
7	5.58782E-06	3.52351E-06	3.79876E-05	3.11302E-05	2.14153E-06	1.9917E-06
8	0.001285944	0.001096446	0.000105332	0.000285643	2.63514E-05	6.0851E-05
9	0.000277408	0.002191646	3.86844E-05	0.000415072	1.45515E-05	0.00012599
10	1.48032E-05	0.000142084	9.32949E-06	1.20237E-05	1.00419E-06	4.2494E-06
MEAN	0.004317494	0.000452603	0.001736338	9.20676E-05	0.000577617	2.5141E-05
SD	0.013019687	0.000714889	0.005394303	0.000141771	0.001804455	3.9681E-05
median	5.56242E-05	0.000126019	2.6797E-05	2.63329E-05	4.89096E-06	9.1388E-06
min	5.58782E-06	3.52351E-06	1.0757E-06	1.77838E-06	9.84427E-07	1.5021E-06
max	0.0413554	0.002191646	0.017088506	0.000415072	0.005713136	0.00012599

TABLE 16- FINGER NITRO FREQUENCY BANDS						
SUBJECT	FINGER NITRO LOW FREQ BAND PRE	FINGER NITRO LOW FREQ BAND POST	FINGER NITRO INTERMEDIATE FREQ BAND PRE	FINGER NITRO INTERMEDIATE FREQ BAND POST	FINGER NITRO HIGH FREQ BAND PRE	FINGER NITRO HIGH FREQ BAND POST
1	1.75291E-05	0.000107778	0.000107778	1.15721E-05	1.8499E-05	7.12234E-06
2	3.19867E-05	0.000327441	4.81822E-06	6.68372E-05	2.55548E-06	1.02487E-05
3	4.01445E-05	0.000159734	5.03476E-06	4.23115E-05	1.5365E-05	2.76726E-05
4	1.7061E-05	0.000329429	6.49765E-06	0.000208551	1.59516E-05	4.3457E-05
5	0.000317087	0.00281988	1.15429E-05	0.000515224	1.83789E-05	5.17014E-05
6	0.006798816	0.008085137	0.000298998	0.000246301	0.000135497	4.99673E-05
7	0.004471654	0.012267326	0.000591854	0.001551734	0.000351921	0.000123079
8	0.001604435	0.00154584	0.000140052	0.000195113	4.68507E-05	3.18929E-05
9	0.01005842	0.002191646	0.001690569	0.000788539	0.000274101	0.000195189
10	0.000269287	0.014502756	0.000178567	0.000784759	6.69528E-05	0.00014961
MEAN	0.002362642	0.004233697	0.000303571	0.000441094	9.46073E-05	6.89939E-05
SD	0.003564035	0.005398995	0.00052063	0.000484648	0.000122786	6.41399E-05
median	0.000293187	0.001868743	0.000123915	0.000227426	3.26748E-05	4.67122E-05
min	1.7061E-05	0.000107778	4.81822E-06	1.15721E-05	2.55548E-06	7.12234E-06
max	0.01005842	0.014502756	0.001690569	0.001551734	0.000351921	0.000195189

The vasodilation associated with each drug was accompanied by increases in each frequency band.

Nonparametric Kruskal-Wallis one-way anova was used to test the difference in following variables among three frequency groups (low, intermediate, high). Unadjusted p-values were shown.

Results:

Pre Forehead Nicotine, p= 0.18; Post Forehead Nicotine, p = 0.054

Pre Forehead Nitroglycerin, p = 0.25; Post Forehead Nitroglycerin, p = 0.23

Pre Finger Nicotine, p = 0.23; Post Finger Nicotine, p = 0.04

Pre Finger Nitroglycerin, p = 0.42; Post Finger Nitroglycerin, p = 0.003

The results show no statistical significance for pre measurements among three frequency groups. For post- measurements, there was significant difference in post Finger nitro among three groups ($p = 0.003$). There was nominal difference in Post finger nicotine ($p = 0.04$), which however became non-significant at all if we apply multiple test adjustments.

DISCUSSION

Both transdermal nicotine and nitroglycerin resulted in vasodilation of the forehead microvasculature resulting in a statistically significant increase in blood flow as measured by the laser Doppler. Nicotine resulted in a larger increase in baseline blood flow than nitroglycerin (285% (0.07→ 0.27) vs 122% (0.081 → 0.18)). It also resulted in a larger increase in height for nicotine 200% (0.03→ 0.09) vs nitroglycerin 104% (0.024 → 0.049). It is not clear whether this effect is dose dependent. In addition, both drugs caused a significant increase in the baseline and height of the waveform when compared with placebo. Nicotine produced a greater increase in both baseline and height of the waveform compared with nitroglycerin.

The vasodilation due to the transdermal nicotine can be explained by nicotine stimulating cholinergic activity. While the forehead microvasculature receives dual innervation from the parasympathetic and sympathetic nervous systems, transdermal nicotine only was able to act upon the ganglionic junctions of the parasympathetic system since these synapse near the end organ(i.e the blood vessels). Since nicotine was not able to access the synapses of the sympathetic system located in the thoracolumbar chain it did not stimulate the sympathetic nervous system to produce vasoconstriction. Nicotine was associated with increased oscillatory activity at every frequency band.

It is possible, but very unlikely that nicotine was able to gain access to the nicotinic receptors of the sympathetic system given the low doses used and the short time frame < 30 minutes in which to act. It is more likely that feedback mechanisms in place to regulate blood flow were activated resulting in the sympathetic system counterbalancing the direct activation of the parasympathetic system.

At the finger, there is no appreciable parasympathetic innervation and hence there was no satistically significant vasodilation associated with transdermal nicotine at the finger.

Nitroglycerin is a known vasodilator which works via a different mechanism from nicotine. It is able to produce vasodilation without activating the parasympathetic nervous system. In 1977 Murad discovered that nitric oxide release caused by nitrates relaxes smooth muscle cells. In addition, in 1986 Ignarro proposed that endothelium derived relaxing factor (EDRF) is identical to NO. These Nobel prize winners established the involvement of endothelium in the coordination of numerous vasoactive factors that play an important role in the regulation of blood vessels.

ANALYSIS OF RHYTHMIC SIGNALS OF THE AUTONOMIC NERVOUS SYSTEM

In other to maintain the delicate homeostasis required for sustaining life, the body must be able to respond appropriately to both external and internal stimuli. These responses are carried out by the autonomic nervous system through dynamic interactions of rhythmically discharging neuronal networks. This physiological control system consists of both the sympathetic and parasympathetic branches which are each characterized by rhythmic activity. These biologic rhythms include slow and fast components and the cycle times can be measured and characterized using various mathematical manipulations to extract the wealth of information hidden within. Each branch of the autonomic nervous system oscillates within a specific range. The existence of a rhythm in the autonomic nervous system is important in that it allows the coordination of the numerous components of the system and it also gives us the ability to predict future events. Moreover, each component oscillates at a particular frequency and hence this allows characterization of the response.

Sympathetic activity is associated with the low frequency range 0.05- 0.11 Hz while parasympathetic activity is associated with intermediate range (0.11-0.19 Hz) and high range (0.2-0.3Hz) oscillations, with the later associated with respiration. Hence, a number of different analytical methods were employed for this analysis. This involved

analyzing the data in both the time and frequency domains as we looked for changes in amplitude and frequency as well as measuring the changes in mean blood flow. Chartview, the software used to acquire the data has built in functions for this analysis.

Hence spectral analysis allows us to determine whether the response was predominantly parasympathetic or sympathetic. This involved converting the data from the time domain into a frequency domain which allowed us to measure the magnitude of the change in power at a given frequency.

It is very common for information to be encoded in the sinusoids that form a signal. This is true of naturally occurring signals, as well as those that have been created by humans. Many things oscillate in our universe. The shape of the time domain waveform is not important in these signals; the key information is in the frequency, phase and amplitude of the component sinusoids. A fourier transform can be utilized to extract these data allowing us to look more closely at the frequencies. Hence spectral analysis allows us to determine whether the response was predominantly parasympathetic or sympathetic.

In our case this was used to study the amplitude, frequency and the power of the signals. It is clear that there is oscillatory activity in the forehead nicotine with an increase in amplitude

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The differences in responses to transdermal nicotine and nitroglycerin support the hypothesis that there are differences in the innervations to the finger and the forehead. Moreover, the different degrees of vasodilation in response to nitroglycerin in the forehead and finger suggest that the differences also exist in the vasculature itself. The forehead produced a much larger increase in blood flow compared with the finger for both nicotine and nitroglycerin.

Given the mechanism of action of nicotine, it is not surprising that transdermal nicotine failed to produce a significant change in blood flow as detected by laser doppler when applied to the finger. This is due to the fact that the autonomic innervation of the vasculature in the limb skeletal muscles is primarily sympathetic. In addition, this is mostly vasoconstrictive and noradrenergic. As mentioned earlier, nicotine binds to nicotinic receptors at the pre-to post ganglionic junctions. In the case of the sympathetic system to the upper limb, these synapses are found in the middle to caudal thoracic spinal segments and possibly from the lower part of the cervical portion of the sympathetic ganglionic cord. Hence, the low dose of transdermal nicotine applied in this study was unable to access them and activate the sympathetic response. Despite this theory, Nicotine was associated with an increase in blood flow in 2/10 subjects which may suggest that there may be some receptors in the smooth muscle that nicotine can activate.

It is well known that there is no parasympathetic innervation to the upper and lower extremities. The primary role of the parasympathetic nervous system is internal maintenance, digestion and excretion. Therefore, there were no parasympathetic synapses near the end organ (i.e microvasculature) as was the case in the forehead for the transdermal nicotine to activate.

The forehead microvasculature is richly innervated by both sympathetic and parasympathetic fibers. These include the cranial parasympathetic, superior cervical sympathetic and the trigeminal sensory nerves.

NICOTINE AS A MEANS TO ASSESS THE MICROVASCULAR FUNCTION

The role of transdermal nicotine patches in smoking cessation is well established and hence these patches are already widely used for that purpose. However, as elucidated in this study, transdermal nicotine patches may well be an ideal tool for testing and assessing the function of the microvasculature. In order for transdermal nicotine to produce a vasodilatory response, the parasympathetic fibers innervating the microvasculature must be intact. Hence transdermal nicotine assesses both the innervation (i.e the post ganglionic pathway) as well as the vessel itself. Many diseases affect the microvasculature such as diabetes, hypertension and preeclampsia.

This test would be valuable in assessing diabetic neuropathy. Current diabetic clinical guidelines recommend annual screening for neuropathy but the literature is unable to provide specific support for a particular screening test due to a lack of evidence for its validity in the medical literature. In fact, the optimal method for screening for neuropathy has been controversial and is based on expert opinion rather than clinical evidence. [8] The development of diabetic neuropathy is an insidious and progressive process which is not well correlated to the development of symptoms. Diabetic neuropathy leads to significant morbidity and mortality as it leads to painful paresthesias, sensory ataxia, ulceration and amputation. Screening and early identification allows an early opportunity to intervene by improving glycemic control and initiating measures

such as early foot care before the effects start to unfold. The current screening modalities in use include mainly the Semmes-Weinstein 5.07/10g monofilament, vibration testing with a 128 Hz tuning fork and superficial pain sensation testing. [8]

The main advantage of using laser Doppler flowmetry in conjunction with transdermal nicotine patch is that it would be non invasive, able to detect dysfunction at an earlier stage, would be user independent and patient independent in the sense that the patient will not have to subjectively qualify the decreases in sensation. Like any other quantifiable measurement such as blood pressure, we would be able to better stratify risks through studies and make recommendations based on those values with the clinical picture in mind. Clinical trial evidence for the efficacy of screening strategies demonstrated reduced outcomes of amputation and ulceration and, consequently, screeningfor neuropathy is recommended in clinical practice guidelines [8].

NITROGLYCERIN AS A MEANS TO ASSESS THE ENDOTHELIUM

The endothelial cells release a wide number of molecules that regulate homeostasis, vasomotor activity, hemostasis and inflammation. Nitric oxide is one of these molecules and has numerous effects. Nitric oxide has vasodilatory, anti-inflammatory, antithrombotic effects, antioxidant, antiapoptotic and antithrombotic effects. Hence endothelial dysfunction has the potential to affect many different regulation pathways. Endothelial dysfunction is important in a number of disease states. This dysfunction leads to a pro-inflammatory and pro-thrombotic state that is thought to play a major role in the development of atherosclerosis and its accompanying clinical complications. Moreover, it is also thought to be a predictor of an increased rate of adverse cardiovascular events.

The endothelium is often evaluated in terms of its vasodilatory function. Several methods are available for assessing endothelial function by measuring endothelial dependent vasodilation. Vasodilation can be induced by the direct infusion of a vasodilator such as acetylcholine or non invasively by the increase in blood flow velocity caused by post occlusive hyperemia. Using a transdermal patch with a Laser Doppler probe provides a quick, non invasive way of assessing the endothelial function which is also quantifiable. It is clear that nitroglycerin produced in measurable response compared to placebo and the forehead and finger.

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