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Measurement of the effectiveness of enhanced external counterpulsation on heart rate variability for patients with myocardial ischemia

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ABSTRACT

MEASUREMENT OF THE EFFECTIVENESS OF ENHANCED EXTERNAL COUNTERPULSATION ON HEART RATE VARIABILITY FOR PATIENTS WITH MYOCARDIAL ISCHEMIA

by
Kripa Jayaraman

This thesis is a study to measure the changes in heart rate variability due to the activity of the autonomic nervous system caused by the Enhanced External Counterpulsation treatment. The treatment is a non surgical, mechanical procedure that can reduce the symptoms of angina or Congestive Heart Failure, presumably by stimulating the opening, or formation of, small branches of blood vessels (collaterals) to create a natural bypass around narrowed or blocked arteries. It has been proved that rhythms can be markers of normal functional states. Even in the absence of external perturbations, the normal heartbeat is not characterized by clockwise regularity. This fluctuation around the mean heart rate is called heart rate variability. The study was conducted on patients who had myocardial ischemia and had been prescribed enhanced external counterpulsation treatment by the physician. One of the patients was a non-ischemic heart failure patient who had heart failure secondary to dilated cardiomyopathy. Myocardial ischemia is a condition in which oxygen deprivation to the heart muscle is accompanied by inadequate removal of metabolites because of reduced blood flow or perfusion.

The study was conducted on three subject groups – 7 patients, 5 controls and 3 normals. Normals had no history of myocardial ischemia, but underwent the EECP treatment while controls were healthy and did not undergo the treatment. The subjects followed paced breathing at the rate of 12 breaths per minute. The data were collected during five minute paced breathing before and after the EECP treatment except for the

controls. The waveforms of ECG, respiration and blood pressure were collected as data from the subjects. Frequency domain-power spectral analysis was performed on the data obtained using the LabVIEW 5.0 software. The time domain-SDNN analysis was also performed using MATLAB.

The results of this study fail to indicate conclusively that the EECP treatment affects the heart rate variability of the patient in a significant way. This was evidenced by conducting Power Spectral Analysis and Standard Deviation of Normal to Normal intervals on the analyzed data for the research.

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by
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APPROVAL PAGE

MEASUREMENT OF THE EFFECTIVENESS OF ENHANCED EXTERNAL COUNTERPULSATION ON HEART RATE VARIABILITY FOR PATIENTS WITH MYOCARDIAL ISCHEMIA

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This thesis is dedicated to my beloved family and friends.

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CHAPTER 1

OVERVIEW

This research was focused on measuring the changes in heart rate variability due to the activity of the autonomic nervous system caused by the Enhanced External Counterpulsation treatment. The patients involved in the research had a history of myocardial ischemia. One of the patients was a non-ischemic heart failure patient, i.e., heart failure secondary to dilated cardiomyopathy. To treat the patients, the physicians prescribed the Enhanced External Counterpulsation treatment. To better understand the changes occurring in the body during the duration of the counterpulsation treatment, it is important to understand the functioning of the heart, the changes it undergoes because of the diseased state and the changes observed after this treatment. This change was quantified by measuring the heart rate variability of the subjects. At the beginning of the research, it was hypothesized that the heart variability of the patients would increase over the seven weeks of the treatment. Since heart rate variability is known to be reduced in patients who survived myocardial ischemia, it was hypothesized that the treatment would lead to an increase in the HRV.

In order to analyze and compare the data, there were three groups of subjects-patients, normals and controls. Patients were the subjects who had a history of myocardial ischemia and underwent the EECP treatment, normals were healthy but underwent the EECP treatment, while the controls were healthy but did not undergo the treatment. Tools like power spectral analysis, standard deviation of R-to-R interval (SDNN) and T tests were used to analyze the data.

CHAPTER 2

PHYSIOLOGICAL BACKGROUND

This chapter will serve as a brief overview of the biological basis of the electrocardiogram, respiration and blood pressure signals used in this study. The overview also covers the physiological background of the heart, its functions and its diseased state. Also, the chapter details the physiological systems evaluated.

2.1 Statement of Objective

The objective of this research was to measure the heart rate variability of patients after the enhanced external counterpulsation treatment. These patients had a history of myocardial ischemia. One of the patients had heart failure secondary to ischemic cardiomyopathy. To better understand the changes occurring in the body during the duration of the counterpulsation treatment, it is important to understand the functioning of the heart, the changes it undergoes because of the diseased state and the changes observed after this treatment. To categorize these changes, various analytical and statistical tools like power spectral analysis, SDNN and t tests have been used.

2.2 Functioning of the Heart

Dark blood, low in oxygen, flows through veins to the heart and enters the right atrium. It passes through the tricuspid valve into the right ventricle. The right ventricle pumps the blood under low pressure through the pulmonary valve into the pulmonary artery. The

pulmonary artery branches into the left and right pulmonary arteries, which lead to the lungs [1]. The lungs provide fresh oxygen to the circulating blood.
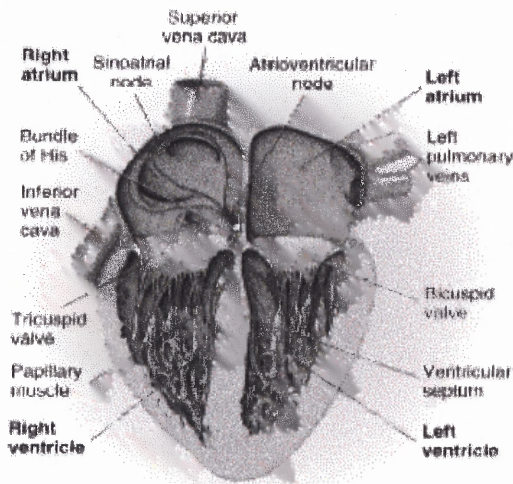
Figure 2.1 Structure of the heart.

Figure 2.1 shows the structure of the heart. Blood returns to the heart from the lungs by the left and right pulmonary veins. It enters the left atrium. From there it flows through the mitral valve and enters the left ventricle. This strong chamber pumps the red oxygen-rich blood out through the aortic valve into the aorta. The aorta takes blood to the body's general circulation, as shown in Figure 2.2. The heart, just like all other muscles in the body, needs its own supply of oxygen in order to function properly. Although its chambers contain blood, the heart receives no nourishment from the blood inside the chambers. The heart gets its blood supply from the coronary arteries. The two major coronary arteries branch off the aorta, and then divide into many smaller arteries that lie in the heart muscle and feed the heart [2].
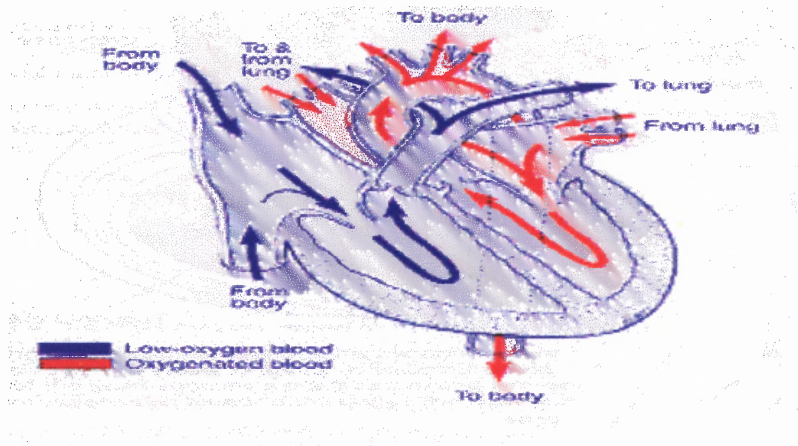
Figure 2.2 Anatomy of the heart.

The cardiac cycle (heart beat) consists of cardiac muscle contraction (systole) and cardiac muscle relaxation (diastole). Blood pressure represents the force (pressure) exerted by blood against the arterial walls during a cardiac cycle. Systolic blood pressure, the higher of the two pressure measurements, occurs during ventricular contraction (systole) as the heart pumps blood into the aorta [1]. After systole, the ventricles relax (diastole), arterial pressure declines and the heart refills with blood. The lowest pressure reached during ventricular relaxation represents the diastolic blood pressure.

Heart rate is normally determined by the rate of depolarization of the cardiac pacemaker. The pacemaker is found in the sinuatrial node, the atrioventricular node and the Purkinje tissue. However, because the rate of depolarization is faster than that of the other pacemaker tissue and the depolarizing impulse spreads via the heart's conducting mechanism to other pacemakers before they spontaneously depolarize, it is the sinuatrial node which determines the rate [3].

2.3 Myocardial Ischemia

Insufficient blood supply to the myocardium can result in myocardial ischemia, injury or infarction, or all three. Myocardial ischemia generally appears first and is more extensive in the sub-endocardial region since these deeper myocardial layers are farthest from the blood supply, with greater need for oxygen. Myocardial ischemia is a condition in which oxygen deprivation to the heart muscle is accompanied by inadequate removal of metabolites because of reduced blood flow or perfusion. During ischemia, an imbalance occurs between myocardial oxygen supply and demand.

2.4 Causes of Myocardial Ischemia

Myocardial ischemia can occur as a result of increased myocardial oxygen demand, reduced myocardial oxygen supply, or both. In the presence of coronary obstruction, an increase of myocardial oxygen requirements caused by exercise, tachycardia, or emotion leads to a transitory imbalance. This condition is frequently termed "demand" ischemia and is responsible for most episodes of chronic stable angina. In other situations, the imbalance is caused by acute reduction of oxygen supply secondary to increased coronary vascular tone or by marked reduction or cessation of coronary flow as a result of platelet aggregates or thrombi [6]. This condition, termed "supply" ischemia, is responsible for myocardial infarction and most episodes of unstable angina. In many circumstances, ischemia results from both an increase in oxygen demand and a reduction in supply.

The heart is an aerobic organ and therefore relies almost exclusively on the oxidation of substrates for generation of energy. It can develop only a small oxygen debt and still have enough energy to function normally. Thus, in a steady state, determination

of the rate of myocardial oxygen consumption provides an accurate measure of its total metabolism. Myocardial oxygen consumption is the product of systolic blood pressure and heart rate [7]. The small fraction of MVO2 in the non-contracting heart is required for those physiologic processes not directly associated with contraction. Increases in the frequency of depolarization of the non-contracting heart are accompanied by only small increases in myocardial oxygen consumption.

Medically, the way to identify Myocardial Ischemia is (1) Anginal discomfort, (2) ST-segment deviation on the ECG (Fig 2.2) (3) Reduced uptake of thallium 201 or technetium 99 in myocardial perfusion images, or (4) Regional or global impairment of ventricular function [8]. Usually, a S-T depression is associated with myocardial ischemia when the S-T segment is 1mm or more below the isoelectric line *at* the J point [6]. Notice in the picture that it is the S-T segment, not just the T wave that is important.
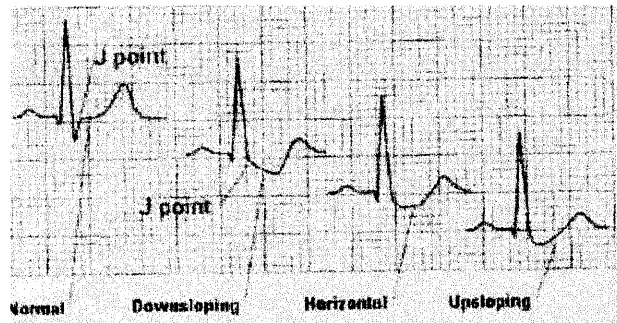
Figure 2.3 S-T depression in the ECG of the Heart.

As the heart undergoes depolarization and repolarization, the electrical currents that are generated spread not only within the heart, but also throughout the body. This electrical activity generated by the heart is generally measured by an array of electrodes placed on the body surface and the resulting tracing is called an electrocardiogram (ECG,

or EKG). The P-wave represents the wave of depolarization that spreads from the SA node throughout the atria and is usually 0.08 to 0.1 seconds (80-100 ms) in duration. The period of time from the onset of the P-wave to the beginning of the QRS is termed the PR interval and normally ranges from 0.12 to 0.20 seconds [9].
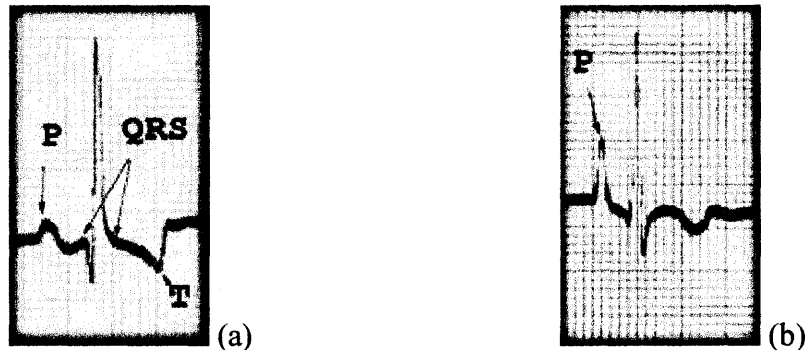
Figure 2.4(a) & (b) In Figure 2.4(a), the normal EKG wave deflection is shown here. In Figure 2.4 (b), the EKG shows slight changes in the P wave, which indicates heart disease and enlargement.

This interval represents the time between the onset of atrial depolarization and the onset of ventricular depolarization. The QRS complex represents ventricular depolarization. The duration of the QRS complex is normally 0.06 to 0.1 seconds indicating that ventricular depolarization normally occurs very rapidly. The isoelectric period (ST segment) following the QRS is the time at which the entire ventricle is depolarized and roughly corresponds to the plateau phase of the ventricular action potential. The ST segment is important in the diagnosis of ventricular ischemia or hypoxia because under those conditions, the ST segment can become either depressed or elevated. The T-wave represents ventricular repolarization and is longer in duration than depolarization (i.e., conduction of the repolarization wave is slower than the wave of

depolarization) [8]. RR interval is used as a measure of heart period as the R peak is more identifiable on the ECG tracing and the PR interval is constant in the absence of conduction disorders.

CHAPTER 3

ENHANCED EXTERNAL COUNTERPULSATION

This chapter will serve as an overview of the counterpulsation technique, as well as an overview of the physiological background and procedure. This chapter details both the physiological systems evaluated and the method by which the data was acquired for this research.

3.1 Introduction

Enhanced External Counterpulsation (EECP) is a non-invasive procedure that has shown to reduce the symptoms of angina pectoris by increasing the coronary blood flow in the ischemic areas of the heart [10]. It is an outpatient procedure that relieves myocardial ischemia by improving perfusion in areas of the heart deprived of adequate blood supply. Counterpulsation is a strategy for increasing the blood flow back to the heart during a specific part of the heartbeat i.e. diastole when the heart needs oxygen rich blood [10]. This strategy not only increases the amount of oxygen available to the heart, but it also decreases the heart's workload, improves circulation and strengthens the cardiopulmonary system. The patients receive 35 sessions, with each session consisting of a one-hour treatment, which is aimed at provoking long lasting beneficial changes in the circulatory system [11]. The goal of the procedure is to increase oxygen rich blood flow to the heart and to reduce the heart's workload by stimulating the opening, or formation of, small branches of blood vessels to create natural bypass around narrowed or blocked arteries [10]. This theory will be explained in detail in Section 3.4.

3.2 History of Enhanced External Counterpulsation

Since the advent of bypass surgery in 1966 and coronary angioplasty in 1977, there has been an explosion in the arena of coronary revascularization for atherosclerotic disease [12]. Even with the successes of reoperation and catheter-based revascularization techniques, the population of patients with intractable angina and no conventional revascularization options is increasing.

Enhanced external counterpulsation was used as a treatment for angina in China for two decades [12]. This noninvasive technique provides augmentation of diastolic blood flow and coronary blood flow similar to the intra-aortic balloon pump, utilizing the serial inflation of three sets of cuffs which wrap around the calves, thighs and buttocks. Inflation and deflation is timed to the patient's ECG. The overall hemodynamic effect is to provide diastolic augmentation and thus increase coronary perfusion pressure; to unload systolic cardiac workload and therefore decrease myocardial oxygen demand; and to increase venous return and subsequently, cardiac output. This exciting new therapy is being advised more and more for patients who have chronic angina, acute ischemic syndromes and cardiac dysfunction [12].

In 1953, Kantrowitz and Kantrowitz initially described the concept of diastolic augmentation as a technique to improve coronary flow, which had been known to be primarily diastolic [13]. Early work by Birtwell and others showed that the ECG QRS complex could be utilized to time an external pumping device that provided a synchronous pulse wave thereby increasing the development of coronary collaterals in experimental models. Also, it was described how these types of assist devices could not

only produce increased coronary flow, but also reduce left ventricular work and oxygen demand.

The term counterpulsation was first coined to describe the two-fold effect of the rapid displacement of the blood through the vessels and reduced resistance to the volume of the blood in the lower arterial circuit. The principle thought to be in effect is that, via persistent augmentation of diastolic flow, stimulation of collaterals to ischemic territories occurs with improvement in symptoms and clinical measures of ischemia. Initial experience with a crude external counterpulsation device used in stable angina saw relief of angina symptoms with angiographic evidence of increased vascularity [12]. In the early 1980's, a Chinese group lead by Z.S. Zheng began reporting a large experience using a sequential three-cuff external counterpulsation system, which provided a pressure wave by sequentially inflating from calf to thigh to buttock. Their clinical experience led to the development and refinement of the EECP technique and device [12].

3.3 Physiological Background

The basic concept is based on the response of the left ventricle to reduce arterial pressure during the systolic period so that the heart can be rested. The heart can be rested if the oxygen demand can be reduced. Oxygen can be reduced if the left ventricular pressure can be reduced. The net perfusion pressure must be maintained to meet the metabolic needs of the body. Under the circumstance of decreased systolic pressure, the diastolic pressure must be increased to maintain that effective perfusion. To achieve this, a system is required that can be synchronized and phased with the cardiac activity.

Repeated and pulsed increases in diastolic pressure during the therapy with EECP enhance or stimulate the opening of collateral channels in the coronary vascular system, increasing perfusion of ischemic areas. The earlier external counterpulsation systems employed non-sequenced pulsation, i.e., compression of the vessels was performed simultaneously along the full length of the compression element. Results could have been improved if the blood was expressed from the extremities in a sequential manner. After developing and testing these techniques, it was determined that greater cardiac output and increase of the ratio of diastolic to systolic pressures were achieved with sequenced systems than with the non-sequenced systems [14]. The EECP treatment employs a three-cuff compression configuration and sophisticated computerized control of the inflation and deflation sequence.

The pulsation is coordinated with the heartbeat. The systolic phase of the heart muscle is the contraction of the ventricles that drives the blood into the aorta and pulmonary artery. The diastolic phase is the resting time between the contractions, when the ventricle fills with blood. The external counterpulsation equipment includes large blood pressure cuffs wrapped around the legs that operate in synchrony with the person's electrocardiogram [15]. The cuffs inflate during the diastolic phase of the heartbeat, wrapped around the legs and pushing the venous blood sequentially from the calves, thighs and buttocks towards the heart. This also increases oxygenated blood flow upwards, supplying greater blood flow to the coronary arteries, brain, liver and kidneys.

The cuffs then deflate while the heart is contracting, which allows blood to flow easily into the legs [15]. In fact, with the EECP returning more blood to the heart from the lower extremities, the left ventricle is pumping against less pressure in the legs.

Therefore it takes less effort and less oxygen demand for the left ventricle to pump more blood to the system, hence reduced workload or reduced ventricular after load. The increased blood flow to the coronary arteries enlarges the smaller blood vessels of the heart and this enlargement gradually increases their blood carrying capacity [13].
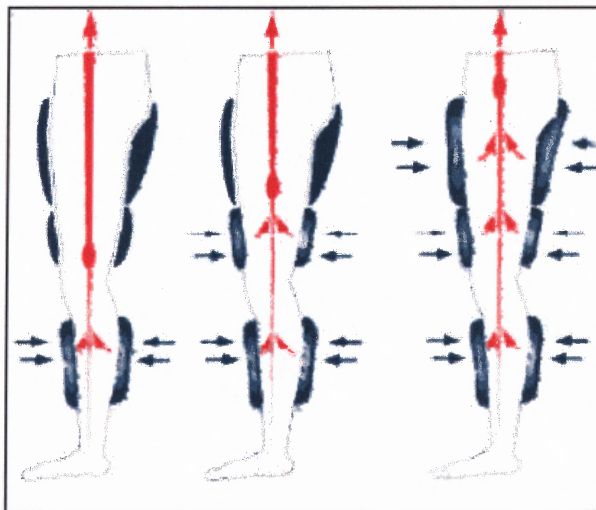
Figure 3.1 Sequential applications of cuff inflations. Series of three cuffs inflate at the calf, thigh and buttock level timed with the cardiac cycle.

The EECP treatment has a marked effect on cardiovascular hemodynamics. Sequenced (from the calves upward) compression of the lower extremities during diastole drives arterial blood backward, augmenting aortic diastolic pressure and causing retrograde flow in the descending aorta. Augmenting aortic diastolic pressure increases coronary perfusion pressure and, consequently, coronary blood flow. Coronary perfusion pressure is the difference between aortic diastolic pressure and left ventricular intracavitary pressure. The increase in coronary pressure and flow causes an increase in shear stress in the coronary arteries.

3.4 Collateral Circulation

The EECP treatment is a non surgical, mechanical procedure that can reduce the symptoms of angina or CHF, presumably by stimulating the opening, or formation of, small branches of blood vessels (collaterals) to create a natural bypass around narrowed or blocked arteries. Evidence to date suggests that the EECP treatment promotes the growth of new blood vessels in the heart and improves blood flow to the areas that are not getting enough blood and oxygen [11]. The improved blood flow decreases chest pain symptoms.

Significant obstruction in one or more coronary arteries can create a pressure difference between areas of the heart muscle that receive and those that do not receive enough blood. Repeated and pulsed increases in pressure during diastole may stimulate opening collateral channels across this pressure gradient within the heart muscle, resulting in increased blood supply to deprived tissues.

3.5 Benefits

Clinical trials confirm the benefits of EECP treatment. There is an improved blood flow to the heart thereby replenishing the deprived heart muscles. Counterpulsation increases the stroke volume per unit work and; therefore, the efficiency of the left ventricle. There is an elimination or reduction in the medications intake. Common medications for CHF may include an ACE Inhibitor like Fosinopril, Enalapril, Lisinopril and many others; Beta Blockers like Metoprolol, Carvedilol and some others; Diuretics (water pills) like Furosemide, Bumetanide, Torsemide etc [17]. The patient shows an improved ability to

exercise. The patient and their families report a noticeably greater ability to engage in daily activity. It is a viable treatment option if:

1. One has angina or congestive heart failure.

2. Medication does not provide adequate relief.

3. One is not a candidate for surgery or angioplasty.

4. One has already undergone surgery or angioplasty and angina has returned.

5. One wants to explore all available treatment options.

3.6 Equipment

The EECP treatment uses a unique equipment to inflate and deflate a series of pneumatic compressive cuffs around the lower extremities. The treatment is administered on a padded table where three sets of electronically controlled inflation and deflation valves are located. These valves are connected to specially designed adjustable cuffs that are wrapped firmly but comfortably around the patient's calves, lower thighs and upper thighs, as shown in Figure 3.2.
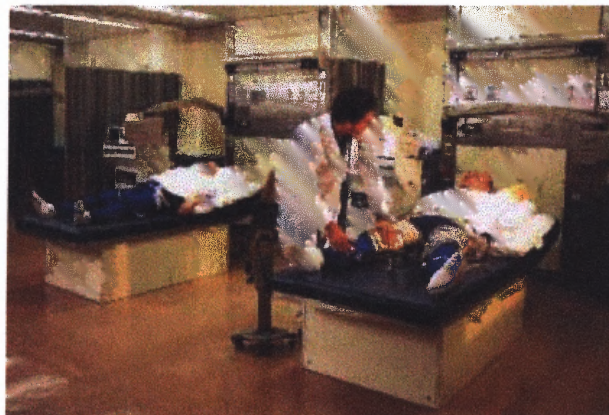
Figure 3.2 The patient lying on the treatment table.

The design of the cuff permits significant pressure to be applied to the arteries and veins at relatively low air pressures. A microprocessor monitors the treatment process. The timing for inflation and deflation is regulated by running the electrocardiogram signals through the microprocessor [11].

3.7 Procedure

The patient receives EECP treatment for 35 hours divided into one sixty-minute session five days per week. In EECP, long inflatable cuffs, similar to large blood pressure cuffs are wrapped around both of the patient's legs; one at calf level, another slightly above the knee and the third on the thigh. The cuffs are larger versions of the familiar blood pressure cuff. While the patient lies on the bed, the leg cuffs are inflated with each heartbeat. A pressure source inflates and deflates these cuffs, moving blood from the lower limbs to the heart. The pressure is applied in sequence from the calves upward and is synchronized with the heartbeat so that the increased blood flow is delivered to the heart at the precise moment it is relaxing and blood flow through the coronary vessels is at its peak. When the heart pumps again, the cuffs are deflated; this lowers resistance in the vascular bed of the legs so that blood may be pumped more easily from the heart. The patient's heart rate and rhythm are constantly measured during the process. The microprocessor reads the patient's ECG and deflates the cuffs at the onset of each heartbeat and inflates as each heartbeat ends. When the cuffs inflate, they do so in a sequential fashion, so that the blood in the leg is pushed upwards towards the heart. During diastole, the cuffs inflate sequentially from the calves proximally. This action results in an increase in diastolic pressure, generation of retrograde arterial blood flow

and an increase in venous return. The cuffs are deflated simultaneously just prior to systole, which produces a rapid drop in vascular impedance, a decrease in ventricular workload and an increase in cardiac output. The venous return and cardiac output is increased by the compression of the vascular bed of the legs. The decompression of the cuffs during systole permits systolic unloading and decreased cardiac workload because of rapid and simultaneous decompression.

3.8 Summary

In the short term, this method of therapy is thought to deliver more oxygen to the ischemic myocardium by increasing coronary blood flow during diastole, while at the same time reducing the demand for oxygen by diminishing the work requirements of the heart. Long-term benefit is expected to result as coronary collateral flow to ischemic regions of the myocardium is increased.

Clinical trials have demonstrated that the beneficial effects of EECP, including increased time until onset of ischemia and a reduction in the number and severity of anginal episodes. These effects are not only sustained between treatments, but may persist for several months to two years after completion of a course of therapy.

The EECP treatment increases blood flow 22-26% to the carotid arteries to the brain, 20-42% to the coronary arteries, and 19% to the renal artery. It increases the heart's output (stroke volume) by 12% and increases blood flow to areas not getting enough oxygen in the heart [15]. The ECP is FDA approved for Coronary Artery Disease and has been shown effective in the treatment of ischemic conditions, including glaucoma, angina, coronary artery disease, stroke and brain injury.

CHAPTER 4
HEART RATE VARIABILITY

This thesis is a study to measure the changes in heart rate variability due to the activity of the autonomic nervous system caused by the EECP treatment. It has been proved that rhythms can be markers of normal functional states [18]. Heart rate is one of the most familiar measures of cardiovascular status. The term heart rate is commonly associated with average heart rate but it is a well-known fact that the heart rate varies on a beat-to-beat basis due to cardiovascular control systems such as the ANS. The mean heart rate is largely determined by a balance between the mean levels of activity in the cardiac sympathetic and para-sympathetic nerves. A decrease in vagal activity can be compensated for by a decrease in sympathetic activity as far as the average rate is concerned.

4.1 History of HRV

Various cardiovascular variables, such as heart rate and blood pressure, fluctuate from one beat to another. HRV is measured on a beat-to-beat basis. Although the temporal fluctuations in cardiovascular signals were noted in ancient times, physicians overlooked for a long time the possible significance of beat-to-beat fluctuation of cardiovascular signals [19]. This variability was treated as noise to be either ignored or averaged out.

With the advent of high-resolution electrocardiography instruments and digital computers with high calculation capacity, it has been possible to measure the subtle beat-to-beat fluctuations in cardiovascular signals. Also, the computation of heart rate variability using various software languages like LabVIEW and MATLAB, and tools like

power time frequency methods, frequency domain methods and time domain methods to assess the frequency and amplitude of the oscillatory components of heart rate behavior has been possible. Recent studies have shown that decreased fluctuation of RR intervals is not noise, but implicates an increased risk for arrhythmic events and an increased mortality rate in patients with a previous myocardial infarction [20]. Time domain and frequency domain measures of heart rate variability have provided prognostic information and also made it possible to perform noninvasive studies on the significance of changes in the regulation of heart rate behavior. In this research the author has used the method of power spectral analysis (frequency domain analysis) and SDNN (time domain analysis) to analyze if the EECP treatment affected HRV in a significant way.

4.2 Background

The heart is designed to match cardiac output with the needs of the body. Sudden changes in heart rate are common and expected in response to physical or mental stress and exercise. Even in the absence of external perturbations, the normal heartbeat is not characterized by clockwise regularity [18]. This fluctuation around the mean heart rate is called heart rate variability (HRV). HRV therefore is a reflection of the cardiovascular control exerted by the sympathetic and parasympathetic divisions of the autonomic nervous system (ANS) on the heart that helps regulate cardiac output [21]. For a healthy heart, the heart rate increases as one inhales and decreases at exhalation. HRV is also associated with the balance of the fight or flight response and the relaxation response branches of the ANS.

4.3 Physiology

The HRV is affected by every system that modulates the autonomic nervous system. The heart rate at any time represents the net effect of the vagus nerves, which slow it and the sympathetic nerves, which accelerate it. In resting conditions, both autonomic divisions are thought to be tonically active with the vagal effects dominant [19]. For the vasomotor system the blood pressure is sensed by the baroreceptors and the sympathetic and the parasympathetic branches integrate this information [20]. The resultant effect is a variation in both heart rate and blood pressure. Therefore, blood pressure and heart rate interact forming a closed loop. This creates an oscillation called the Mayer waves or the 10 seconds rhythm. For the thermoregulatory system, it is reported that the system causes slow drifts in the HRV signal [21]. For the Renin-angiotensin system, the increase or decrease of body fluids and the intake of salt can modify the HRV signal. The bridge between the Renin-angiotensin system and the cardiac system is the sympathetic branch [22]. The effect is a slow variation in the RR time series. For the central nervous system, emotional states can modify the HRV. Intrinsic periodic rhythms that influence the heart rate variability are [23]:

1. Respiratory sinus arrhythmia
2. Baroreceptor reflex regulation
3. Thermoregulation
4. Neuroendocrine secretion
5. Circadian rhythms
6. Other, unknown rhythms

4.4 Applications of HRV

The greater the variability in heart rate, the better is the state of the ANS and cardiac system. HRV is a noninvasive test of cardiovascular autonomic regulation. Specifically, HRV is a measurement of the interaction between sympathetic ("fight or flight" energy mobilization) and parasympathetic (the opposite of the sympathetic activity or "relaxation" response) activity in the nervous system that controls the heart, intestines, and other organs. In general, higher HRV is desirable; lower HRV has been found to be a significant predictor of cardiac mortality and morbidity and other diseases.

The HRV is a promising yet complicated measurement that still has many unresolved issues. In this research the author looked into the high frequency region of the power spectrum as the high frequency (HF, 0.15-0.4 Hz) component is relatively well established as a measure of parasympathetic activity. To remove the possibility of conflicting results arising from different measurements of HRV and data collection methods, the author performed the HRV measurements in standardized conditions in order for the data to be reliable and valid. When collecting data from subjects, data was collected around the same time each day. Other factors like age, sex, position, breathing, smoking and medications which influence HRV were standardized. Breathing was standardized by each subject following paced breathing protocol at 12 breaths per minute. The quantization of the variability in cardiovascular signals provides information about the autonomic neural regulation of the heart and the circulatory system. Several factors have an indirect effect on these signals as well as artifacts and several types of noise are contained in the recorded signal. The dynamics of RR and QT interval time series have

also been analyzed in order to predict a risk of adverse cardiac events and to diagnose them.

Patients with heart disease underwent the EECP treatment for one hour and the ECG reading was collected pre and post treatment for 5 minutes for the purpose of assessing time domain indexes (SDNN) and frequency domain indexes (LF and HF) of HRV. The subjects followed paced breathing, the details of which will be explained in chapter 5. Time and frequency domain indexes of HRV are high in healthy subjects and decline proportionally with the severity of heart failure. Several studies have shown lowered heart rate variability in patients with chronic heart failure (CHF) [24]. The author sought to assess the prognostic value of heart rate variability due to the EECP treatment on patients with heart problems. HRV was assessed from calculation of the mean R-R interval and its standard deviation measured on short-term i.e. 5 minute electrocardiograms. The smaller the standard deviation in R-R intervals, the lower is the HRV.

4.5 Frequency Domain (Power Spectrum) Analysis

Power spectrum analysis decomposes the heart rate variability signal into its frequency components and quantifies them in terms of their relative intensity, or power. This analysis provides the basic information of how power (variance) distributes as a function of frequency. Independent of the method used, only an estimate of the true power spectral density of the signal can be obtained by proper mathematical algorithms; i.e. each mathematical algorithm does not yield an actual value of the power spectral density of a signal, but only an estimate.

The heart rate power spectrum can be analyzed in two major ways: (1) Fast Fourier transformation and (2) autoregressive model estimation, which are generally classified as nonparametric and parametric methods, respectively. As was utilized in this study, Fast Fourier transformation (FFT) describes a signal as a sum of various sinusoids at fixed and equally spaced frequencies; i.e., the output of several sinusoidal oscillators, the amplitudes and phases of which are described by FFT. The advantages of such nonparametric methods include the simplicity of the algorithm used (i.e. FFT), and the high processing speed. The advantages of the parametric methods are smoother spectral components that can be distinguished independent of preselected frequency bands, easy post processing of the spectrum with an automatic calculation of low and high frequency power components with an easy identification of the central frequency of each component, and an accurate estimation of the power spectral density, even on a small number of samples on which the signal is supposed to maintain stationary.

The Fourier transform is a signal processing technique that is used to represent a function in the time domain as a function within the frequency domain. It does this by representing the components of a non-periodic signal as a sum of complex exponentials or sinusoids. The following is the equation for the Fourier transform [24]:

$$X(\omega) = \int_{-\infty}^{\infty} x(t)e^{-j\omega t} dt \qquad (4.1)$$

where $X(\omega)$ represents the Fourier transform of time function $x(t)$, and $e^{-j\omega \tau}$ represents the complex exponentials or sinusoids that make up the signal. Essentially the Fast Fourier transform is a computer algorithm used to efficiently compute the discrete time equivalent of the continuous time Fourier transforms. Once the frequency domain

analysis is completed using the Fast Fourier transform, the frequency content of the power spectrum can be analyzed.

Three major frequency bands can be distinguished in a power spectrum, where each is generally associated with different systems that control heart rate and calculated from short-term recording of approximately 5 minutes (Figure 4.1). The computed spectrum represents the distribution of the power density across the frequency range of interest by decomposing the heart rate variability signal into its frequency components. Power spectral analysis of short segments of five minutes of beat to beat heart rate variability (PS/HRV) reveals three distinct peaks: A very low frequency range (VLF, 0.02 - 0.05Hz), a low frequency range (LF, 0.05 - 0.15Hz) and a high frequency range (HF, 0.15 - 0.4Hz) as shown in the Figure 4.1. The energy in the HF is vagal mediated, the energy in the LF and VLF are due to both sympathetic and parasympathetic systems.
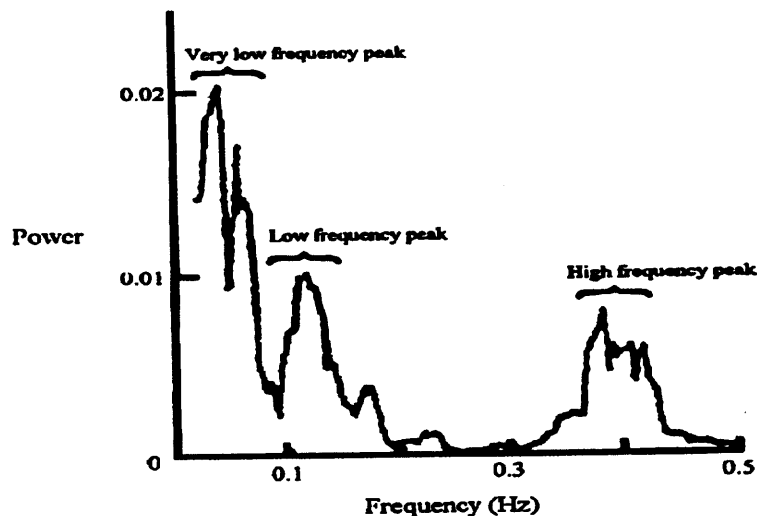
Figure 4.1 Frequency spectrum of an interbeat interval signal. (From M. V. Kamath and E. L. Fallen, "Power Spectral Analysis of Heart Rate Variability: A Noninvasive Signature of Cardiac Autonomic Function." *Clinical Reviews in Biomedical Engineering*, vol. 21, pp. 245-311, 1993.)

The high frequency (HF) band, ranging between 0.15 Hz and 0.4 Hz, is associated with parasympathetic activity and is influenced by respiratory sinus arrhythmia. A predominant peak usually occurs at the respiration frequency; therefore, depending on respiratory rate, the respiratory peak may not be within this HF range. For example, the respiration peak may reside around or above 0.15 Hz for respiratory rates below 9 breaths per minute or above 0.4 Hz for rates above 24 breaths per minute. Consequently, the maximum HF range is not limited to 0.4 Hz because for respiration rates above 24 breaths per minute, the respiratory peak begins to move into a higher frequency region.

The low frequency (LF) band ranges between 0.05 Hz and 0.15 Hz. The LF component is, to some extent, generated by baroreceptor modulation of sympathetic and vagal nervous tone [25]. It has been widely accepted that the low frequency band can serve as an indicator of both parasympathetic and sympathetic nervous system activity [26].

The third major frequency band that can be distinguished is the band of frequencies lying between 0.02 Hz and 0.05 Hz known as the very low frequency (VLF) band. The physiological origin of these very slow fluctuations is still to be determined; however, it has been suggested that variations in the activity of the renin-angiotensin system and thermoregulation are of importance.

Since the LF band is modulated by both parasympathetic and sympathetic activity, there is no frequency band in the power spectrum analysis of heart rate variability that yields a direct assessment of the sympathetic nervous system. As a result, an indirect measure has been established. The ratio of LF to HF power (LF/HF power

ratio) may be considered to be a measure of sympathovagal balance, a conclusion supported by many experimental and clinical studies [27].

The measurement of VLF, LF, and HF power components is usually made in absolute values of power (milliseconds squared). In this study, the LF and HF have been normalized in two ways. One, the LF and HF values were normalized to five minutes as 5LF and 5HF respectively, to standardize the data, as the length of each subject file was different. LF and HF may also be measured in normalized units, which represent the relative value of each power component in proportion to the total power minus the VLF component. The representation of LF and HF in normalized units emphasizes the controlled and balanced behavior of the two branches of the autonomic nervous system. Moreover, the normalization tends to minimize the effect of the changes in total power on the values of LF and HF components. Hence, LF and HF have been normalized to normalized low frequency (NLF) and normalized high frequency (NHF), respectively. Nevertheless, normalized units are always quoted with absolute values of the LF and HF power in order to describe completely the distribution of power in spectral components.

4.6 Time Domain (SDNN) Analysis

The most reliable score in HRV, and the measure of variability itself, is the SDNN (standard deviation of normal to normal intervals). This index is the one used to predict mortality [28]. The quantification of HRV in time-domain analysis is based on several statistical measures or indexes (such as the mean or the standard deviation) of the RR time series. Time-domain indexes fall in two categories: indexes for short and long time measurements. One of the most important and popular time-domain HRV parameters is

SDNN, which reflects the overall variability over a specified time interval [25]. However, the SDNN value depends on the length of the recording.

Due to the standardized steady measurement condition of any 5-minute HR recording, its SDNN value is typically significantly lower than the SDNN measured during a 24-hr period, which includes the night sleep hours and periods of physical and mental activity with significantly higher HR readings. In this study the SDNN was calculated for five-minute recordings. The time domain measures of HR variability are calculated by statistical analyses (means and variance) from the lengths of successive R-R intervals. SDNN is considered to reflect both the sympathetic and the parasympathetic influence on HRV. SDNN mostly reflects the very-low-frequency fluctuation in heart rate behavior. It cannot detect subtle changes in heart rate dynamics, because the fast changes in heart rate occurring within a few seconds or minutes are lost under the majority of slower changes. All time and frequency domain measures of HR variability could be affected by artifacts, and these measures require data from which these artifacts have been eliminated.

CHAPTER 5

EXPERIMENTAL PROCEDURES AND METHODS

The data used in this experiment was obtained at the University Hospital, Department of Cardiology, The University of Medicine and Dentistry of New Jersey (UMDNJ) in Newark, New Jersey. This chapter provides the details of the experiment, including subject background, equipment configuration, and data analysis methods. In order to analyze and compare the data, there were three groups of subjects- Patients, Normals and Controls. The study for the controls was conducted at New Jersey Institute of Technology (NJIT) while the data for the normals and patients was acquired at UMDNJ.

5.1 Subject- Patient

5.1.1 Patient Background and Preparation

In this research, studies were conducted on people who had myocardial ischemia or heart failure. The patients had been recommended for EECP treatment. The preparation for the treatment involves almost no preparation on the subject's part except for wearing light fitting pants to avoid chafing and skin irritation from the natural movement of the pressurized cuffs. There were seven patients who had the EECP treatment recommended to them. Of the seven patients, six were myocardial ischemic patients and one was a heart failure patient. The patients had to be non-smokers. The patients were labeled as A, B, C, D, E, F and G. The data was collected from patients who had no caffeine intake for at least three hours and no food intake at least two hours before the data acquisition.

5.1.2 Data Acquisition

Subjects received 35 one-hour EECP treatments over a seven-week period. Data of patients having heart conditions were collected for the pre and post one-hour treatment period. The electrocardiogram, respiration and blood pressure signals were collected for 5 minutes pre and post treatment. The patients followed paced breathing at 12 breaths per minute. Details of the paced breathing equipment and procedure are given in section 5.5.

5.2 Subject- Normal

5.2.1 Normals Background and Preparation

To compare the data obtained from the patients who underwent the EECP treatment, there were another group of subjects, who were healthy but underwent the EECP procedure. This set of people was defined as Normals. They underwent a total of three EECP sessions during three consecutive days. Normals had no history of myocardial ischemia known to the physician. They were healthy individuals. There were three Normals. In this research the normals were labeled as H, I and J. They also followed paced breathing at the rate of 12 breaths per minute. The normals were instructed on:

1. No caffeine intake for at least three hours before the data collection.

2. No food intake at least two hours before the data acquisition.

3. They should be non-smokers.

4. Healthy individuals.

5.2.2 Data Acquisition

Normals received 3 one-hour EECP treatments over a period of three days. The data were collected for the pre and post one-hour treatment period. As with the patients, the electrocardiogram, respiration and blood pressure signals were collected for 5 minutes pre and post treatment. The normals followed paced breathing at the rate of 12 breaths per minute, the details of which are given in Section 5.5.

5.3 Subject- Control

5.3.1 Controls Background and Preparation

Other than the patients and normals that underwent the EECP treatment, there was another group of subjects, who had no presence of heart disease and did not undergo the EECP procedure. The ECG, respiration and blood pressure signals were collected from these subjects. These set of people were defined as Controls. There were five controls. The prerequisites for the controls were:

1. No caffeine intake for at least three hours before the data collection.

2. No food intake at least two hours before the data acquisition.

3. They should be non-smokers.

4. Healthy individuals.

5. In the same age group of 23-27 years.

The average age group for the controls was 25 years. The data was collected in the biosignals research laboratory at NJIT. The data from the 5 controls were collected on the same day, one control following the other.

5.3.2 Data Acquisition

The electrocardiogram, respiration and blood pressure signals were collected for 5 minutes before and after an hour. In the intermediate hour, they rested or indulged in non-stressful activity as any kind of activity increases the sympathetic activity. An increase in the sympathetic activity increases the low frequency activity of the HRV spectrum.

5.4 Testing Procedure

A RESP 1 impedance pneumograph (UFI, Morrow Bay CA) was used to convert both respiration and ECG data into analog signals. The Resp1 is a self balancing, stand-alone signal conditioner which converts changes in thoracic impedance resulting from respiration, into a high-level respiration signal. A front panel meter shows respiration activity for easy checkout. A 9V battery powered the Resp1 impedance pneumograph. The ECG recordings were obtained using surface silver-silver chloride disposable electrodes. (Myotronics Research, Seattle, WA). The skin underlying the electrodes was dried with alcohol and the electrodes were placed on the surface of the upper left chest and upper right chest and were affixed to the skin. The exact placing of the electrodes on the chest depends on the strength of the ECG waveform, which is monitored through the Resp1's output to the computer. The ECG and respiration recordings were taken through the Resp1 impedance pneumograph. The ground electrode was placed on the left arm and approximately 20 centimeters away from the elbow towards the hand. Real time blood pressure data were collected as analog signals through the Colin 7000 (San Antonio, TX) noninvasive arterial tonometry device. This device consisted of a sensor positioned over the left radial artery at the wrist and an occlusion cuff positioned around

the upper left arm. The Colin 7000 was calibrated before each data acquisition. Data were collected using LabVIEW v.5.0 software, a data collection program including a BNC 800 interface board and a 12-bit analog to digital (A/D) DAQ Card-700 board (National Instruments, Inc., Austin TX). The data were imported as three channels. The LabVIEW software was also used to analyze the data using power spectral analysis. The converted digital data were imported to a Dell Notebook via the National Instruments data acquisition card, NI PCI-6025E.

5.5 Paced Breathing

While recording the data, the patients, normals and controls followed paced breathing. With each subject following paced breathing, i.e., controlled breathing at 12 breaths/minute, the data were standardized, so that the respiratory peak occurred in the HF region at 0.2 Hz. Paced breathing was done using a paced breathing box, which has a series of LED lights, flashing in sequence at the rate of 12 breaths per minute. Figure 5.1 shows the plot of a patient who followed paced breathing.

Figure 5.1 is the plot showing the heart rate variability of patient MS. Referring to the left hand side of the plot, SF gives the value of the sampling frequency at the rate of 250 sample points per second. HF area shows the value of high frequency power in the region of 0.15-0.4 Hz in the heart rate power spectrum. The LF area shows the value in the low frequency region. Referring to the second graph of the respiration power spectrum in figure 5.1, the peak at 0.2 Hz validates the fact that the patient was breathing at 12 breaths/min. The third graph shows respiration as a function of time. It can be easily

identified by its periodical waveform. Time for each data file in minutes was also calculated.
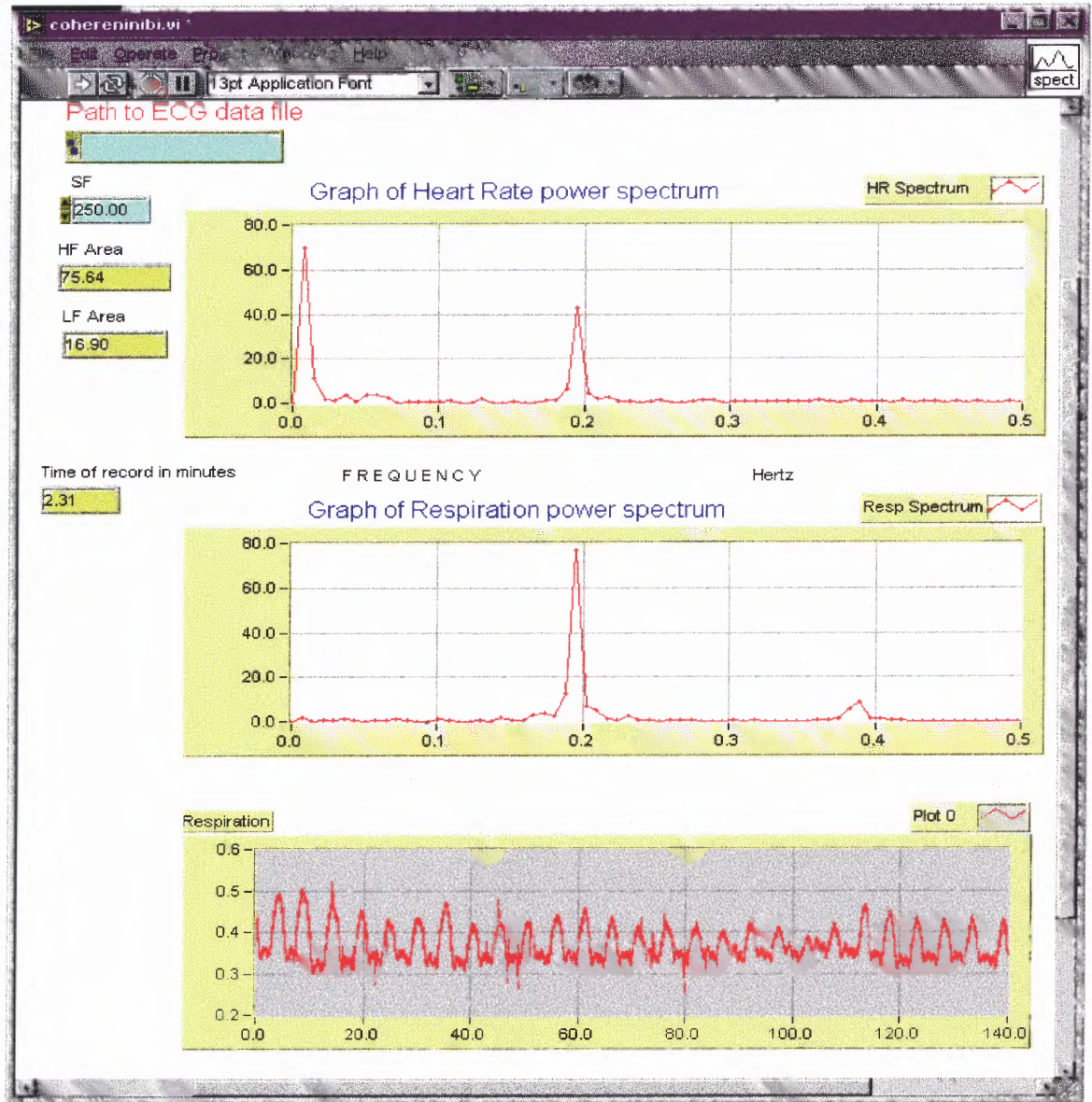
Figure 5.1 Plot shows the peak at respiration frequency for patient D.

5.6 Data Analysis

5.6.1 Programs

The ECG, BP and RESP data were the inputs to three programs used to calculate the HRV power spectrum. A sample data file is given in the Appendix A. LabVIEW 5.0 was used to analyze the data.
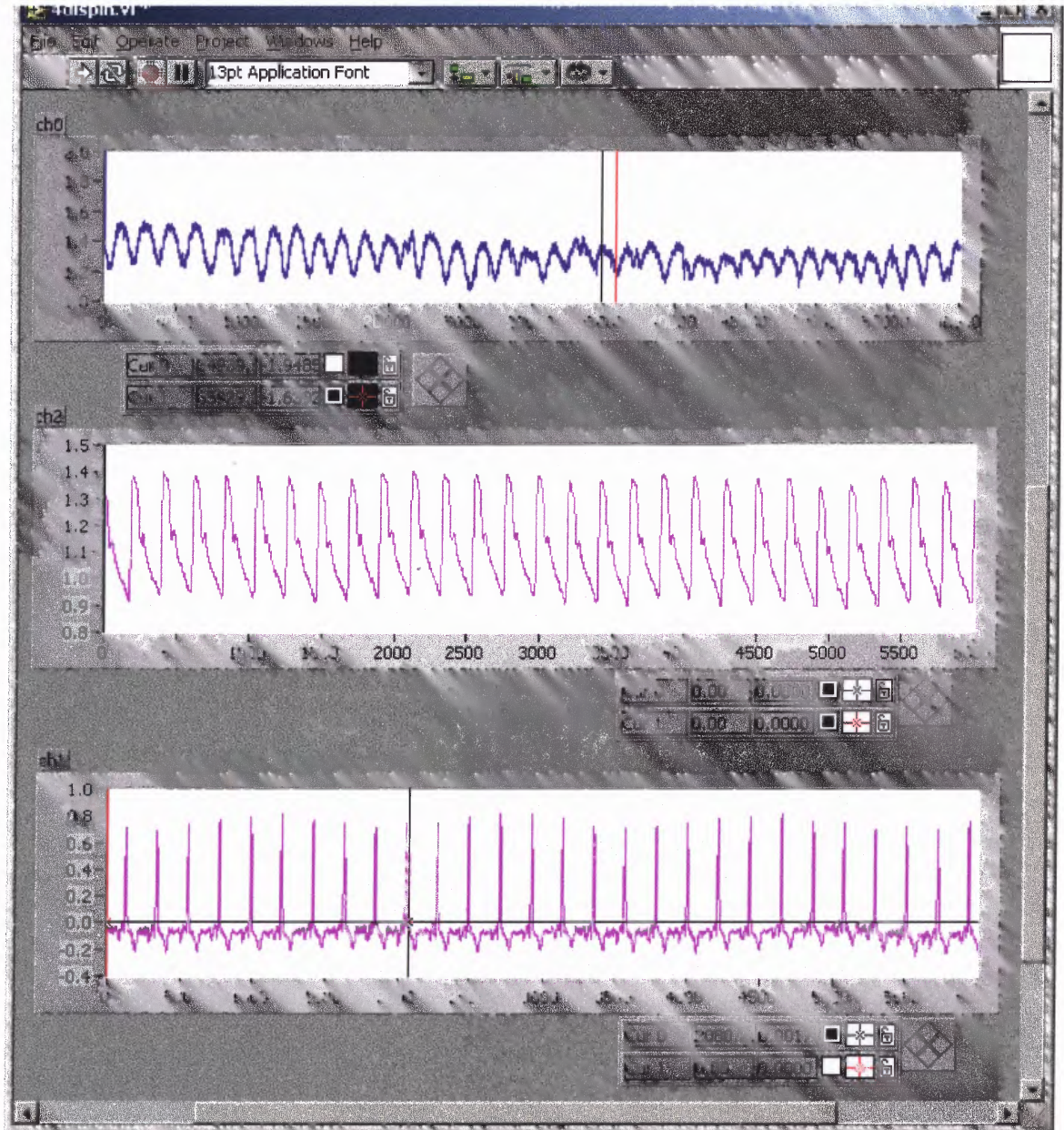
Figure 5.2 Plot shows the channels of respiration, blood pressure and ECG, respectively.

Program A was used to identify the channels of ECG, respiration and blood pressure. Figure 5.2 shows the plots generated by program A. Program A is given in Appendix B. In Figure 5.2, channel 0 was respiration, channel 2 was blood pressure and channel 1 was ECG.

The output from program C generated heart rate and respiration power spectra versus frequency. Program C is given in Appendix F. The basic requirement for the computation of the HRV spectrum is a noise free record of the signal lasting five minutes. Noise in the data usually occurs from improper placing of the electrodes, coughing or movement by the subject or not following the pacing signal. Each data file is approximately five minutes in length. Since the data was real time data, in some cases, there was presence of noise in the 5-minute file.

The criterion for an acceptable segment was a relatively sinusoidal or periodic respiration waveform. Hence, an acceptable segment of minimum 15000 sample points i.e. 1 minute was used as a criterion to extract signals from the original file. For this purpose, a data extraction program (Program B) was used. The program is given in Appendix B. Figure 5.3 and Figure 5.4 show the original and the extracted file of patient B, respectively. Channel 0 shows the respiration waveform of patient B in both the figures. In the original file, the sample points are 108000. Due to the presence of noise in the data, the power spectral analysis program detected the noise as peaks and gave incorrect values for LF and HF power. Regions in channel 0 where there was discontinuity (sample points 0 to 13000) and irregular peaks (sample points 39000 and 103000) could not be used for data extraction. A sudden high peak at point 39500 and an irregularity at 71000, made it impossible to use the segment of 25000 to 100000. Since

the criterion was at least a segment of 15000 sample points, hence points 40000 to 70000 were extracted from the original data file to perform the spectral analysis. Figure 5.4 shows the extracted data file with 30000 sample points.
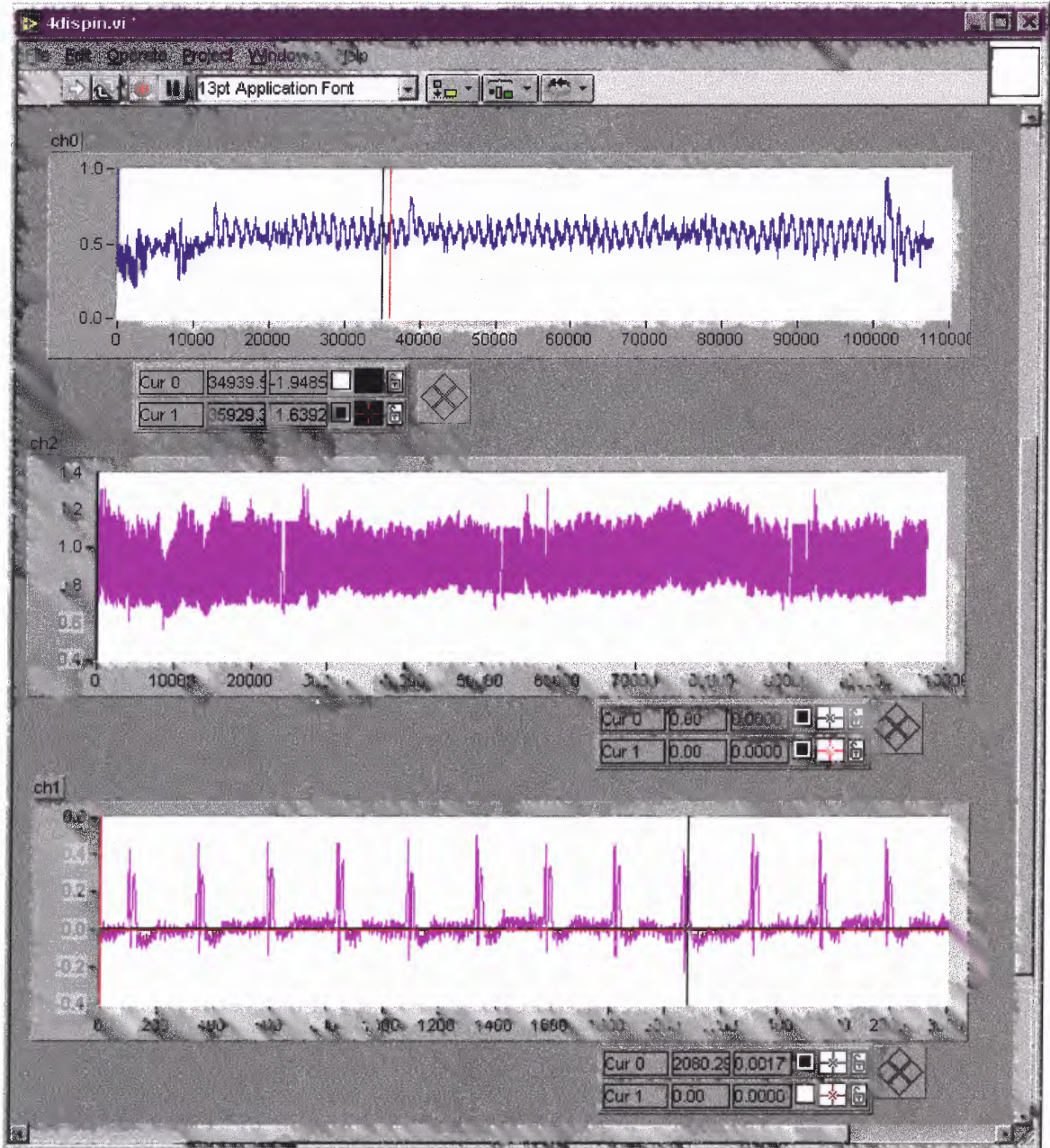
Figure 5.3 Plot for patient B with original data points of 108000.
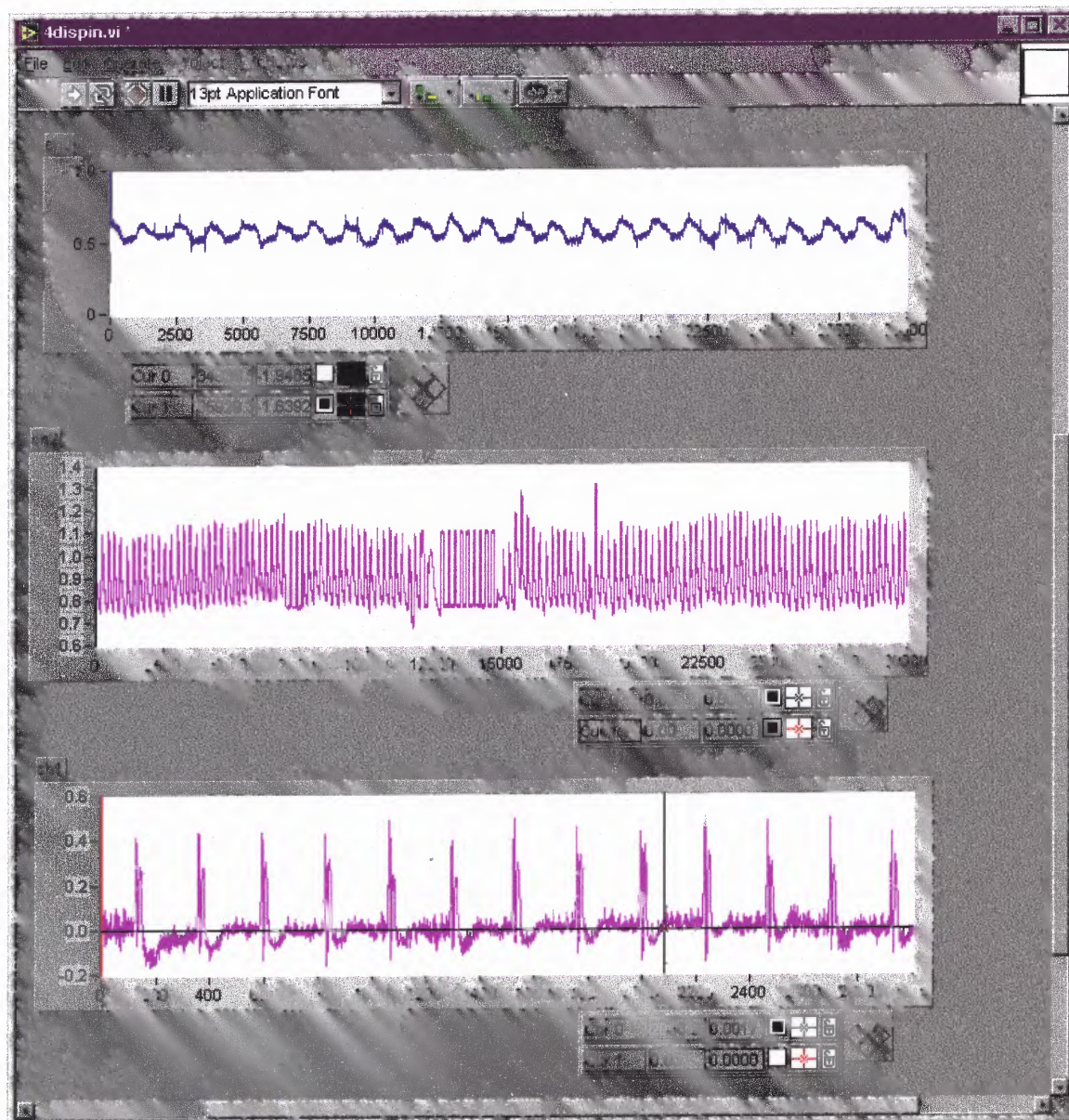
Figure 5.4 Plot for patient B with extracted data points of 30000.
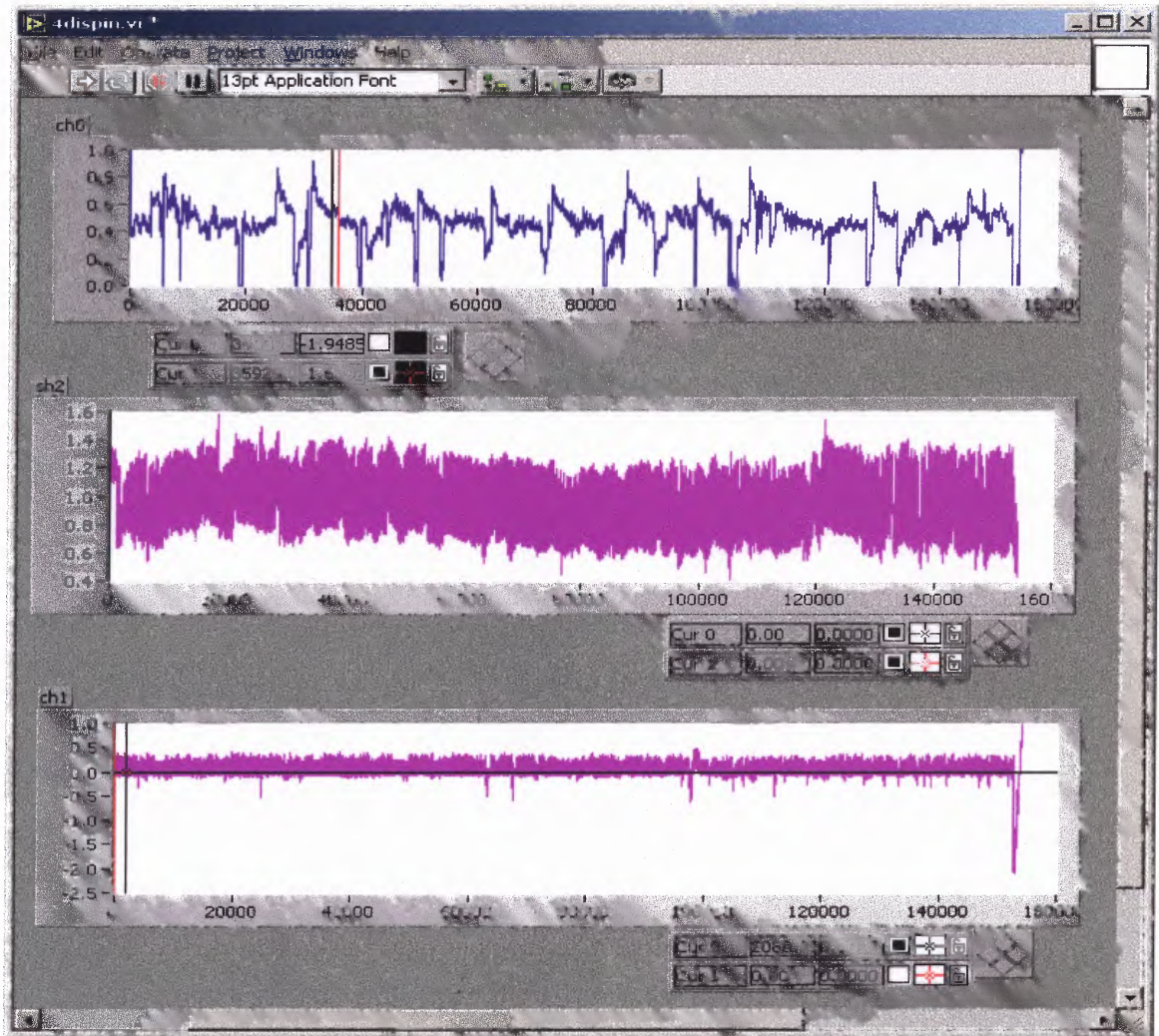
Figure 5.5 Plot for patient B showing a data file for patient B where no data could be extracted.

Figure 5.5 shows a data file of patient B, where no acceptable region of minimum 15000 sample points could be found. The presence of innumerable irregular respiration peaks observed from channel 0 in Figure 5.5 and absence of periodic respiration waveform made it impossible for the author to extract a reasonable segment to perform the data analysis. Files with these problems could not be used for spectral analysis, but were still counted as session numbers when performing the statistical analysis.

The recorded ECG, BP and respiration files were approximately five minutes in length, taken before and after each treatment. The recorded ECG signal was sampled at 250-samples/sec to precisely locate the QRS complex. The blood pressure was also sampled at 250-samples/sec. Subjects followed paced breathing at approximately 12 breaths per minute.

5.7 Power Spectral Analysis

Spectral analysis (frequency-domain analysis) quantifies the frequency content. It detects periodic oscillations (amplitude and frequency) and it has been employed in a great variety of time series processing [28]. It is well known that HRV has oscillations at different frequencies that are originated by different physiological systems [29]. These oscillations are transmitted to the heart by the sympathetic and parasympathetic systems. It is also well established that high frequency oscillations (0.15-0.4 Hz) are vagally mediated. Low frequency oscillations (0.05-0.015 Hz) are due to both parasympathetic and sympathetic systems. The starting point in every frequency-domain analysis is to perform the power spectral estimation of the signal [30]. The next step is to quantify the result of spectral estimation. One way to do this quantification is to divide the power spectrum in bands and to quantify the energy (by integration) in each one.

The location of the R peaks was detected using a peak detection algorithm incorporated in program C to compute the LF and HF power. Figure 5.6 shows the execution window of the peak detection algorithm. The troughs in the program show the missed peaks and the crests show the non-real peaks detected.

The respiratory signal was analyzed to determine the respiration peak. In Figure 5.7, the second graph shows that the respiration peak is close to 0.2 Hz. The HF peak has been associated with respiration frequency and is mediated by parasympathetic pathways. The results were displayed as a power spectrum, in which the area under the curve represents the degree of variability. The peak of the curve occurs at or near the respiration frequency.
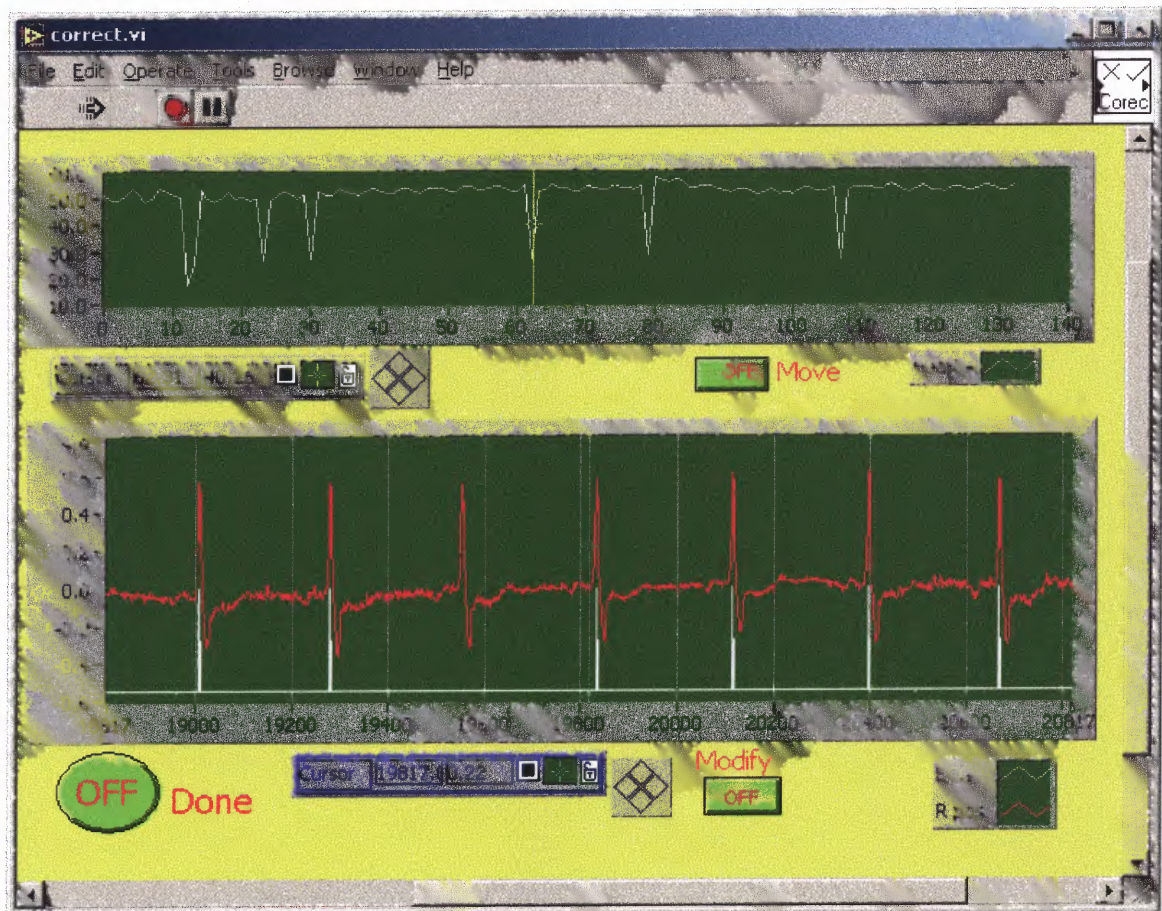
Figure 5.6 Window showing the peak detection. The troughs in the first plot are the missed peaks in the second plot.

From Figure 5.7, the LF and HF were computed as LF area and HF area. The length of the file was generated as the time of record in minutes. Using these basic values, calculations were conducted for normalized high frequency (NHF) and

normalized low frequency (NLF). Since every file was of a different length, depending on the length extracted from the original data file, the LF and HF generated from the program were normalized to five minutes as 5LF and 5HF, respectively. An example of the calculations involved for one subject is shown in Table 5.1.
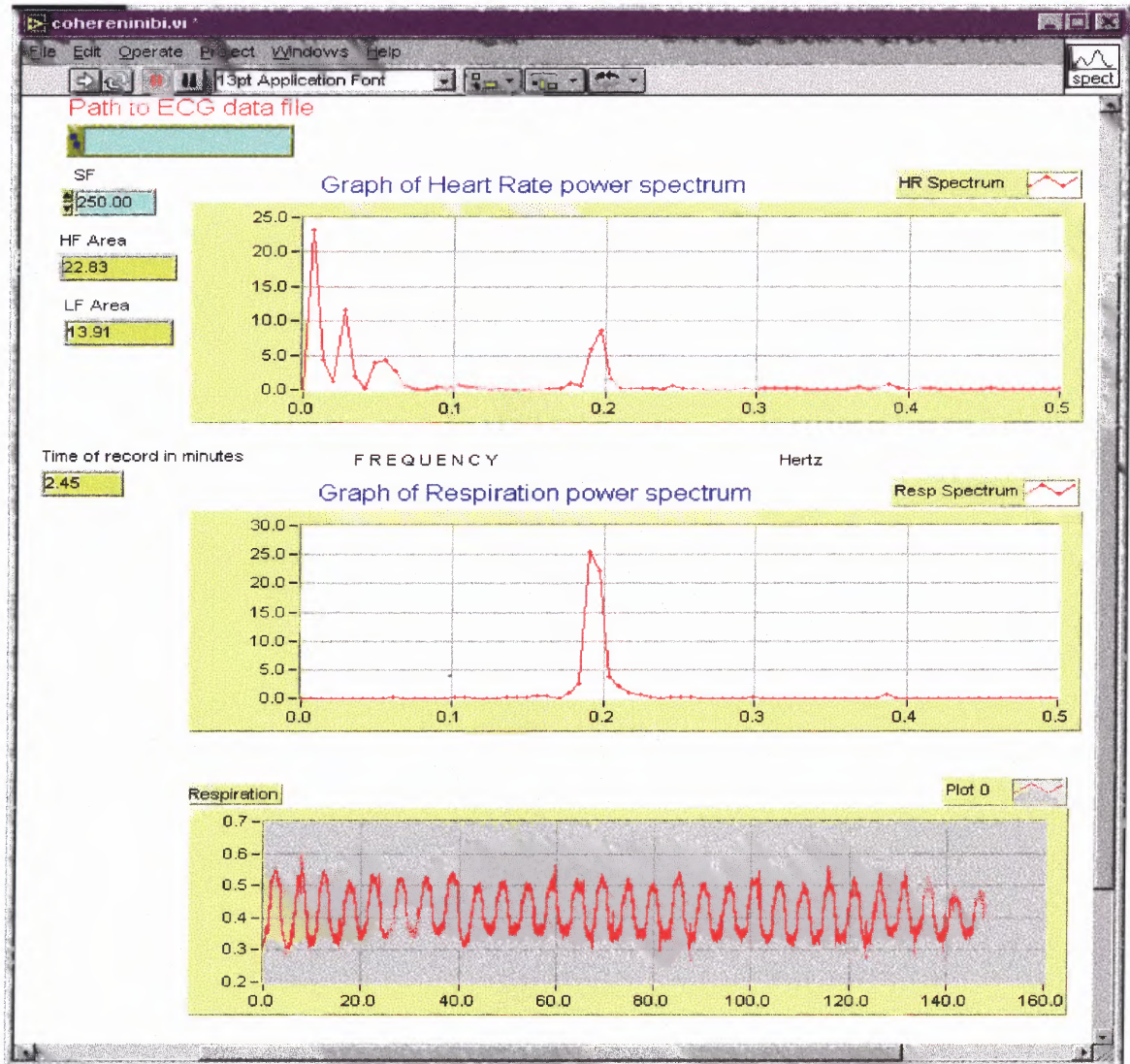
Figure 5.7 Window showing the power spectral analysis.

Low Frequency normalized to 5 minutes (5LF) was defined by (5.1)

$$5LF = (LF * 5) \ / \ \text{Time of record} \qquad (5.1)$$

High Frequency normalized to 5 minutes (5HF) was defined by (5.2)

$$5HF = (HF * 5) / \text{Time of record} \tag{5.2}$$

Normalized High Frequency (NHF) is defined by (5.3)

$$NHF = 5HF / (5LF + 5HF) \tag{5.3}$$

and, Normalized Low Frequency (NLF) is defined by (5.4)

$$NLF = 5LF / (5LF + 5HF) \tag{5.4}$$

Table 5.1 Table of Calculations Involved for Patient F Generated from Using Equation 5.1 Through Equation 5.4

TIME	LF	HF	5LF	5HF	NLF	NHF	NLF/NHF
1.65	67.19	11.64	203.6061	35.2727	0.8523	0.1477	5.7723
1.82	17.62	16.78	48.40659	46.0989	0.5122	0.4878	1.0501
1.58	75.64	14.43	239.3671	45.6646	0.8398	0.1602	5.2419
1.98	57.27	28.49	144.6212	71.9444	0.6678	0.3322	2.0102
0.99	33.26	7.41	167.9798	37.4242	0.8178	0.1822	4.4885
1.32	79.5	95.38	301.1364	361.288	0.4546	0.5454	0.8335
1.65	71.08	24.22	215.3939	73.3939	0.7459	0.2541	2.9348
1.99	57.22	15.8	143.7688	39.6985	0.7836	0.2164	3.6215
1.19	54.19	12.33	227.6891	51.8067	0.8146	0.1854	4.395
2.06	10.01	10.76	24.29612	26.1165	0.4819	0.5181	0.9303
1.99	47.75	19.17	119.9749	48.1658	0.7135	0.2865	2.4909
1.99	56.11	49.33	140.9799	123.945	0.5322	0.4678	1.1374
0.99	51.84	55.6	261.8182	280.808	0.4825	0.5175	0.9324
1.38	26.05	10.22	94.38406	37.029	0.7182	0.2818	2.5489
3.99	33.18	8.25	41.57895	10.3383	0.8009	0.1991	4.0218
1.51	31.81	13.41	105.3311	44.404	0.7034	0.2966	2.3721
2.65	106.2	26.63	200.3774	50.2453	0.7995	0.2005	3.988
3.09	50.3	15.91	81.39159	25.7443	0.7597	0.2403	3.1615
1.06	52.85	16.47	249.2925	77.6887	0.7624	0.2376	3.2089
0.98	39.22	17.68	200.102	90.2041	0.6893	0.3107	2.2183

The calculated values of NLF and NHF were used as a basis for statistical analysis. Sometimes, the patients missed some of the sessions. In that case, the particular session was not used in the calculations of the session numbers. The author was unable to

analyze some of the data files due to the unavailability of a good segment of respiration waveform. Therefore, the data file for the day was valid only if analyzed data files were available for pre and post session. The values of NLF pre and post treatment were compared against the sessions. Figure 5.8 is a graph plotted to observe any trends for NLF versus session numbers. The criteria were same for NHF, NLF/NHF. Also, graphs were plotted for NHF and NLF/NHF against session numbers as shown in Figure 5.9 and Figure 5.10. Table 5.2 is the table of NLF values for patient F used for plotting Figure 5.8. Similarly Figure 5.9 and Figure 5.10 are graphs for NHF and NLF/NHF against session numbers, respectively.

Table 5.2 Sample Table for Plot Between NLF and Session Number for Patient F

SESSIONS	NLF
1	0.85234048
2	0.512209302
3	0.839791273
4	0.667793843
5	0.81780182
6	0.454597438
7	0.745855194
8	0.783620926
9	0.814642213
10	0.481945113
1	0.713538553
2	0.532150986
3	0.482501862
4	0.718224428
5	0.800868936
6	0.703449801
7	0.799518181
8	0.759703972
9	0.762406232
10	0.689279438
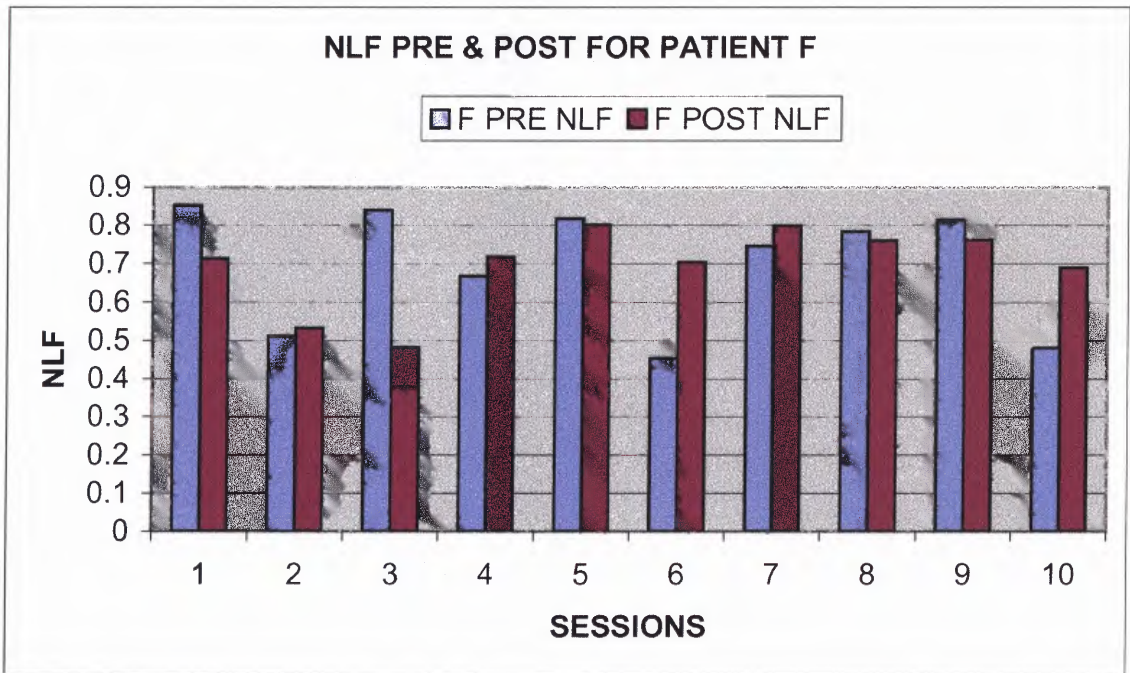
Figure 5.8 Graph showing the plot of NLF versus session number for patient F.
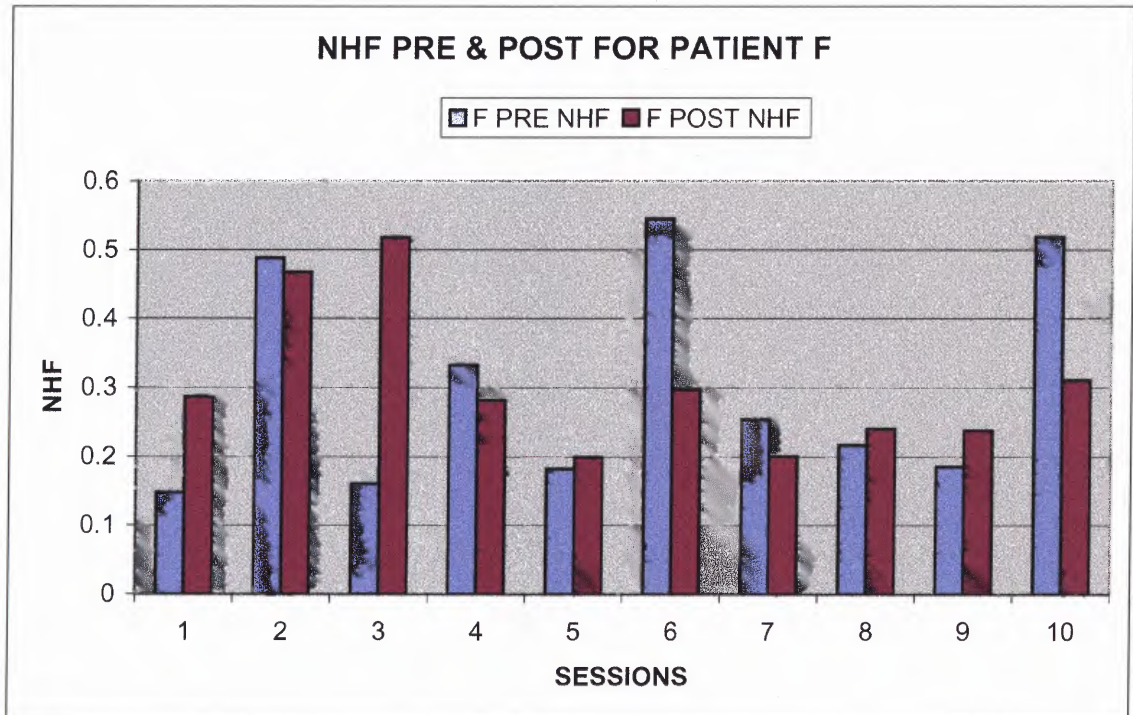
Figure 5.9 Graph showing the plot of NHF versus session numbers for patient F.
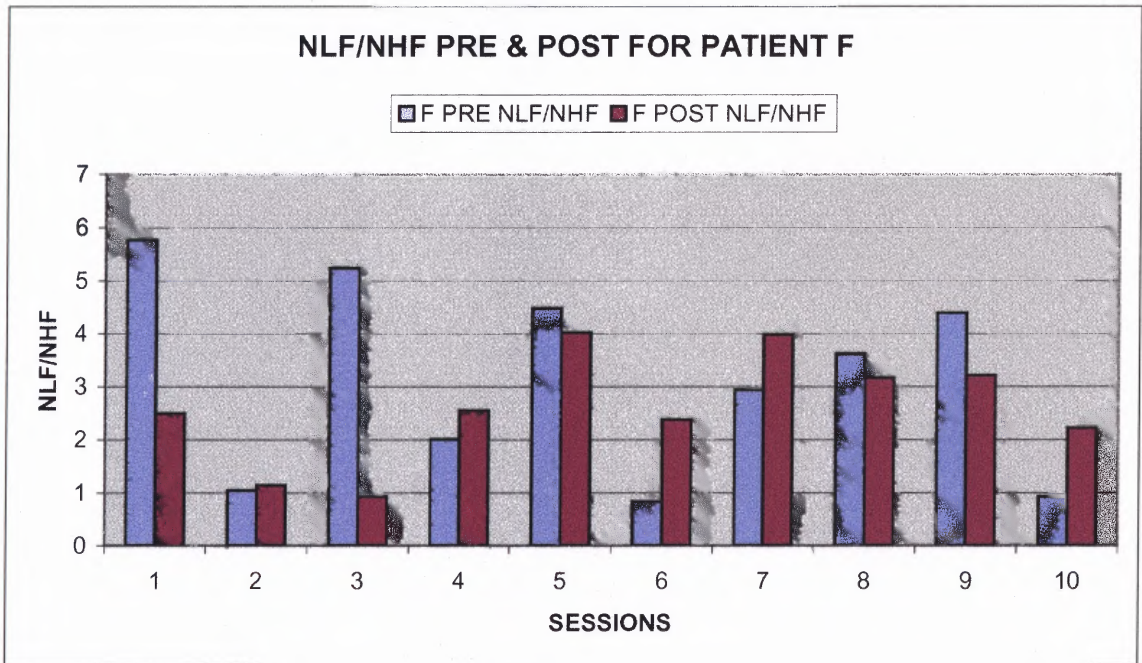
Figure 5.10 Graph showing the plot of NLF/NHF versus session numbers for patient F.

5.8 Standard Deviation of Normal to Normal Intervals

After performing the R wave peak detection in the spectral analysis of the data file in LabVIEW 5.0, the filtered file obtained was stored as an interbeat interval (IBI) file. This file was then executed through a MATLAB program to generate the standard deviation of normal-to-normal (SDNN) intervals. The algorithm for calculating the SDNN values is given below:

1. Calculate the average of the original data values (observations).

2. Subtract the average from each original data value. This is the deviation or deviation from mean.

3. Square the resulting number. This gives the set of squared deviation values. It also eliminates any negative values from the equation.

4. Sum the squared values (simply add them all together), which gives the sum of squared deviations.
5. Count the total number of observations (N) MINUS 1. It is called N-1.

6. Divide the sum of squared deviations by (N-1).

7. Take the square root of the whole thing. This value provides the standard deviation.

The program also calculated the number of sampling points. The code for the MATLAB program is given in Appendix B as program D. Table 5.3 shows the SDNN values for the patient F pre and post treatment. These values were plotted against the session numbers, the graph of which is shown in Figure 5.11.

Table 5.3 Table for Calculation of SDNN Values

SDNN	N
0.0492	25000
0.055	27500
0.2622	24000
0.1448	30000
0.0586	15000
0.0145	20000
0.0685	25000
0.0383	30000
0.0515	18000
0.0362	31000
0.0542	30000
0.0242	30000
0.0894	15000
0.0483	21000
0.0913	60000
0.3138	23000
0.0678	40000
0.069	46450
0.0798	16000
0.0193	15000
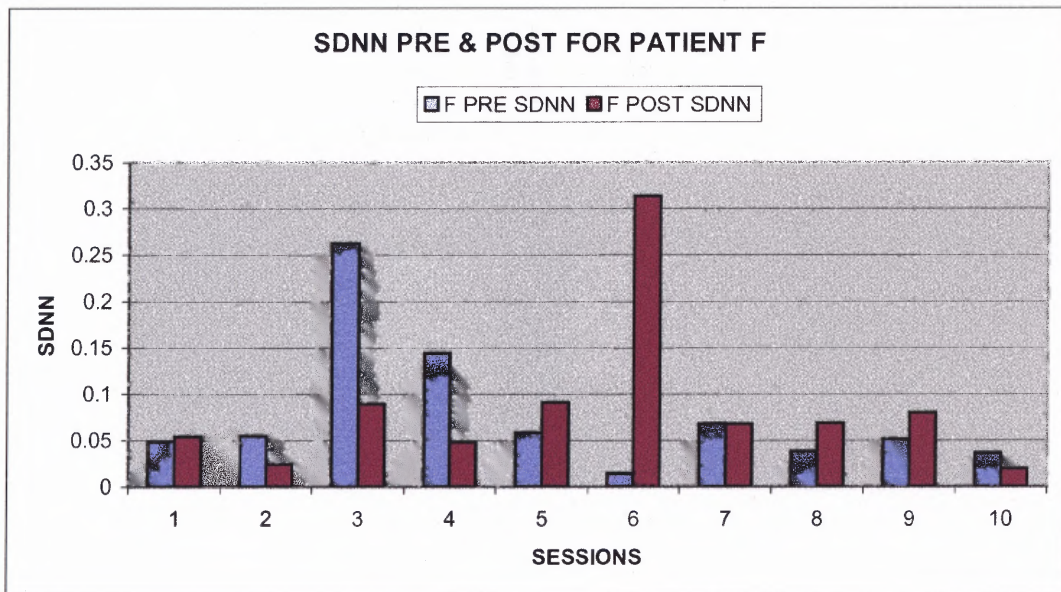
Figure 5.11 Plot for SDNN and session number for patient F.

5.9 t-test Analysis

The t-test is a parametric test of data analysis. The t test assumes that the research involves the estimation of at least one variable [31]. It involves certain assumptions about the variables, which is, that the variable is normally distributed in the overall population. It tests whether two group means are different or not. Both groups must be independent i.e. nothing in one group is related to the other group. It is also used to show difference among groups. If the groups are related, and the differences are compared, a variation of the t test, called a paired t or a correlated t test can be used. Table 5.4 and Table 5.5 show the t-test values conducted on the patient F's NLF pre and post values. For the Hypothesized Mean Difference, the test asks what is the probability of obtaining the given results by chance if there is no difference between the means. In the Table 5.4, the t stat value shows the t value calculated from the data. The p (T<=t) two-tail value shows the probability of getting the calculated t value by chance alone. That probability is

extremely low, so the means are significantly different. The t Critical two-tail shows the t value that would needed to exceed in order for the difference between the means to be significant.

Table 5.4 Table Showing the Values of T-Test using Paired Two Sample for Means

t-Test: Paired Two Sample for Means		
	Variable 1	Variable 2
Mean	0.30294024	0.303835761
Variance	0.024761219	0.011501263
Observations	10	10
Pearson Correlation	0.214398079	
Hypothesized Mean Difference	0	
Df	9	
t Stat	-0.01662188	
P(T<=t) one-tail	0.49355046	
t Critical one-tail	1.833113856	
P(T<=t) two-tail	0.987100921	
t Critical two-tail	2.262158887	

Table 5.5 Table Showing the Values of T-Test using Paired Two Sample for Means

t-Test: Two-Sample Assuming Equal Variances		
	Variable 1	Variable 2
Mean	0.30294024	0.303835761
Variance	0.024761219	0.011501263
Observations	10	10
Pooled Variance	0.018131241	
Hypothesized Mean Difference	0	
Df	18	
t Stat	-0.01487124	
P(T<=t) one-tail	0.49414925	
t Critical one-tail	1.734063062	
P(T<=t) two-tail	0.988298499	
t Critical two-tail	2.100923666	

When comparing two groups, the t-value will be positive if the first mean is larger than the second and negative if it is smaller. Once the t-value is computed, to test the significance, a risk level is set, called the alpha level. The alpha level also called the

significance level is the odds that the observed result is due to chance. In this test, the alpha level was set to 0.05. This means that five times out of a hundred we would find a statistically significant difference between the means even if there were none. A one- or two-tailed t-test is determined by whether the total area of alpha is placed in one tail or divided equally between the two tails.
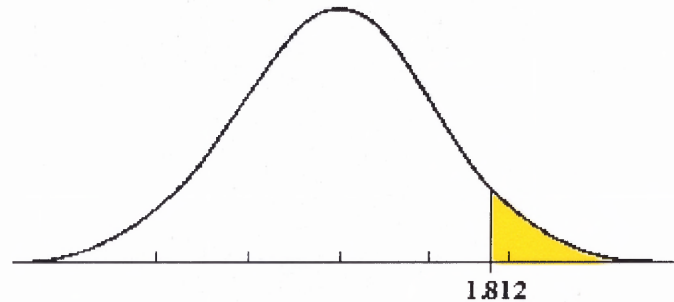
1.812

Figure 5.12 An example of a figure showing the alpha area for a one tail t test. The t-critical value is 1.812 meaning that the critical value of t when there are ten degrees of freedom (df=10) and alpha is set to .05, is $t_{crit} = \pm 1.812$.

-2.228   2.228
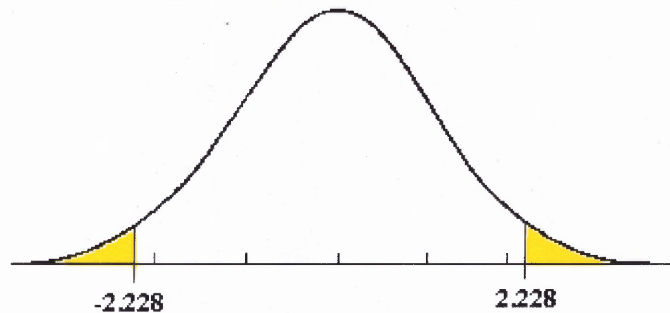
Figure 5.13 An example of a figure showing the t test value for a two tail t-test. The t-critical value is 2.228 meaning that the critical value of t when there are ten degrees of freedom (df=10) and alpha is set to .05, is $t_{crit} = \pm 2.228$.

In a one-tailed t-test, all the area associated with alpha is placed in either one tail or the other. Selection of the tail depends upon which direction the t value would be (+ or -) if the results of the experiment came out as expected. The selection of the tail is made

before the experiment is conducted and analyzed. The one-tailed t-test is performed if the results are interesting only if they turn out in a particular direction. A one-tailed t-test in the positive direction is illustrated in Figure 5.11. The two-tailed t-test is performed if the results would be interesting in either direction. A two-tailed t-test divides alpha in half, placing half in the each tail. Figure 5.12 shows the two-tail t-test.

CHAPTER 6

RESULTS AND DISCUSSION OF ANALYSIS

The analysis of the data was focused in three main categories. First, a comparison of the NLF values for pre and post session for every subject was performed, employing graphs of NLF against the sessions, and t tests. Next, a comparison of NHF values against session numbers was performed for pre and post session of all the subjects. Also, a comparison of NLF/NHF values with the session numbers was made to study the sympathovagal balance as the ratio of Low/High (LF/HF) frequency. Lastly a comparison of the SDNN values was made against the session numbers. This chapter will detail the results of the analyses performed in this research and provide a discussion of these findings in comparison to normals and controls.

As discussed in Chapter 5, a power spectral analysis was performed on the ECG data recordings. For this study, the blood pressure data was not used. The blood pressure data can be used to study the blood pressure variability as a part of future research. The tables for the calculations and graphs of all the patients, normals and controls are given in Appendix C, D and E, respectively.

6.1 Results of the NLF Comparison

Table 6.1 shows the t-test analysis conducted on the NLF values of all the patients. Table 6.2 is the table of t-test values for the normals while Table 6.3 is the t-test values for the controls. These t-test values are paired since the two sets of data – NLF pre and NLF post, are independent of each other.

Table 6.1 Table Showing the T Test Values for NLF of All the Patients

t-Test: Paired Two Sample for Means

	A	B	C	D	E	F	G
	Var 1	Var 1	Var 1	Var 1	Var 1	Var 1	Var 1
Mean	0.4870185	0.375743	0.432253	0.436488	0.332407	0.69706	0.5125
Variance	0.0336099	0.046938	0.066499	0.028706	0.048784	0.024761	0.0313
Observations	7	20	10	4	24	10	8
Pearson Correlation	-0.854497	0.217395	-0.00164	-0.54678	0.001655	0.214398	-0.0717
Hypothesized Mean Difference	0	0	0	0	0	0	0
Df	6	19	9	3	23	9	7
t Stat	-0.037166	0.682782	-2.05249	-0.76439	-0.3823	0.016622	1.7430
P(T<=t) one-tail	0.4857792	0.251493	0.035165	0.250129	0.352875	0.49355	0.0624
t Critical one-tail	1.9431809	1.729131	1.833114	2.353363	1.71387	1.833114	1.8945
P(T<=t) two-tail	0.9715583	0.502986	0.07033	0.500258	0.705751	0.987101	0.1248
t Critical two-tail	2.4469136	2.093025	2.262159	3.182449	2.068655	2.262159	2.3646

Table 6.2 Table Shows the T Test Paired Two Sample for Means for the Normals

	H	I	J
NLF for Normals			
t-Test: Paired Two Sample for Means			
	H	I	J
	0.696228	0.307421	0.082664
Mean	0.752899	0.213943	0.264754
Variance	0.001008	0.014357	0.008905
Observations	2	2	2
Pearson Correlation	-1	1	-1
Hypothesized Mean Difference	0	0	0
Df	1	1	1
t Stat	-1.12021	-1.14473	-0.25853
P(T<=t) one-tail	0.231972	0.228552	0.419469
t Critical one-tail	6.313749	6.313749	6.313749
P(T<=t) two-tail	0.463945	0.457104	0.838939
t Critical two-tail	12.70615	12.70615	12.70615

Table 6.3 Table Shows the T Test Paired Two Sample for Means for the Controls

NLF		
t-Test: Paired Two Sample for Means		
	0.312846448	*0.229084264*
Mean	0.272361832	0.348926312
Variance	0.009231194	0.012749337
Observations	4	4
Pearson Correlation	0.702915833	
Hypothesized Mean Difference	0	
Df	3	
T Stat	-1.866696428	
P(T<=t) one-tail	0.079386141	
t Critical one-tail	2.353363016	
P(T<=t) two-tail	0.158772283	
t Critical two-tail	3.182449291	

Comparing the NLF values of the normals and controls with the patients, no significance was observed from the t-test analysis to prove that EECP treatment affected the HRV for the normalized low frequency. A variety of studies have demonstrated that acute interventions that increase sympathetic nervous system activity, such as orthostatic perturbations, mental stress, or handgrip exercise increases the LF spectral power of heart rate [31]. In addition to acute perturbations of cardiac sympathetic nerve activity, feedback oscillations generated by the baroreceptor reflex also appear to contribute to LF spectral power of heart rate.

6.2 Results of the NHF Comparison

In an effort to quantify the changes that are observable via visible analysis for purposes of graphical observation, the NHF data were plotted for pre and post session against the session numbers. Figures 6.1 and 6.2 are the NHF versus session number plots for patients B and D, respectively. Sessions from day 9 through day 14 in Figure 6.1 are

missing. The reason was that the author was unable to analyze some of the data files due to the unavailability of a good segment of respiration waveform. Therefore, the data file for the day was valid only if analyzed data files were available for pre and post session. The total numbers of sessions were the sessions that the patients actually attended. That was the reason why patient D had 24 sessions instead of the 35 sessions.
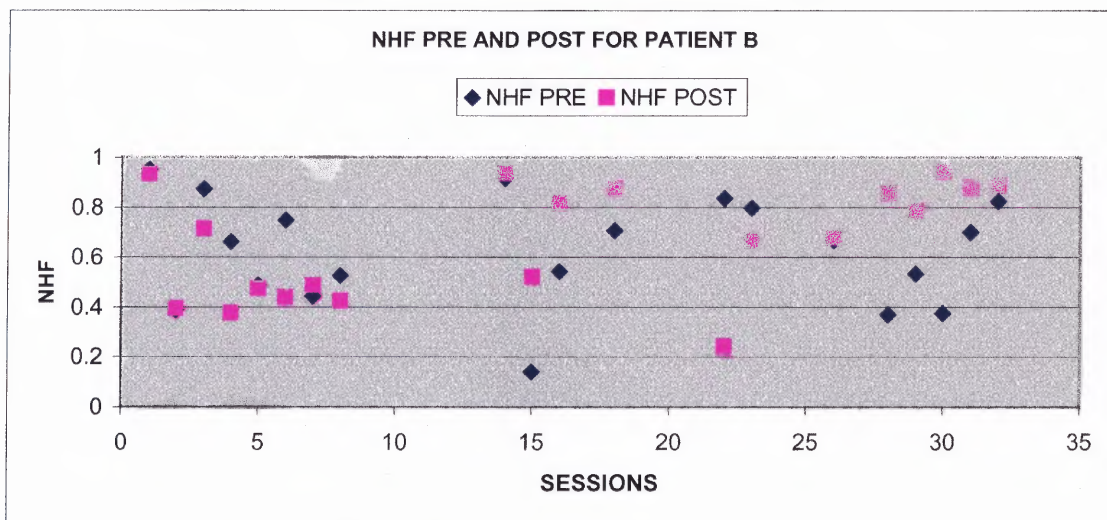

Figure 6.1 Graph showing the comparison of NHF values for pre and post session for heart failure patient B.
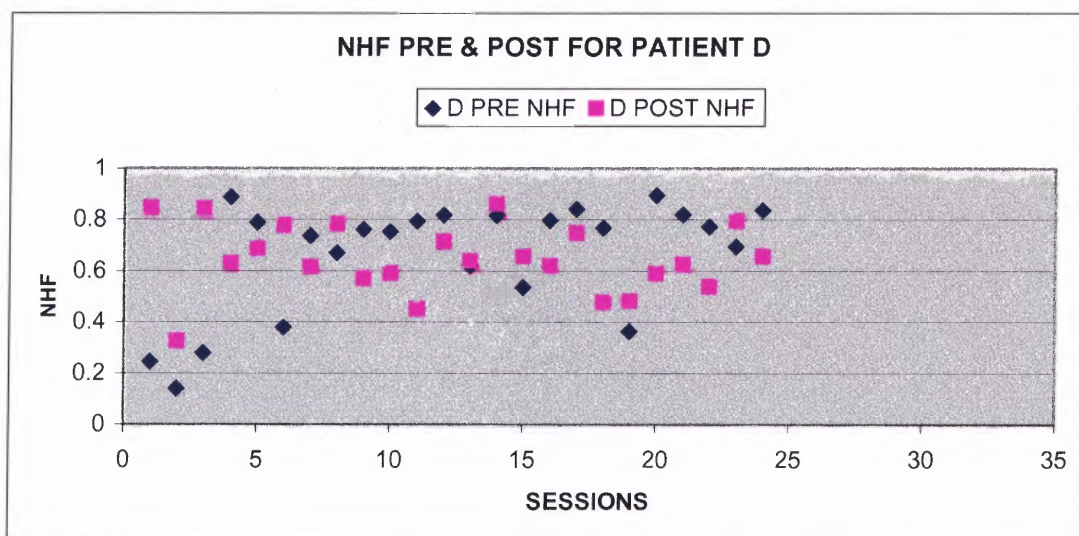

Figure 6.2 Graph showing the NHF values for patient D.

From the plot of NHF against session numbers for pre and post treatment values for heart failure patient B (Figure 6.1), it is observed that the NHF values for post treatment increase in the third to the fifth week period. This might be an indicator that the maximum effect of the treatment happens in the third to fifth week period of the EECP treatment. From then onwards, the post sessions normalized high frequency show no marked increase. This trend was not observed for any of the other patients. Figure 6.2 shows the plot of patient D against the session numbers. No particular trend was observed from the plot to substantiate the trend observed in the heart failure patient. Patients B and D were the subjects who attended the maximum number of EECP treatment sessions for the author to observe any trends. Patient B attended 33 sessions while patient D attended 24 sessions. Since the author was looking at 20 or more session numbers to observe any trends, the database of the remaining patients A, C, E, F and G were not significant enough to observe any trends. The plots showing the variation of NLF, NHF and NLF/NHF against the session numbers for all the patients have been shown in Appendix C.

Tables 6.4, 6.5 and 6.6 show the values of t-test for two-paired sample for means for patients, normals and controls, respectively. In the absence of significance observed from the t-test values, the author is unable to propose statistically that the EECP treatment affects the heart rate variability in a comprehensive way.

Table 6.4 Table Showing the T Test Values for NHF of All the Patients

NHF for Patients   **t-Test: Paired Two Sample for Means**

	A	B	C	D	E	F	G
	Var 1	Var 1	Var 1	Var 1	Var 1	Var 1	Var 1
Mean	0.5129815	0.624257	0.567747	0.563512	0.667593	0.30294	0.487402
Variance	0.0336099	0.046938	0.066499	0.028706	0.048784	0.024761	0.031358
Observations	7	20	10	4	24	10	8
Pearson Correlation	-0.854497	0.217395	-0.00164	-0.54678	0.001655	0.214398	-0.07177
Hypothesized Mean Difference	0	0	0	0	0	0	0
Df	6	19	9	3	23	9	7
t Stat	0.037166	-0.68278	2.052486	0.764391	0.382297	-0.01662	-1.74305
P(T<=t) one-tail	0.4857792	0.251493	0.035165	0.250129	0.352875	0.49355	0.062426
T Critical one-tail	1.9431809	1.729131	1.833114	2.353363	1.71387	1.833114	1.894578
P(T<=t) two-tail	0.9715583	0.502986	0.07033	0.500258	0.705751	0.987101	0.124852
T Critical two-tail	2.4469136	2.093025	2.262159	3.182449	2.068655	2.262159	2.364623

Table 6.5 Table Showing the T Test Values for NHF of All the Normals

NHF for Normals   **t-Test: Paired Two Sample for Means**

	H	I	J
	0.303772	0.692579	0.082664
Mean	0.247101	0.786057	0.264754
Variance	0.001008	0.014357	0.008905
Observations	2	2	2
Pearson Correlation	-1	1	-1
Hypothesized Mean Difference	0	0	0
Df	1	1	1
t Stat	1.120207	1.144733	-0.25853
P(T<=t) one-tail	0.231972	0.228552	0.419469
t Critical one-tail	6.313749	6.313749	6.313749
P(T<=t) two-tail	0.463945	0.457104	0.838939
t Critical two-tail	12.70615	12.70615	12.70615

Table 6.6 Table Showing the T Test Values for NHF of All the Controls

NHF for Controls   t-Test: Paired Two Sample for Means

t-Test: Paired Two Sample for Means		
	0.687153552	*0.770915736*
Mean	0.727638168	0.651073688
Variance	0.009231194	0.012749337
Observations	4	4
Pearson Correlation	0.702915833	
Hypothesized Mean Difference	0	
Df	3	
t Stat	1.866696428	
P(T<=t) one-tail	0.079386141	
t Critical one-tail	2.353363016	
P(T<=t) two-tail	0.158772283	
t Critical two-tail	3.182449291	

6.3 Results of the NLF/NHF Comparison

The LF/HF ratio increases in situations where sympathetic activity is known to be increasing. Tables 6.7, 6.8 and 6.9 show the t-test values of NHF values pre and post treatment for patients, normals and control, respectively. Again, the lack of significance in the t-test values fails to assess any changes in the heart rate variability.

Table 6.7 Table Showing the T Test Values for NLF/NHF of All the Patients

NLF/NHF for Patients   t-Test: Paired Two Sample for Means

	A	B	C	D	E	F	G
	Var 1	*Var 1*	*Var 1*	*Var 1*	*Var 1*	*Var 1*	*Var 1*
Mean	1.2145749	0.956085	1.135489	0.901228	0.887595	3.127802	1.51054
Variance	0.8117888	1.747229	0.931554	0.328409	1.902891	3.428891	2.459457
Observations	7	20	10	4	24	10	8
Pearson	-0.855714	0.050169	0.135194	-0.63168	0.473069	0.19349	-0.12388
H M D	0	0	0	0	0	0	0
Df	6	19	9	3	23	9	7
t Stat	0.0919811	0.666377	-2.35757	-0.668	1.040916	0.846817	1.496847
P(T<=t) one T	0.4648536	0.256591	0.021386	0.275964	0.154367	0.209522	0.089048
t Critical one T	1.9431809	1.729131	1.833114	2.353363	1.71387	1.833114	1.894578
P(T<=t) two-tail	0.9297072	0.513181	0.042771	0.551929	0.308734	0.419044	0.178096
t Critical two T	2.4469136	2.093025	2.262159	3.182449	2.068655	2.262159	2.364623

Table 6.8 Table showing the T Test Values for NLF/NHF of All the Normals

NLF/NHF for Normals   **t-Test: Paired Two Sample for Means**

	H	I	J
	2.291939	0.443879	0.917336
Mean	3.080606	0.287126	0.735246
Variance	0.274793	0.038494	0.008905
Observations	2	2	2
Pearson Correlation	-1	1	-1
Hypothesized Mean Difference	0	0	0
Df	1	1	1
T Stat	-1.04615	-1.04608	0.258534
P(T<=t) one-tail	0.242823	0.242833	0.419469
t Critical one-tail	6.313749	6.313749	6.313749
P(T<=t) two-tail	0.485645	0.485665	0.838939
t Critical two-tail	12.70615	12.70615	12.70615

Table 6.9 Table Showing the T Test Values for NLF/NHF of all the Controls

NLF/NHF for Controls   **t-Test: Paired Two Sample for Means**

	Variable 1	Variable 2
Mean	0.405015276	0.516762968
Variance	0.026240047	0.07289641
Observations	5	5
Pearson Correlation	0.404725219	
Hypothesized Mean Difference	0	
Df	4	
t Stat	-0.989773489	
P(T<=t) one-tail	0.189156972	
t Critical one-tail	2.131846486	
P(T<=t) two-tail	0.378313944	
t Critical two-tail	2.776450856	

6.4 Results of the SDNN Comparison

In order to observe the SDNN values graphically to compare it against each session, the pre and post values were plotted against the session numbers. Figure 6.3 shows the graph. From all the graphs of all the patients, controls and normals; no particular trend was observed.
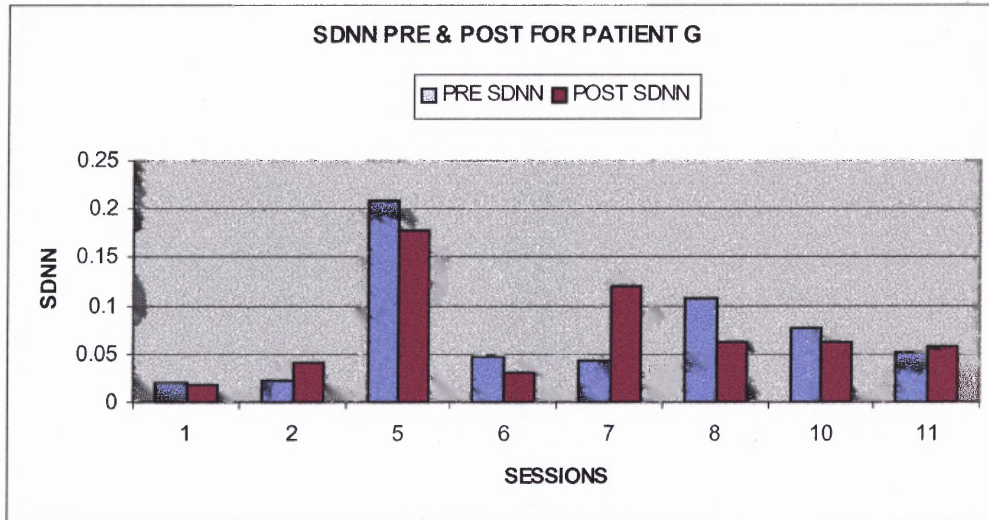

Figure 6.3 Graph showing the variation of SDNN values against the session numbers for patient G.

6.5 Discussion and Conclusions

An ideal method for clinical work would be a single HR variability index that could be calculated reliably on the basis of a simple, widely available analytical method. At the moment, there is no consensus about the best available index of HR variability for clinical use, despite the efforts of the Task Force of the North American Society of Pacing and Electrophysiology and the European Society of Cardiology to unify and standardize the methodology [32]. Noisy data, artifacts, trends and ectopic beats are the major practical problems encountered in HR variability measurements. In this research, a minimum segment of one minute was used, with the respiration waveform as the criterion

to obtain an acceptable length of file. The criterion for an acceptable segment was a relatively sinusoidal or periodic respiration waveform (refer section 5.6.1, page 35). Simple statistics of heart rate variability, such as the standard deviation of R-R intervals in the ECG, can reliably predict the prognosis of cardiovascular diseases [33].

Sympathetic outflow is increased in case of heart failure [34]. Congestive Heart Failure (CHF) is usually treated with medication. If medication is ineffective or the risk of heart attack is high, doctors may usually recommend Coronary artery bypass surgery. In cases where the medication is not effective or the bypass surgery failed, the patients are prescribed the EECP treatment by their physicians. The major advantages of EECP include the non-invasive nature of this therapy, the overwhelming rate of success and lack of any significant complications associated with it. An increase in high frequency post treatment is an indicator of increase in vagal activity and HRV.

Due to the lack of significance observed from the t-tests, the author failed to prove that the EECP treatment affected the HRV. One of the possible factors that caused the HRV test to fail was the fact that subjects were already in the normal range. If the five minutes normalized LF and HF values were greater that 250, then the subject was assumed to be in the normal range. Table 6.10 shows the values of LF and HF for patient C who was in the normal range. In that case, an increase in variability would not be expected. Subjects A, B, C, G, I and J were already in the normal range before the study began, hence any trend or significance was not observed.

Table 6.10 Table Showing the 5 Minute Normalized LF and HF Values for Patient E

5LF	5HF
680.8119658	2158.418803
1971.161616	1223.838384
1066.196581	1390.512821
34.59150327	117.1895425
124.2084942	201.7181467
43.43691149	94.00188324
113349.7407	27921.7037
3354.101307	3096.062092
86.93396226	426.745283
1213.14433	2004.536082
4028.535354	2413.787879
1550.204082	1348.826531
8541.686747	4754.834337
1471.36	1414.32
631.5189873	584.5886076
1229.619048	916.5238095
3977.525773	4069.226804

Over the period of the treatment, an increased HR variation was noticed for heart failure patient B. A marked increase in the NHF was observed between weeks three and five of the treatment. The remaining two weeks in the treatment, weeks six and seven, showed no marked increase in value. As evident from Fig 6.1,which displays an example of one subject data, the post NHF starts increasing around the third week till the fifth week suggesting that there may be a shift in pre post ratios of NHF starting in week 5. This was observed in the non-ischemic heart failure patient only. Future research would help to follow-up this initial finding.

Since the controls rested for an hour before the post readings were collected, there should not have been much variation between the pre and post LF and HF. For controls 1, 3 and 4 (Figure 6.4), drastic HR variation was observed. When collecting data from

subjects, data was collected around the same time of the day. Other factors like age, sex, position, breathing, smoking and medications which influence HRV were standardized. Breathing was standardized by each subject following a paced breathing protocol at 12 breaths per minute. Mental stress could be one of the possible causes for the HRV variation. If the controls became very relaxed over the period of data collection, then the HF value would increase, which could be another causes for the HRV variation. This can be verified with a longer database.
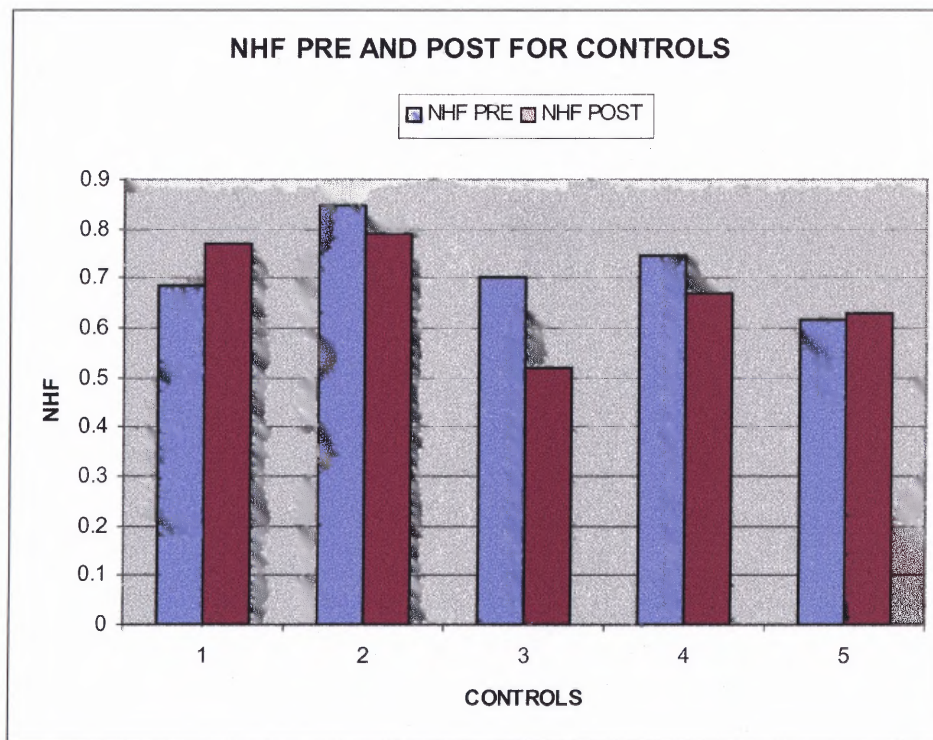

Figure 6.4 Plot showing the NHF pre and post data collection for controls.

The reliability of the heart rate variability study depends on a number of factors. One set of variables is the physiological condition of the subject, such as, the diseased state of the subject, type of disease, age, gender, medication or the treatment that the subject is undergoing. The other set of variables include the technical aspects of data

collection, such as, the position of the subject at the time that the data was collected, caffeine, food and nicotine intake, the length for which the data was collected etc. Hence, the reliability of heart rate variability depends on a number of variables. Once these variables have been eliminated, with the help of different analysis tools like non-linear method of analysis, wavelets on a substantial database, the heart rate variability and its reliabilty can be measured.

CHAPTER 7

SUGGESTIONS FOR FUTURE RESEARCH

7.1 Suggestions for Future Work

As is often the case, this clinical study raises more questions than it is capable of answering. In this research, the number of patients who underwent the EECP treatment was seven. With a more extensive database, EECP could probably prove conclusively whether it affects HRV substantially or not. Also, the number of normals was only three, so it could not be concretely used as a comparison basis between the patients and normals. It is highly recommended to have an extensive database for analysis of the effect on heart rate variability by the EECP treatment, for the data pool was not as large as that required of a medical conclusion. Though the author did not come to any novel conclusions about the underlying data or the spectral and time-domain analyses, the author is confident that additional research in this area would prove useful and medically important.

Studying the increase in NHF over the period of 5-6 weeks for the heart failure patient ES, with more detailed study, it can possibly be verified conclusively that EECP treatment increases NHF within six weeks.

Currently, the primary indication for EECP treatment is chronic stable angina. With more in depth research, maybe decreased HRV in people with heart conditions could be used a marker that the EECP treatment may be used as a potential treatment modality for heart failure patients, in whom invasive revascularization procedures do not offer a survival. The EECP treatment may be particularly useful in patients considered

high risk for revascularization procedures or in whom revascularization is not technically possible.

The literature suggests that the variation and fluctuation of the heart rate in relation to the mean heart rate is a function of cardiorespiratory physiology [34]. It has been known for several decades that enhanced vagal tone has a salutary effect on the electrophysiologic properties of the ventricle to prevent the emergence of life-threatening ventricular arrhythmias in certain situations, particularly in post infarction and diabetic patients. Hence, heart rate variability may offer information about sympathetic and parasympathetic autonomic function and could serve as a measure of risk stratification for serious cardiac arrhythmias and possible sudden cardiac death. Notably, continuous changes in the sympathetic-parasympathetic balance can be reflected in variations of the sinus rhythm that oscillate around the mean heart rate.

Future work should include a more thorough examination of the data with more analysis tools like wavelets, time frequency methods and non-linear methods. Since the heart rate variability research lab at NJIT has excellent software tools in MATLAB, LabVIEW and Wavelets, an extensive data pool would allow for a detailed study and reach a fruitful conclusion.

A difficulty in analyzing data of this nature is that the patient tends to move or shift while the data is collected. Even a slight movement can affect the data recorded. To this effect, it is recommended that a marker system be developed to indicate in the data files when events, such as coughing, shifting, moving hand etc occur during the experiment. This would allow the researcher to properly identify motion artifact in the data. This may be implemented using a button push marker system, with specific codes

inserted into the data for various activities. A camera system may also be implemented effectively to coordinate movements with data recordings.

7.2 Unanswered Questions

Two major questions concerning heart rate variability remain to be clarified. First, many methods to measure heart rate variability have been reported, and it is very difficult to conclude which one is most appropriate for establishing normal values and for particular patient subgroups. There is a need to standardize the measurement of heart rate variability and to quantify normal values under various circumstances, including patient age and gender. Second, the sensitivity, specificity and predictive accuracy of this test require much more prospective investigation. Correlations of this test with other risk stratification measurements will be necessary to evaluate its independent predictive value. The preliminary data available from this research does not allow for definitive conclusions.

Furthermore, the value of heart rate variability or any other risk stratification test in patients who undergo revascularization of infarcting myocardium requires assessment. It appears that mortality after myocardial infarction is decreasing with the new and aggressive therapeutic methods being used, and heart rate variability will have to be evaluated in this setting. Similar evaluations need to be performed in other pathophysiologic states such as congestive heart failure.

APPENDIX A

SAMPLE DATA FILE

A sample data file of patient ES is given in this appendix. The first column represents channel 0 that is, respiration. The second column shows channel 1 which is ECG, while the third column shows channel 2, which is blood pressure. In some cases, during R wave detection the r peaks were inverted, which meant that the ECG values were negative in value, which was an indication that the ECG leads were switched. To counter this, the program to analyze the ECG signal was multiplied by a factor of –1.

0.947	-0.042	0.918
0.962	-0.061	0.918
0.945	-0.012	0.906
0.950	-0.027	0.911
0.967	-0.071	0.913
0.947	-0.139	0.901
0.955	-0.137	0.901
0.967	-0.076	0.891
0.957	-0.012	0.889
0.994	-0.022	0.884
0.994	-0.044	0.881
0.906	-0.090	0.879

0.901	-0.090	0.876
0.964	-0.051	0.889
0.950	-0.042	0.867
0.952	-0.046	0.833
0.969	-0.071	0.850
0.952	-0.098	0.842
0.955	-0.105	0.840
0.969	-0.095	0.835
0.959	-0.059	0.828
0.959	-0.105	0.823
0.974	-0.078	0.818
0.959	-0.088	0.818
0.967	-0.090	0.813
1.033	-0.076	0.813
0.994	-0.044	0.806
0.913	-0.049	0.803
0.920	-0.068	0.796
0.959	-0.032	0.813
0.959	-0.078	0.796
0.977	-0.066	0.771
0.964	-0.095	0.776
0.964	-0.107	0.779

APPENDIX B

PROGRAMS FOR THE STUDY

Three LabVIEW programs were used in the study to analyze the data. LabVIEW has two panels in a program- Front panel, where the waveforms are generated and a back panel where the graphical programming is executed. A Matlab program was used to calculate the standard deviation of normal-to-normal intervals.

Program A was used to identify the three channels of respiration, ECG and blood pressure. This program was also helpful to execute the original data file and obtain an extracted data file by using a relatively periodic respiration waveform as criteria to extract the data. Inputs to the program were the sampling rate and the name of the file to be executed. Once the segment of the file was identified, it was extracted with the help of program B. The inputs to the program were the start and end points of the segment of the data file. The program then wrote to another spreadsheet file, which was then used for spectral analysis. Then, Program C was used to obtain the values of LF and HF power as well time of record. It also plotted the respiration and heart rate spectrum.

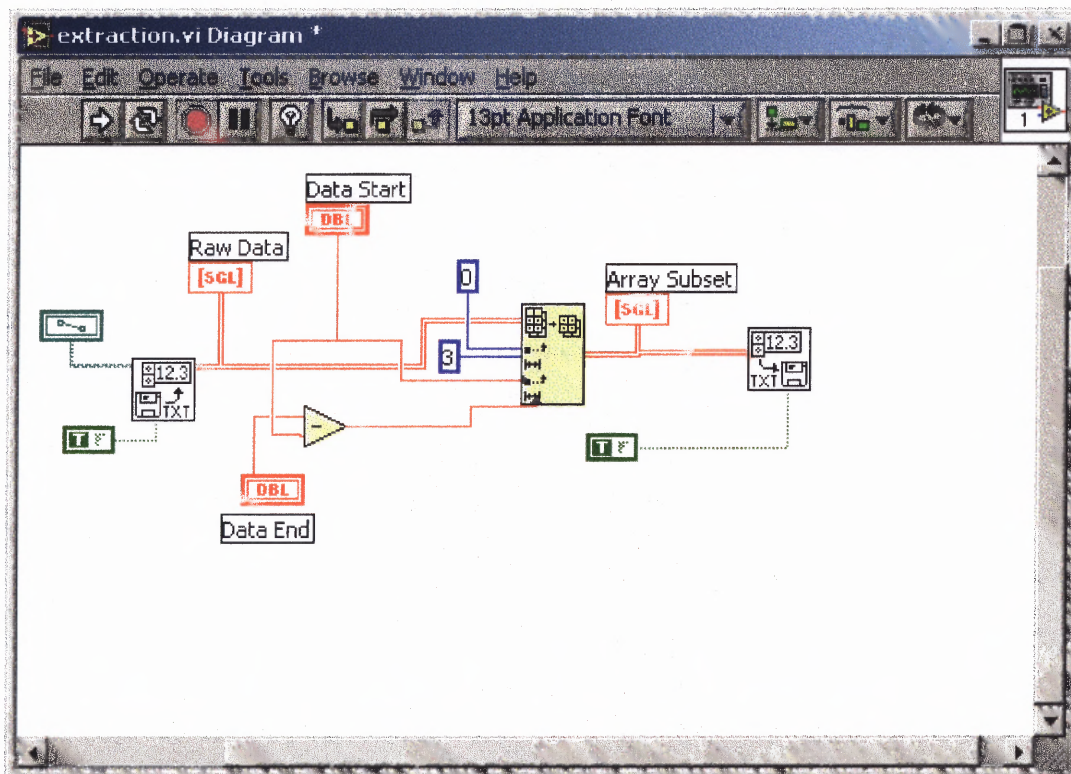**Program A –** To identify the channels.
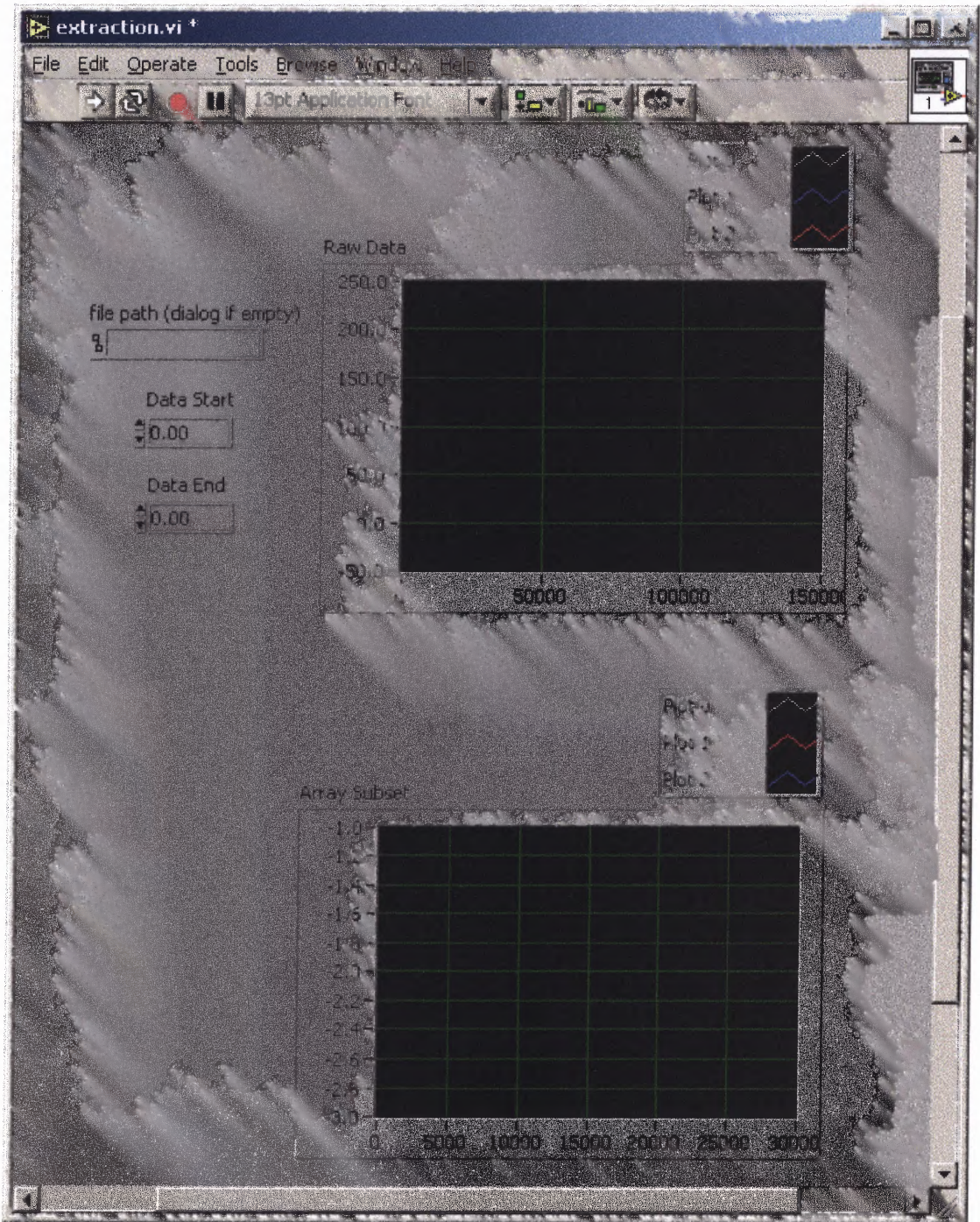
Main Diagram:
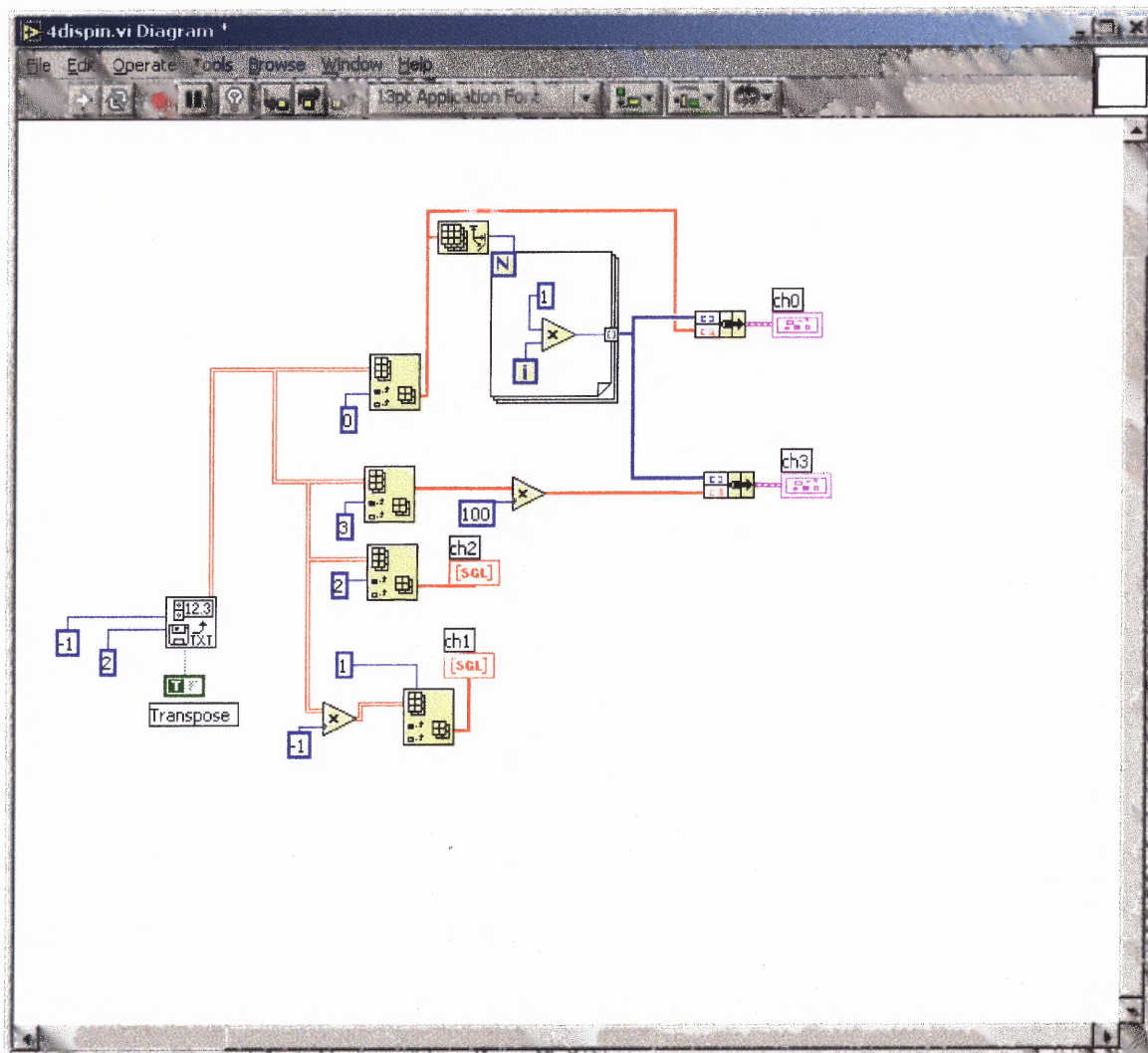
Front Panel:
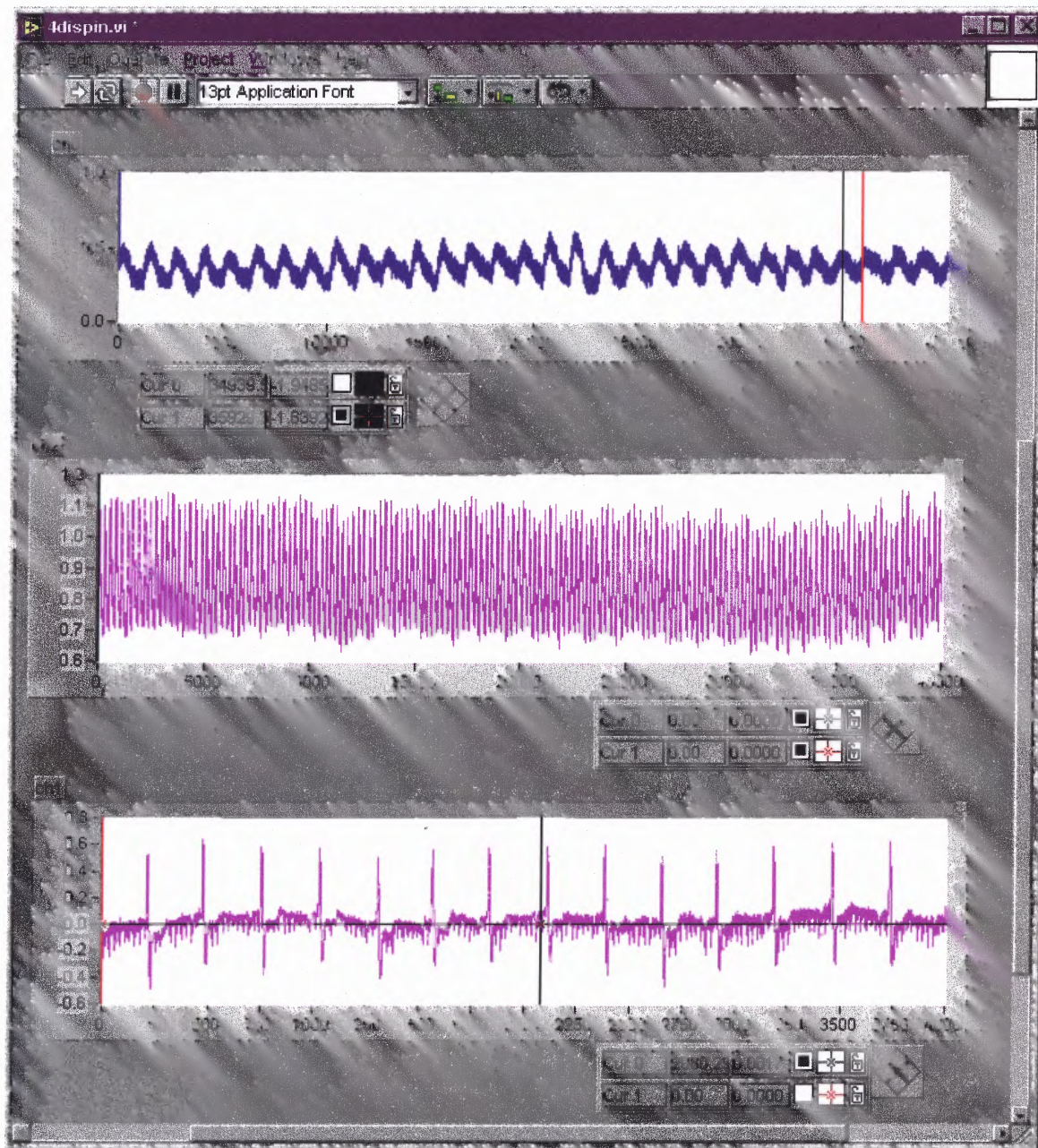
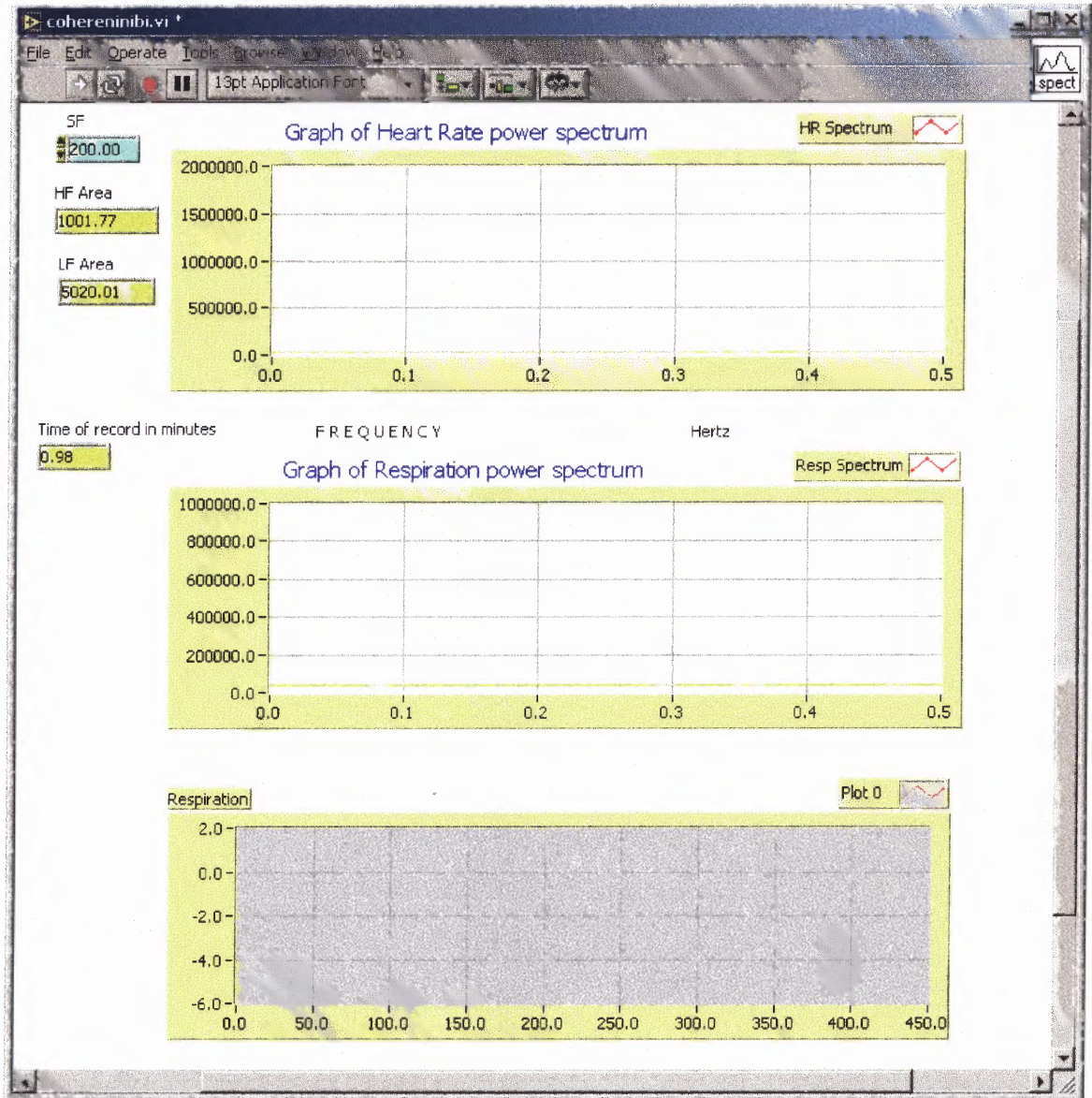**Program B** – To extract the data for the spectral analysis.

Main Diagram:

Front Panel:

Program C – To calculate the LF and HF power.

Main Diagram:

Front Panel:

Program D- Matlab Code for calculating SDNN values.

****

```matlab
% Ask the user to select the signal that is to be analyzed.
[fname,pname]=uigetfile('*.*','Please select the IBI file that will be analyzed with
SDNN:');
```
****
```matlab
if isstr(fname) == 0
        disp(' Cannot find file')
        dbquit
 end

Filename = [pname fname];
load(Filename) ;             % load file
fname = strtok(fname,'.');   % drop extension
k=eval(fname);                      % evaluate fname
k = transpose(k);
signal=k(1:length(k));

y = detrend(signal,'constant');

z = y.^2;

n=length(z)

s=sum(z);

varnn=s/(n-1);

SDNN=varnn^0.5
```

****

APPENDIX C

PATIENT DATA ANALYZED IN THE RESEARCH

This Appendix contains the data table and plots for patients A, B, C, D, E, F and G. The tables contain the values of NLF, NHF, NLF/NHF and SDNN for pre and post session of each patient. The sessions that had the data for pre and post treatment for the particular day has been included. The plots show a comparison of pre and post session values for each patient for NLF, NHF, NLF/NHF and SDNN.
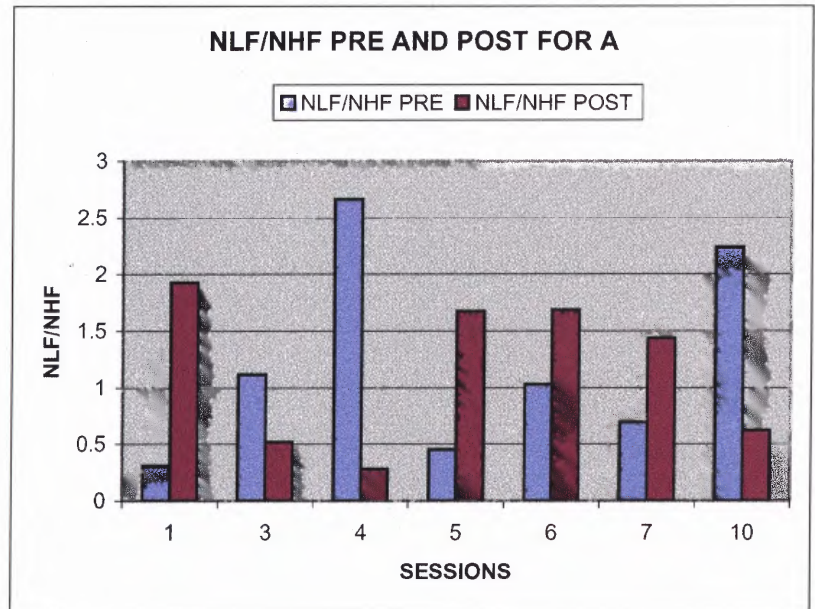
PATIENT A

NLF	
PRE	POST
0.235440296	0.658139203
0.527188013	0.34098604
0.727054244	0.217827168
0.310683814	0.625790951
0.507078926	0.627337781
0.41031842	0.589259853
0.691365557	0.383683982

NLF PRE AND POST FOR PATIENT A
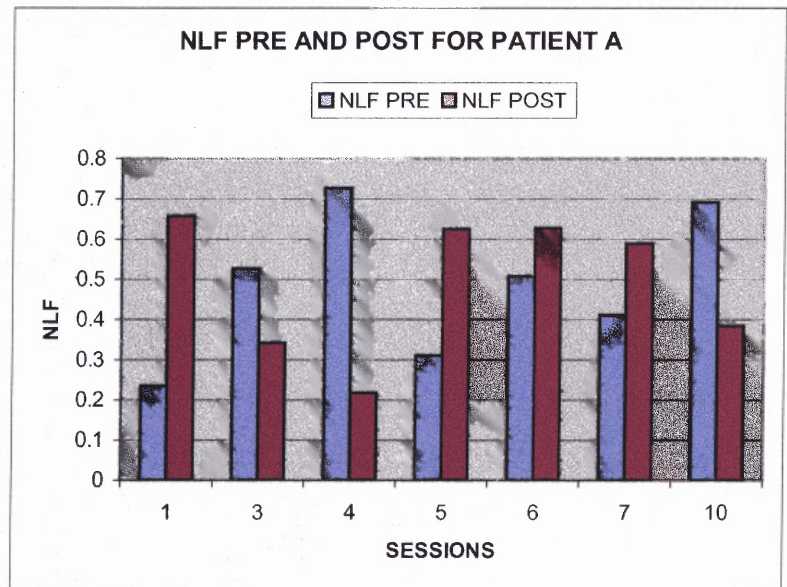
NHF	
PRE	POST
0.764559704	0.341860797
0.472811987	0.65901396
0.272945756	0.782172832
0.689316186	0.374209049
0.492921074	0.372662219
0.58968158	0.410740147
0.308634443	0.616316018
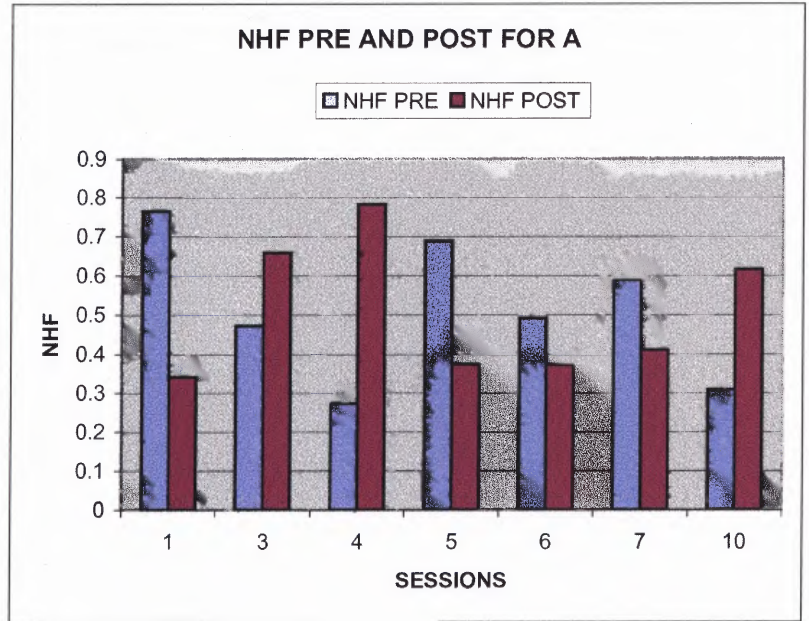
NLF/NHF	
PRE	POST
0.307942329	1.925167224
1.115005599	0.517418538
2.663731638	0.278489816
0.450713068	1.672303098
1.028722351	1.683395176
0.695830485	1.434629311
2.240079075	0.622544231

SDNN	
PRE	POST
0.0343	0.0921
0.1059	0.1576
0.027	0.0886
0.036	0.0801
0.0292	0.0687
0.0232	0.0087
0.0193	0.0198

SDNN PRE AND POST FOR A
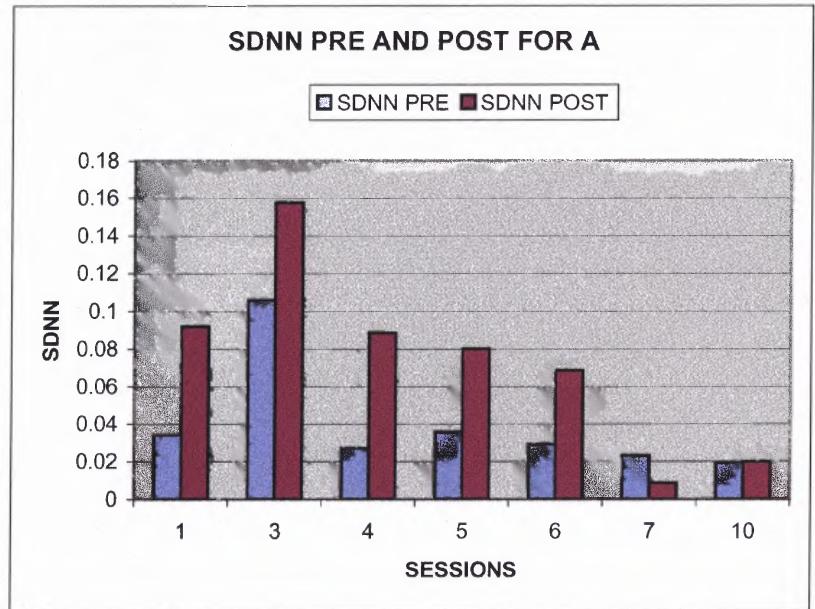
PATIENT B

NLF	
PRE	POST
0.048646966	0.066492403
0.612134444	0.604736492
0.126096797	0.28704762
0.337716529	0.622992789
0.510827537	0.524937296
0.252701475	0.560996635
0.556582147	0.513865381
0.474445499	0.575163863
0.085108545	0.064621692
0.859062936	0.478444883
0.457361958	0.183144044
0.292953603	0.124073134
0.163750644	0.756391743
0.203049836	0.33304782
0.333985379	0.323878763
0.630960192	0.142952194
0.46727918	0.215952616
0.62543554	0.056863569
0.300281182	0.121730097
0.176478536	0.111957747

NLF PRE AND POST FOR B
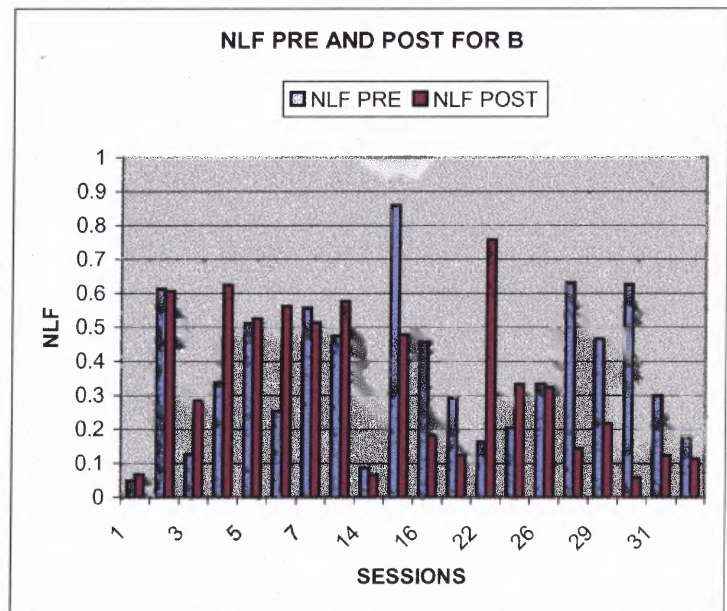
NHF	
PRE	**POST**
0.951353034	0.933507597
0.387865556	0.395263508
0.873903203	0.71295238
0.662283471	0.377007211
0.489172463	0.475062704
0.747298525	0.439003365
0.443417853	0.486134619
0.525554501	0.424836137
0.914891455	0.935378308
0.140937064	0.521555117
0.542638042	0.816855956
0.707046397	0.875926866
0.836249356	0.243608257
0.796950164	0.66695218
0.666014621	0.676121237
0.369039808	0.857047806
0.53272082	0.784047384
0.37456446	0.943136431
0.699718818	0.878269903
0.823521464	0.888042253

NHF PRE AND POST FOR B
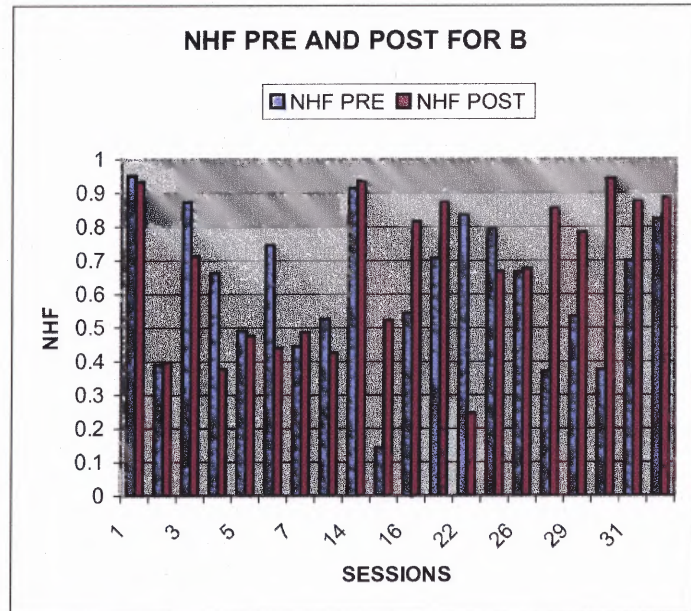
NLF/NHF	
PRE	**POST**
0.051134505	0.071228561
1.578212955	1.529957815
0.144291492	0.402618223
0.509927462	1.652469162
1.044268791	1.104985281
0.338153317	1.277886869
1.25520915	1.057043379
0.902752233	1.353848724
0.093025839	0.069086157
6.095365611	0.9173429
0.84284905	0.224206046
0.414334342	0.141647823
0.19581557	3.104951177
0.254783606	0.499357869
0.501468538	0.479024686
1.709734772	0.166796056
0.877155843	0.275433118
1.669767442	0.060291987
0.4291455	0.138602151
0.214297433	0.126072545

NLF/NHF PRE AND POST FOR B
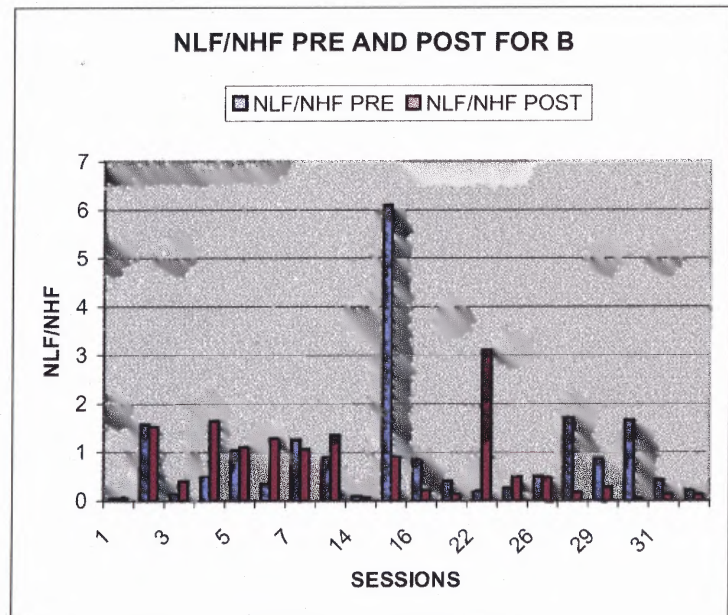
SDNN	
PRE	**POST**
0.1377	0.0599
0.1045	0.1767
0.0759	0.0721
0.2826	0.2826
0.9287	0.1397
0.0506	0.0506
0.0711	0.0455
0.0261	0.0743
0.0688	0.0661
0.055	0.0306
0.053	0.0539
0.0296	0.048
0.237	0.1446
0.0564	15.609
0.0558	15.37
0.0154	0.0589
0.064	0.2402
0.0223	0.0602
0.0196	0.0351
0.0379	0.0255

SDNN PRE AND POST FOR B
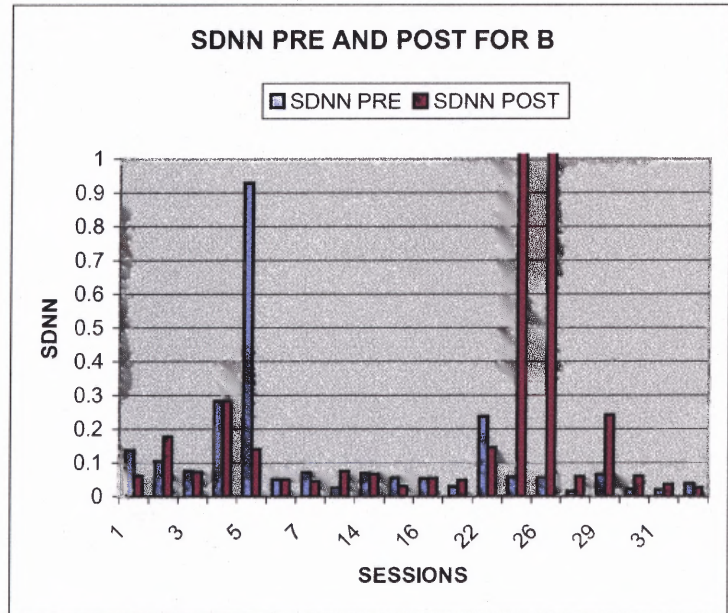
PATIENT C

NLF	
PRE	POST
0.6169519993	0.377024494
0.2279039972	0.534731877
0.3810934408	0.642400121
0.5200025333	0.572943709

NLF PRE & POST FOR C
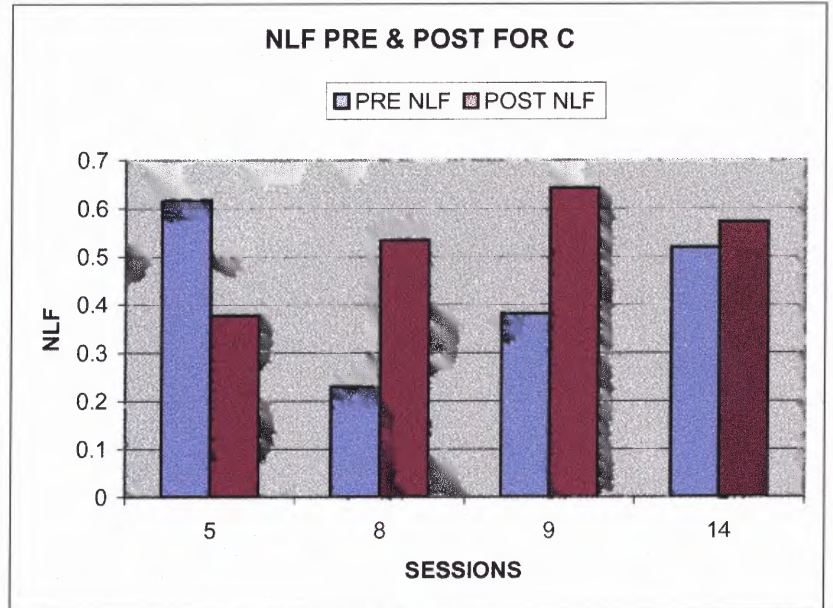
NHF	
PRE	POST
0.3830480007	0.622975506
0.7720960028	0.465268123
0.6189065592	0.357599879
0.4799974667	0.427056291
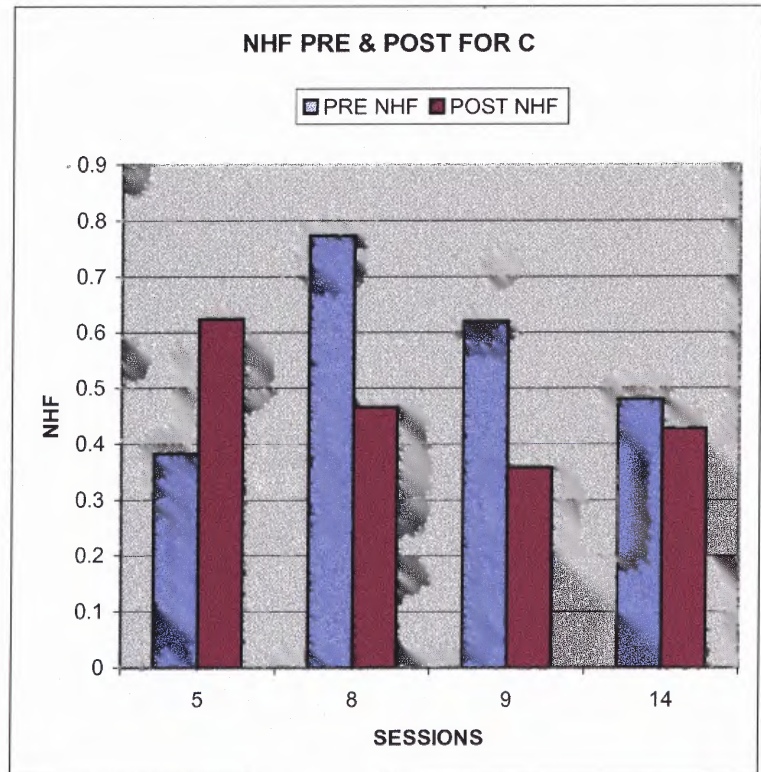
NHF PRE & POST FOR C

NLF/NHF	
PRE	POST
1.6106388825	0.605199547
0.295175683	1.149298332
0.615752704	1.796421524
1.083344328	1.34161168

NLF/NHF PRE & POST FOR C
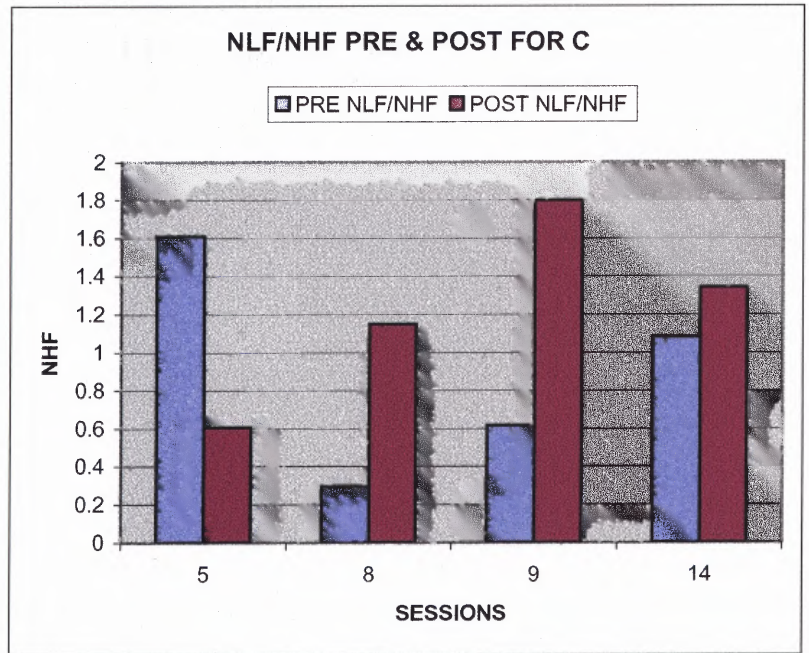
SDNN	
PRE	POST
0.0848	0.1005
0.221	0.1226
0.2033	0.1226
0.1675	0.1624

SDNN PRE & POST FOR C
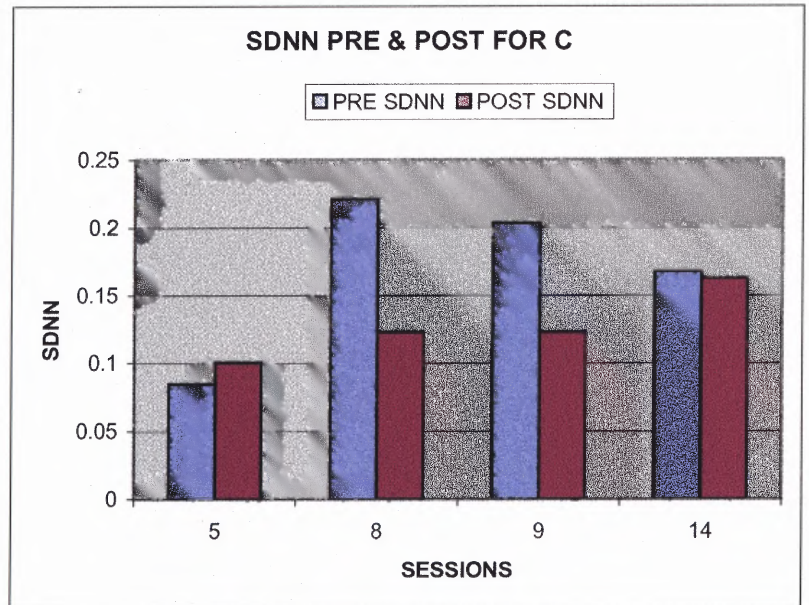
PATIENT D

NLF	
PRE	POST
0.75484249	0.15418258
0.860800594	0.674086094
0.72129872	0.157282105
0.111514866	0.369477912
0.210874509	0.310955961
0.620862309	0.221364842
0.263079814	0.383070301
0.329073482	0.216648291
0.238241309	0.428525812
0.246391407	0.408712459
0.206337903	0.548936824
0.180716544	0.285863692
0.378606424	0.36128273
0.18262373	0.139193371
0.462965827	0.341387856
0.202890283	0.379609145
0.159450555	0.25100573
0.230977851	0.522153674
0.63640434	0.516812514
0.105864857	0.40922531
0.179410258	0.374661613
0.226624473	0.460590671
0.304983883	0.205013073
0.162938407	0.342504476

NLF PRE & POST FOR D
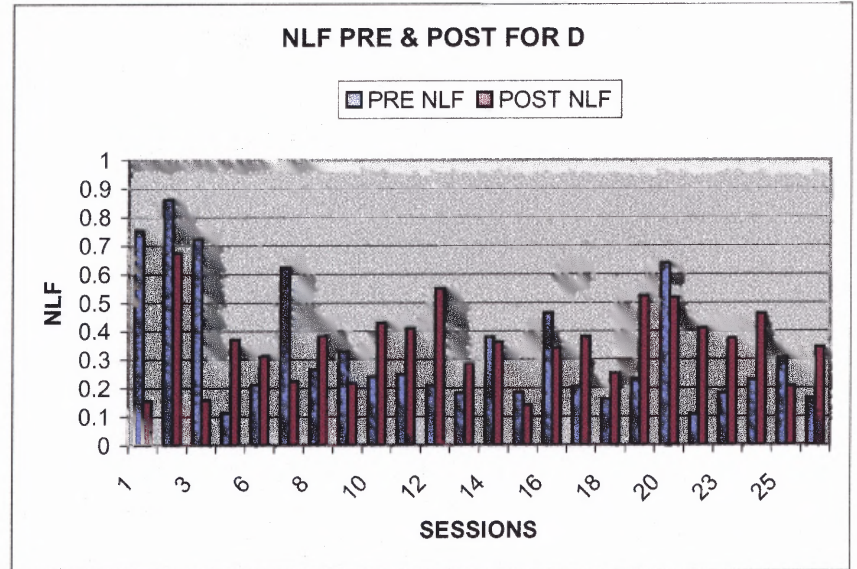
NHF	
PRE	**POST**
0.24515751	0.84581742
0.1391994406	0.325913906
0.278701028	0.842717895
0.8884851134	0.630522088
0.7891254491	0.689044039
0.3791376911	0.778635158
0.7369201866	0.616929699
0.6709265186	0.783351709
0.7617586911	0.571474188
0.7536085931	0.591287541
0.7936620971	0.451063176
0.8192834566	0.714136308
0.621393576	0.63871727
0.81737627	0.860806629
0.5370341731	0.658612144
0.7971097171	0.620390855
0.840549445	0.74899427
0.7690221491	0.477846326
0.36359566	0.483187486
0.894135143	0.59077469
0.8205897421	0.625338387
0.7733755271	0.539409329
0.6950161171	0.794986927
0.8370615931	0.657495524
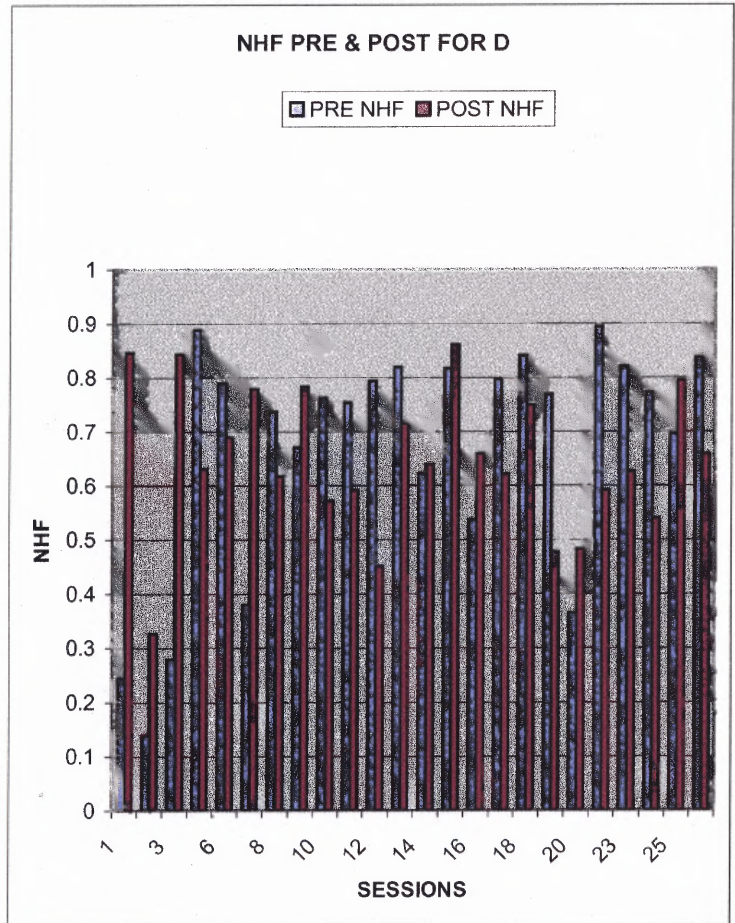
NHF PRE & POST FOR D

NLF/NHF	
PRE	**POST**
3.079010263	0.182288253
6.183938687	2.068294974
2.588071066	0.186636721
0.125511234	0.585987261
0.267225557	0.451286048
1.637564197	0.284298544
0.356999061	0.620930233
0.49047619	0.276565799
0.312751678	0.749860309
0.326948775	0.691224541
0.259982055	1.216984343
0.220578778	0.400292897
0.609286027	0.565637955
0.223426758	0.161701091
0.862078894	0.518344309
0.254532443	0.611887073
0.189698007	0.335123698
0.300352665	1.092723005
1.750307855	1.069590023
0.118399168	0.692692693
0.218635756	0.599134199
0.293032899	0.853879691
0.438815555	0.257882319
0.194655218	0.520922901

NLF/NHF PRE & POST FOR D
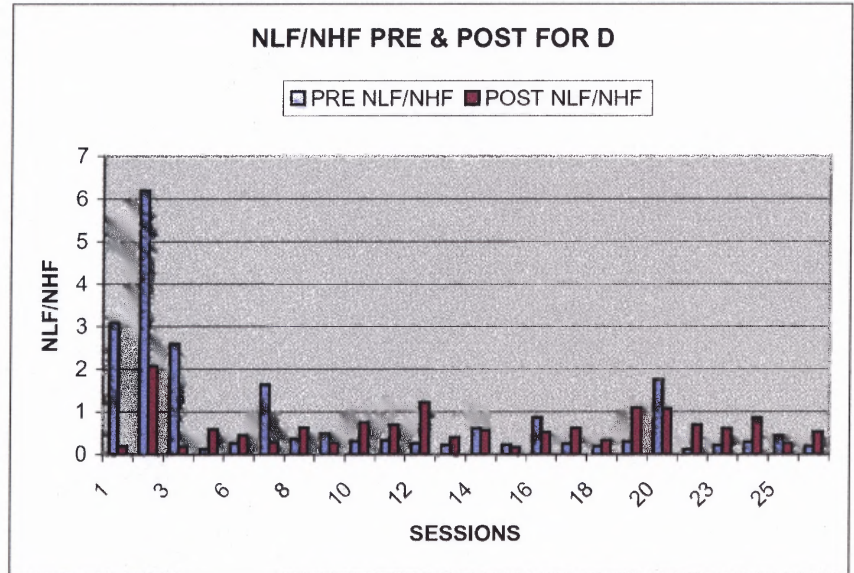
SDNN	
PRE	POST
0.0561	0.3697
0.1241	0.0949
0.102	0.1473
0.0465	0.0479
0.2562	0.0675
0.0565	0.0484
0.0678	0.0608
0.0864	0.0481
0.0755	0.0745
0.0454	0.0568
0.0393	0.0471
0.069	0.0571
0.0669	0.045
0.0396	0.0188
0.0423	0.0476
0.0435	0.0513
0.096	0.2785
0.0608	0.1548
0.1	0.1406
0.0258	0.183
0.015	0.0095
0.0101	0.0283
0.1015	0.1316
0.0433	0.0225

SDNN PRE & POST FOR D
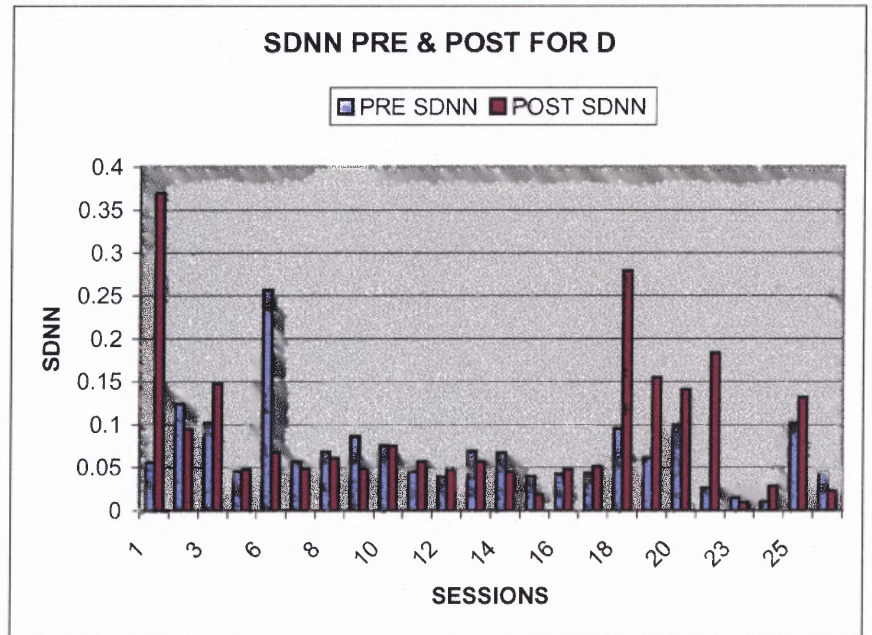
PATIENT E

NLF	
PRE	POST
0.144354635	0.684915025
0.612014603	0.397664612
0.561970087	0.361748295
0.750647111	0.833033303
0.654092072	0.740289414
0.118991794	0.877508651
0.115777839	0.745780969
0.623814197	0.868999551
0.561810795	0.621518987
0.179055887	0.345808383

NLF PRE & POST FOR E
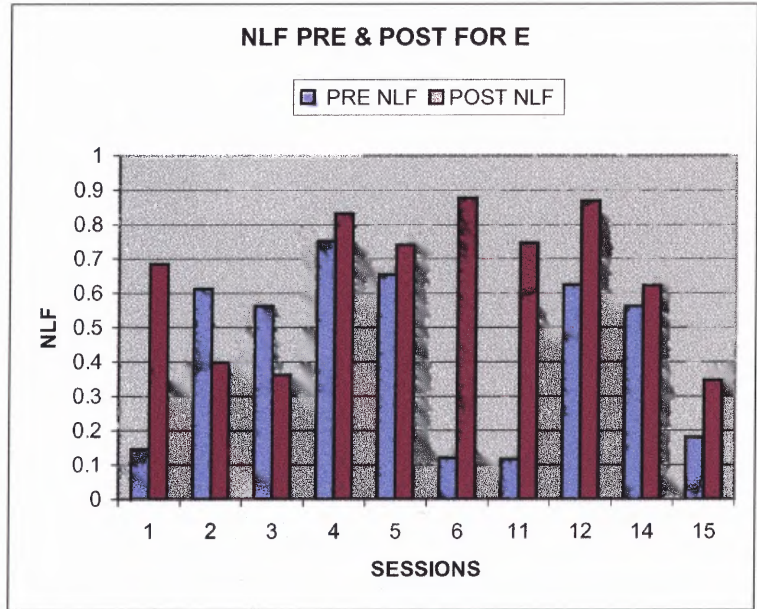
NHF	
PRE	POST
0.855645365	0.315084975
0.387985397	0.602335388
0.438029913	0.638251705
0.24935289	0.166966697
0.345907928	0.259710586
0.881008206	0.122491349
0.884222161	0.254219031
0.376185803	0.131000449
0.438189205	0.378481013
0.820944113	0.654191617
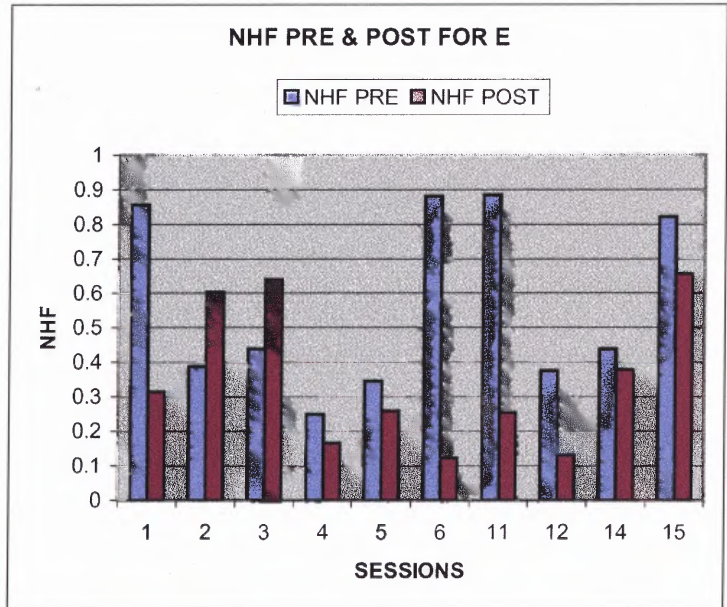
NHF PRE & POST FOR E

NLF/NHF	
PRE	POST
0.168708487	2.173747017
1.577416595	0.660204631
1.282949108	0.56677999
3.010380623	4.989218329
1.890942699	2.850439883
0.135063207	7.163841808
0.1309375	2.933615819
1.65826087	6.633561644
1.282119205	1.642140468
0.218109716	0.528604119

NLF/NHF PRE & POST FOR E
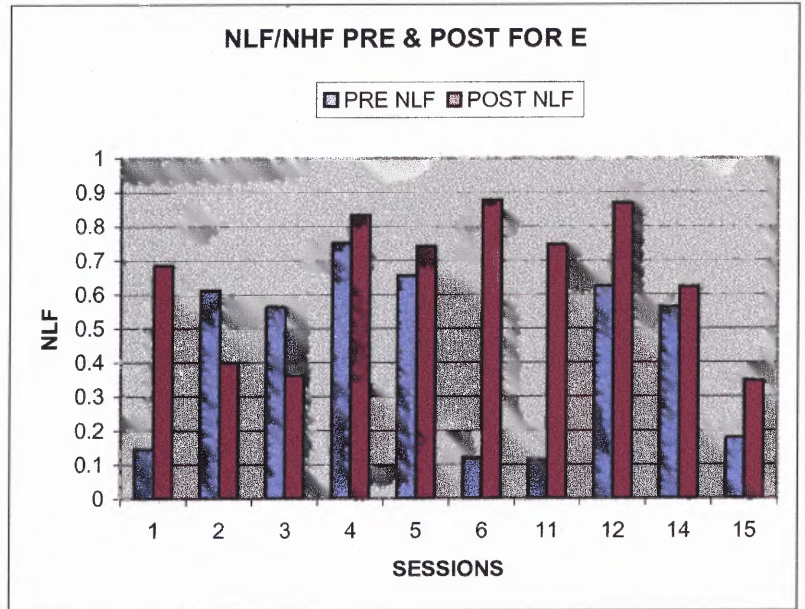
SDNN	
PRE	POST
0.0121	0.0465
0.2232	0.0934
0.0304	0.0279
0.3009	0.4184
0.1973	0.045
0.0743	0.0161
0.0151	0.0192
0.1284	0.0398
0.0153	0.0153
0.0122	0.0117

SDNN PRE & POST FOR E
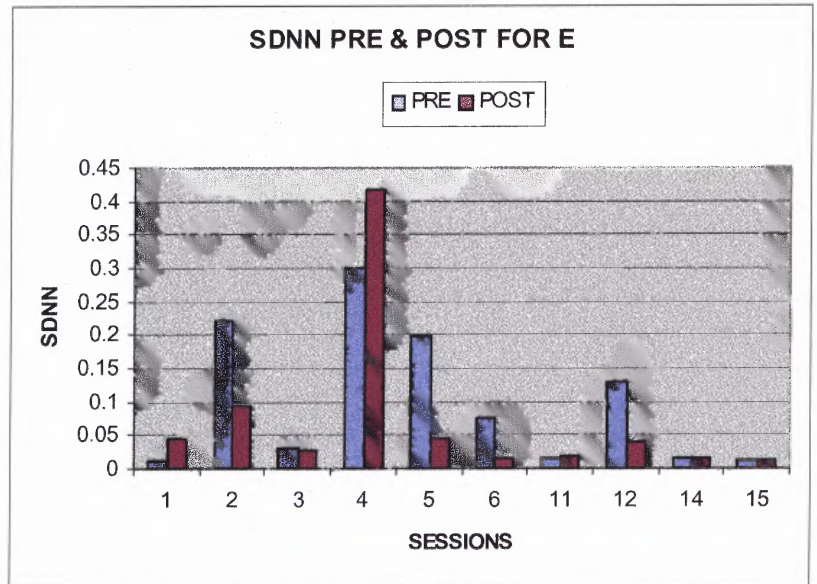
PATIENT F

NLF	
PRE	**POST**
0.85234048	0.713538553
0.5122093020	0.532150986
0.8397912730	0.482501862
0.6677938430	0.718224428
0.81780182	0.800868936
0.4545974380	0.703449801
0.7458551940	0.799518181
0.7836209260	0.759703972
0.8146422130	0.762406232
0.4819451130	0.689279438

NLF PRE & POST FOR F
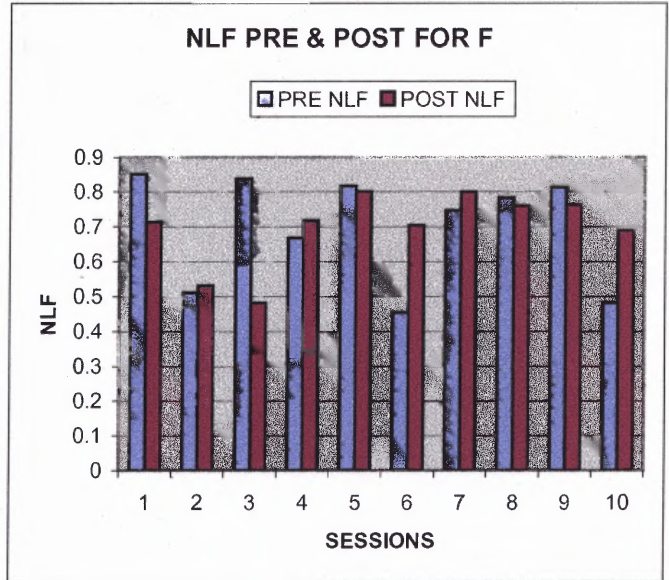
NHF	
PRE	**POST**
0.14765952	0.286461447
0.4877906980	0.467849014
0.1602087270	0.517498138
0.3322061570	0.281775572
0.18219818	0.199131064
0.5454025620	0.296550199
0.2541448060	0.200481819
0.2163790740	0.240296028
0.1853577870	0.237593768
0.5180548870	0.310720562
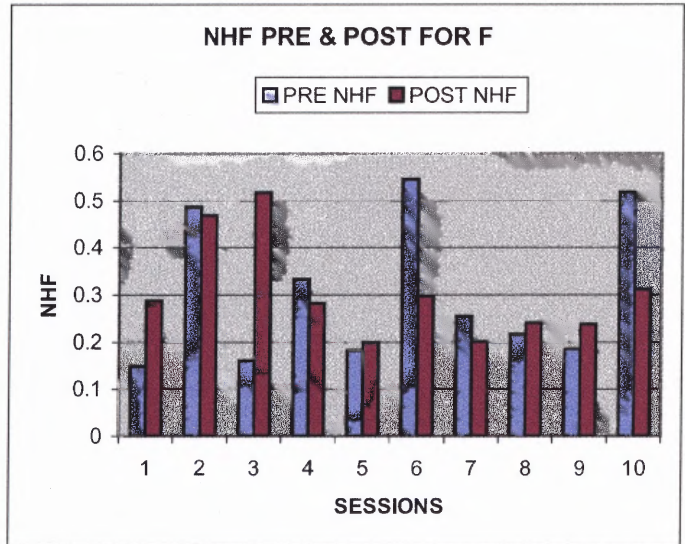
NHF PRE & POST FOR F

NLF/NHF	
PRE	POST
5.772336772	2.490871153
1.050059595	1.137441719
5.241857242	0.932374101
2.010179012	2.548923679
4.488529015	4.021818182
0.833508073	2.372110365
2.934764657	3.987983477
3.621518987	3.161533627
4.394971614	3.208864602
0.930297398	2.218325792

NLF/NHF PRE & POST FOR F
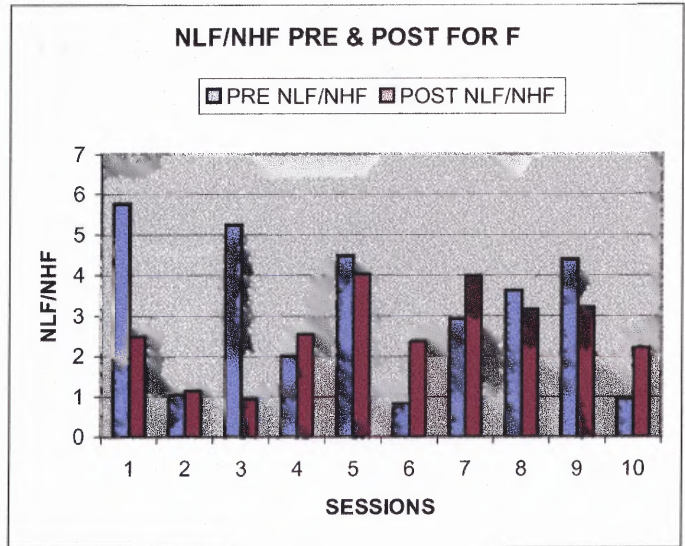
SDNN	
PRE	POST
0.0492	0.0542
0.055	0.0242
0.2622	0.0894
0.1448	0.0483
0.0586	0.0913
0.0145	0.3138
0.0685	0.0678
0.0383	0.069
0.0515	0.0798
0.0362	0.0193

SDNN PRE & POST FOR F
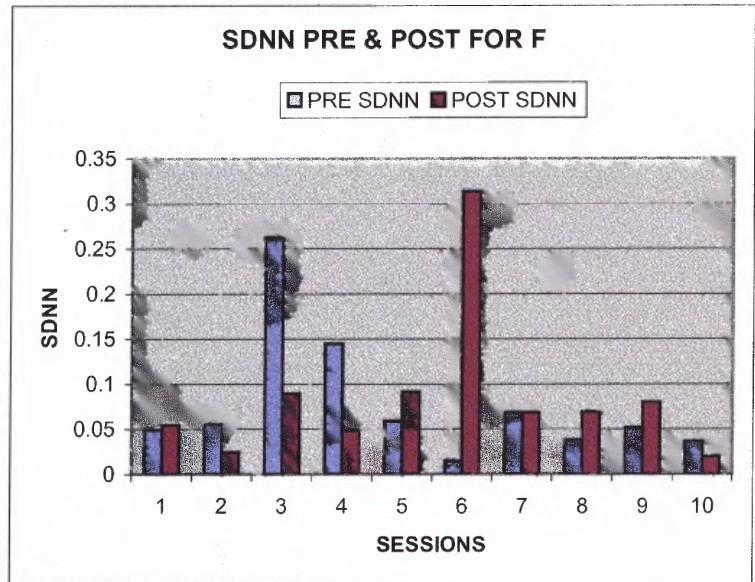
PATIENT G

NLF	
PRE	POST
0.345918278	0.33653183
0.4836622225	0.124005821
0.6806295556	0.352100089
0.4407906180	0.452496298
0.3082424690	0.448055477
0.4315905592	0.423752247
0.8375529630	0.361380611
0.572393954	0.516584836
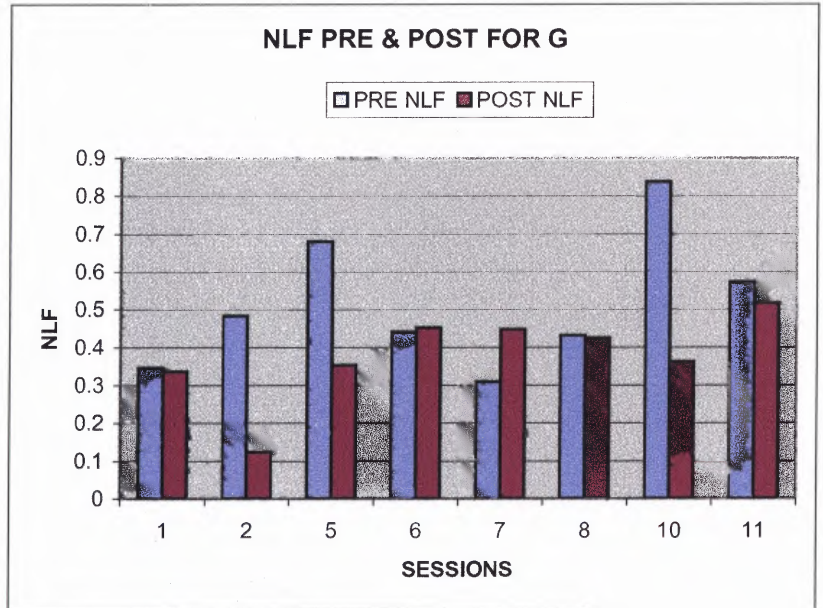
NHF	
PRE	POST
0.654081722	0.66346817
0.5163377750	0.875994179
0.3193704440	0.647899911
0.5592093820	0.547503702
0.6917575310	0.551944523
0.5684094080	0.576247753
0.1624470370	0.638619389
0.4276060460	0.483415164
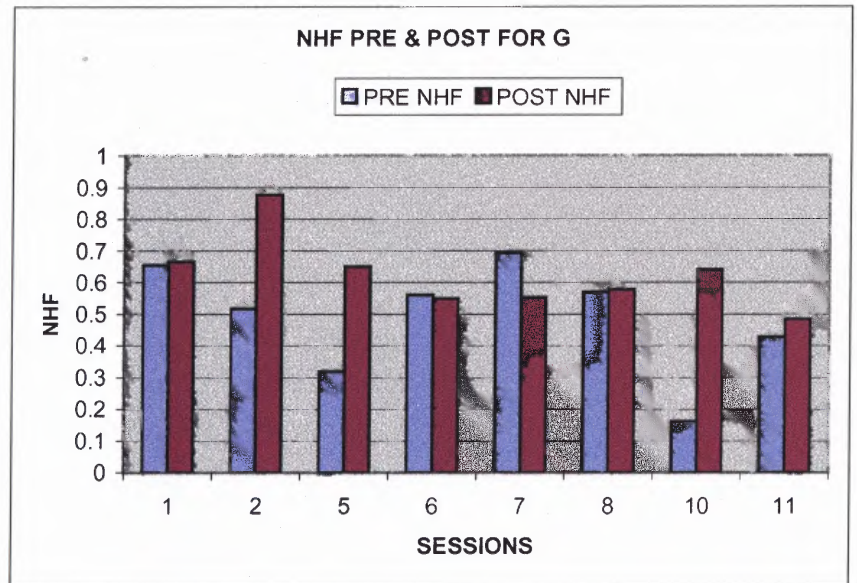
NLF/NHF	
PRE	**POST**
0.528860946	0.507231312
0.936716714	0.141560097
2.131160125	0.543448276
0.788238952	0.826471667
0.445593225	0.811776288
0.759295301	0.735364683
5.155852521	0.565877919
1.338601173	1.068615289

NLF/NHF PRE & POST FOR G
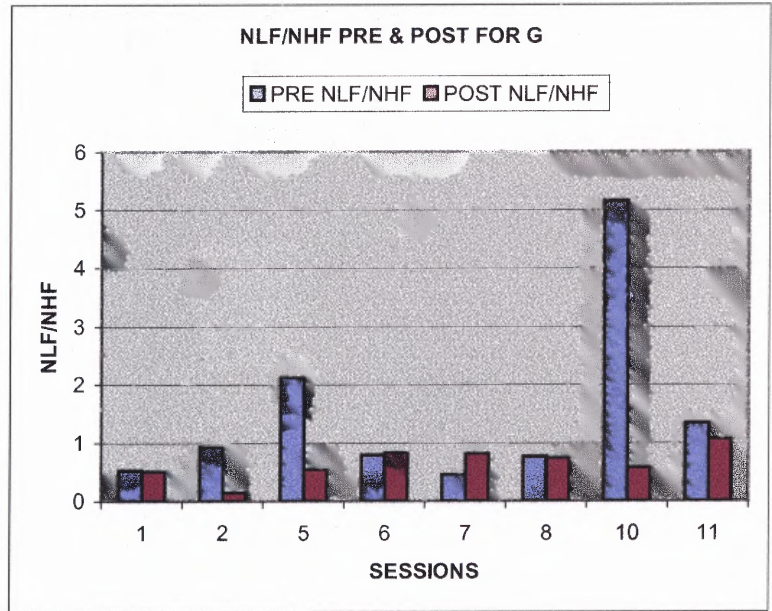
SDNN	
PRE	**POST**
0.0209	0.0185
0.0222	0.041
0.2092	0.1774
0.0468	0.0315
0.0436	0.1202
0.1077	0.0624
0.0764	0.0624
0.0525	0.0577

SDNN PRE & POST FOR G
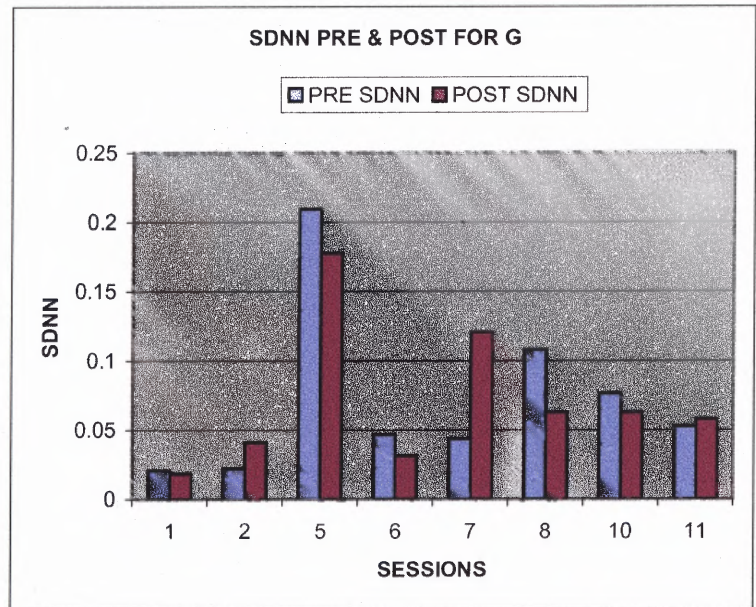
CONTROL DATA ANALYZED IN THE RESEARCH

This Appendix contains the data table and plots for five controls. The tables contain the values of NLF, NHF, NLF/NHF and SDNN for pre and post session of each control. The plots show a comparison of pre and post session values for each control for NLF, NHF, NLF/NHF and SDNN. There were five controls; who have been treated as five sessions.
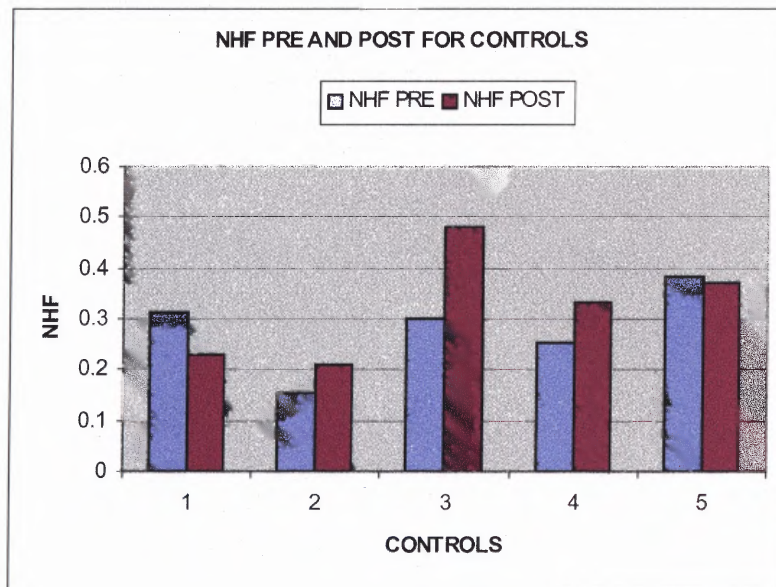
CONTROLS

NLF	
PRE	POST
0.312846448	0.229084264
0.152958277	0.209067123
0.298208035	0.482944362
0.254364521	0.333011777
0.383916497	0.370681986
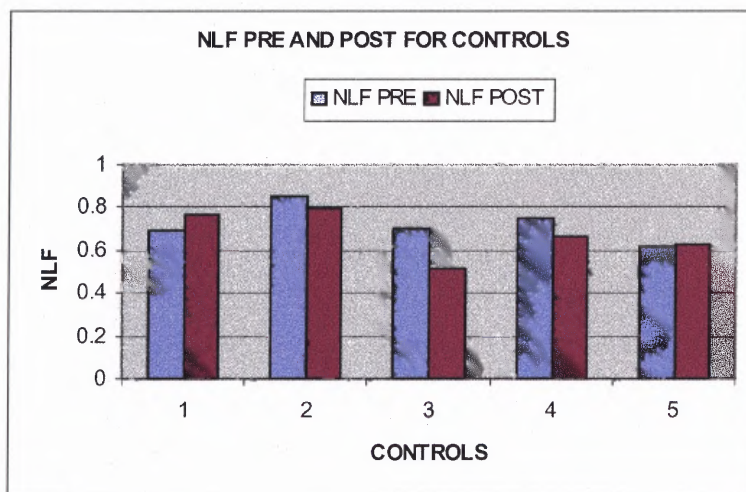
CONTROLS

| NHF	
PRE	POST
0.687153552	0.770915736
0.847041723	0.790932877
0.701791965	0.517055638
0.745635479	0.666988223
0.616083503	0.629318014

NLF/NHF PRE AND POST FOR CONTROLS

CONTROLS

NLF/NHF	
PRE	POST
0.455278805	0.297158629
0.18057939	0.264329792
0.424923695	0.934027841
0.341137898	0.499276846
0.623156593	0.589021732

SDNN PRE AND POST FOR CONTROLS
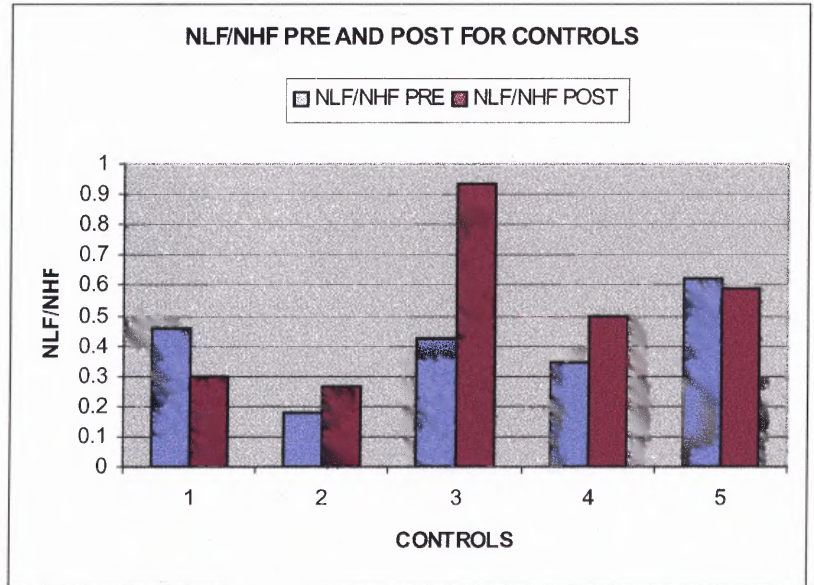
SDNN	
PRE	POST
0.4362	0.3462
0.2342	0.5042
0.3843	0.3311
0.1428	0.2202
0.2578	0.2804
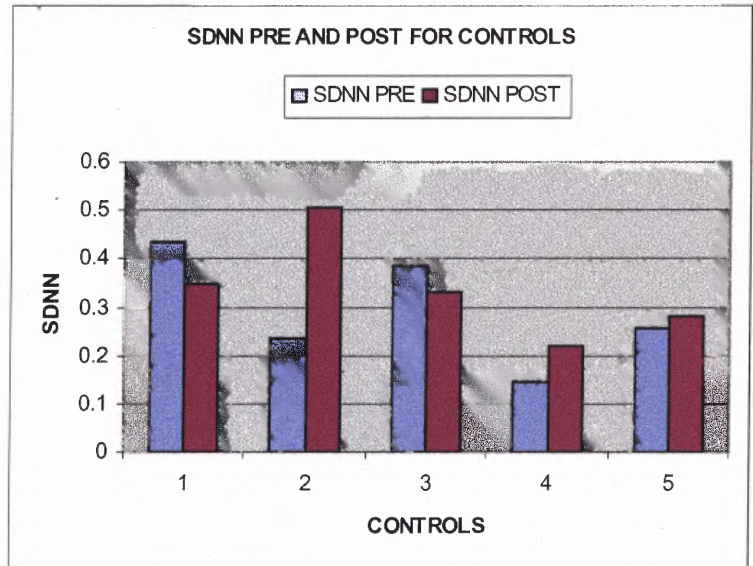
APPENDIX E

## NORMAL DATA ANALYZED IN THE RESEARCH

This Appendix contains the data table and plots for normals H, I and J. The tables contain the values of NLF, NHF, NLF/NHF and SDNN for pre and post session of each normal. The plots show a comparison of pre and post session values for each normal for NLF, NHF, NLF/NHF and SDNN.
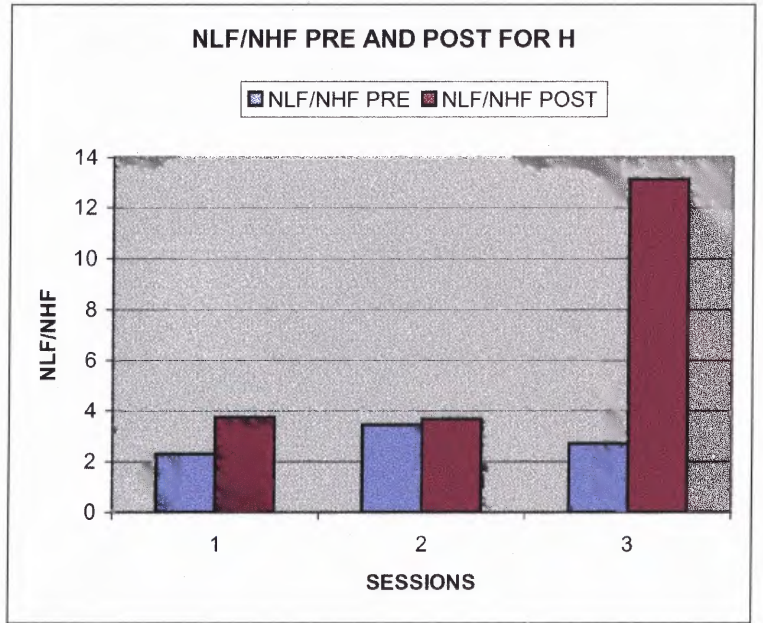
NORMAL H

NLF	
PRE	POST
0.696227671	0.789140338
0.775345339	0.786616601
0.730453513	0.929256219

NLF PRE AND POST FOR H
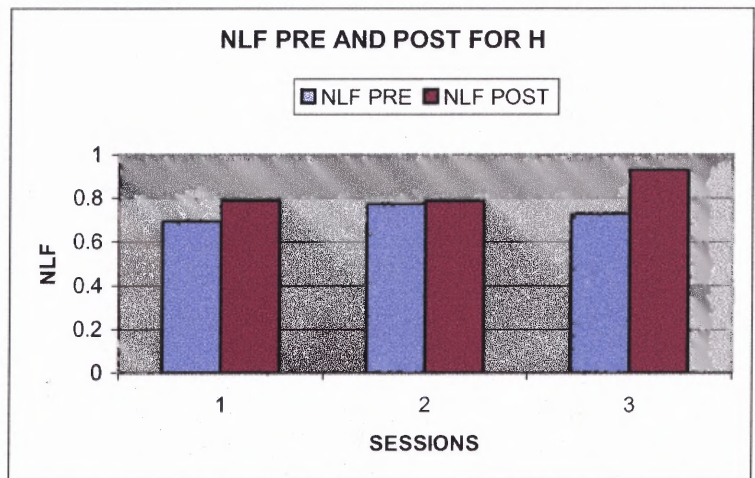
NHF	
PRE	POST
0.303772329	0.210859662
0.224654661	0.213383399
0.269546487	0.070743781

NHF PRE AND POST FOR H
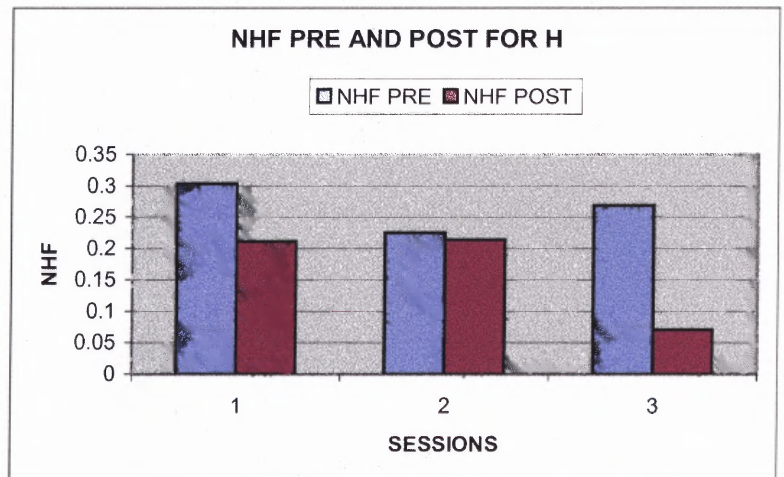
NLF/NHF	
PRE	POST
2.291939077	3.742490773
3.451276448	3.68640018
2.709935205	13.13551808

NLF/NHF PRE AND POST FOR H

■ NLF/NHF PRE ■ NLF/NHF POST

SDNN	
PRE	POST
0.0277	0.2541
0.4602	0.3707
1.5555	0.1563

SDNN FOR PRE AND POST H
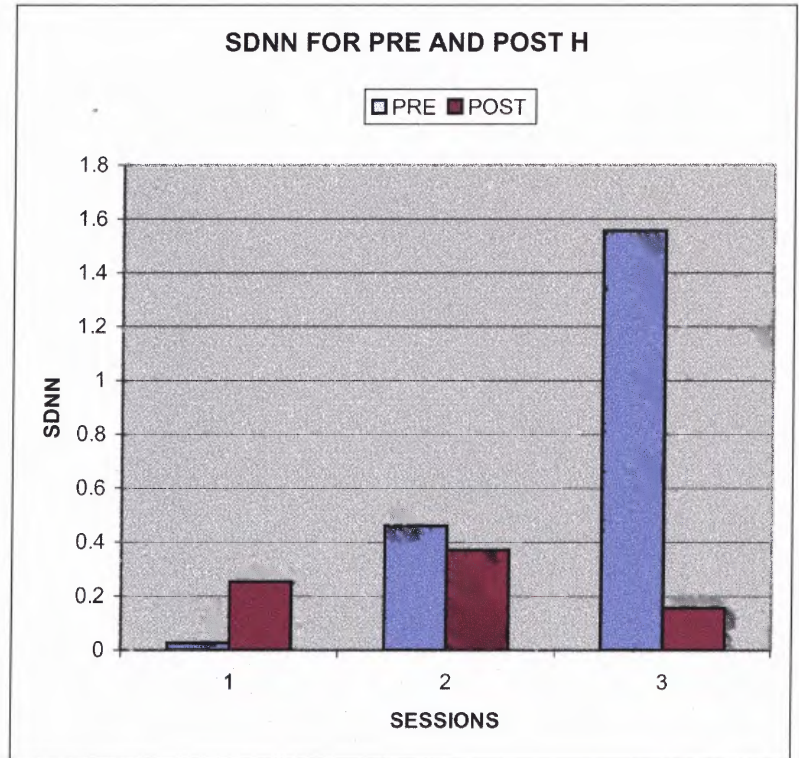
☐ PRE ■ POST

NORMAL I

NLF	
PRE	POST
0.307421298	0.237375986
0.298668273	0.648979034
0.129217768	0.152857806

NLF PRE AND POST FOR I
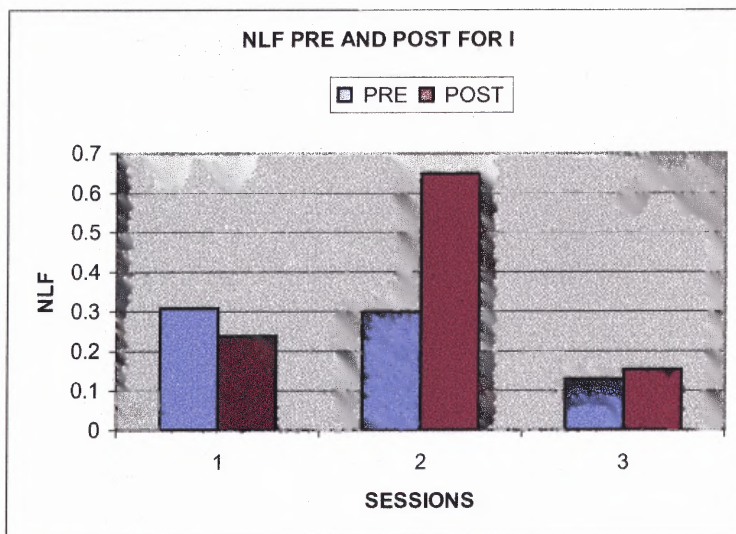
NHF	
PRE	POST
0.692578702	0.762624014
0.701331727	0.351020966
0.870782232	0.847142194

NHF PRE AND POST FOR I
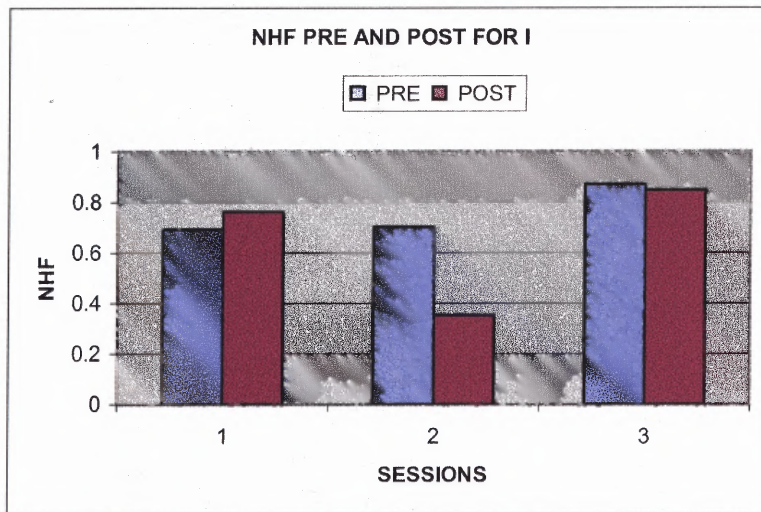
NLF/NHF	
PRE	POST
0.4438792225	0.311262144
0.4258587779	1.848832685
0.1483927470	0.180439373

NLF/NHF PRE AND POST FOR I
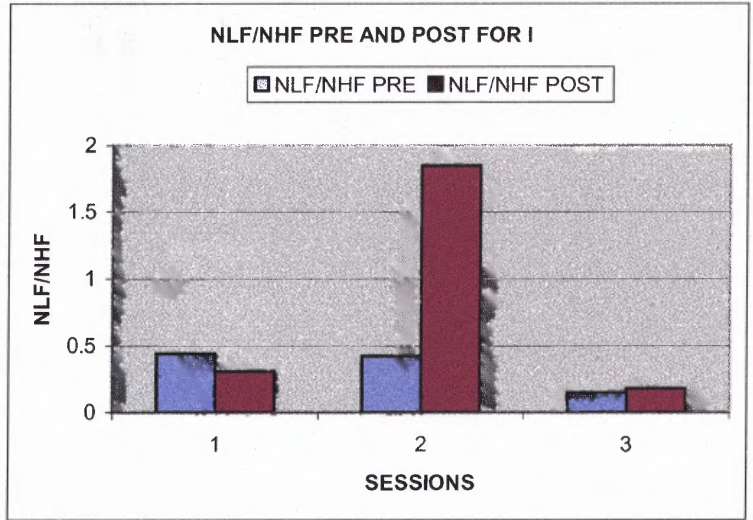
SDNN	
PRE	POST
0.183	0.1707
0.0857	0.0528
0.1651	0.1531

SDNN PRE ANS POST FOR I
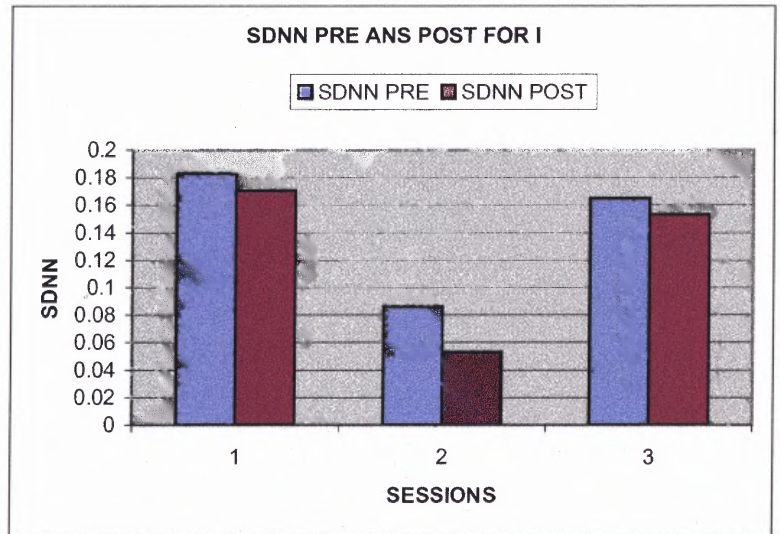
NORMAL J

NLF	
PRE	POST
0.08266	0.088683923
0.33148	0.251192692
0.19803	0.334306658

NLF PRE AND POST FOR J
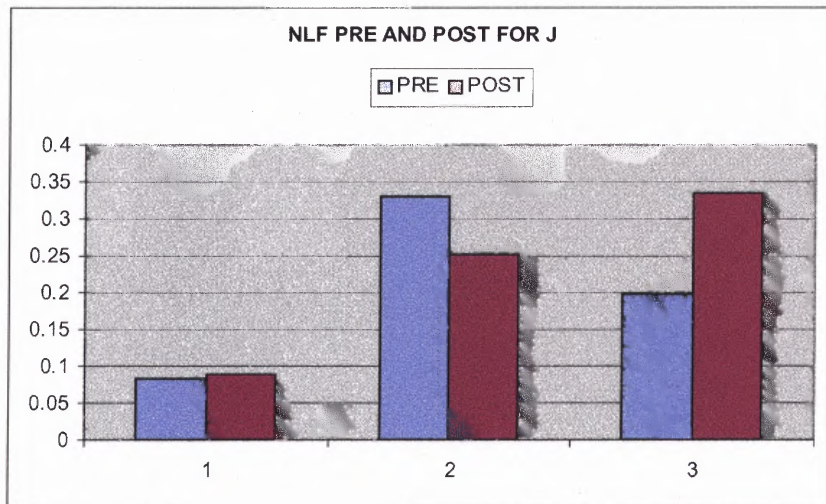
NHF	
PRE	POST
0.91734	0.911316077
0.66852	0.748807308
0.80197	0.665693342

NHF PRE AND POST FOR J
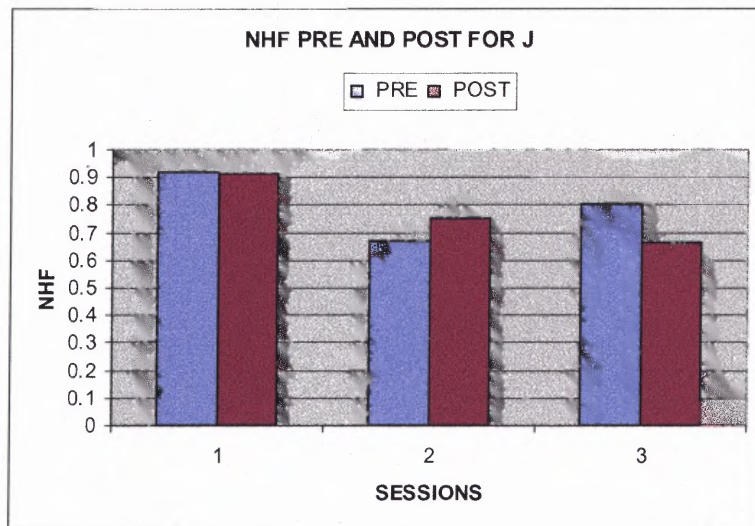
NLF/NHF	
PRE	POST
0.09011	0.09731412
0.49585	0.335457051
0.24692	0.502193183

NLF/NHF PRE AND POST FOR J
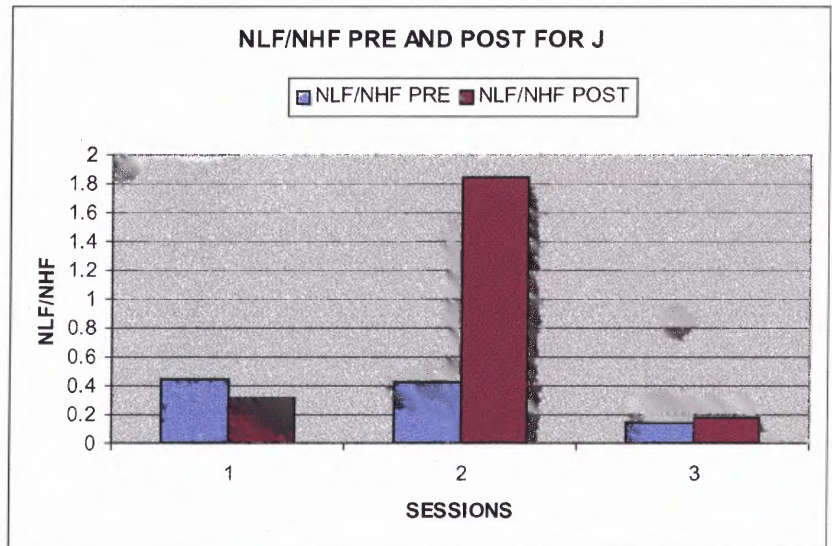
SDNN	
PRE	POST
0.1105	0.1072
0.0342	0.0499
0.0331	0.0184

SDNN PRE AND POST FOR J
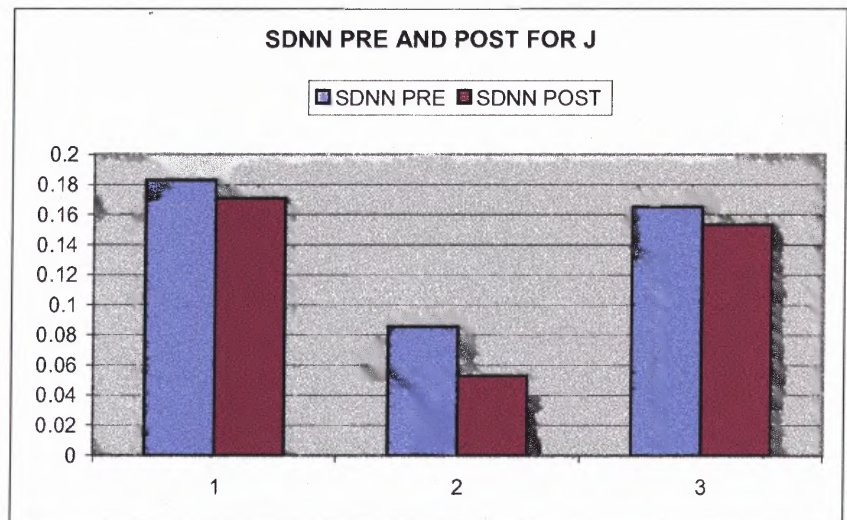
REFERENCES

[1] C. A. Guyton, *Textbook of medical physiology: the function of heart*, Philadelphia: Saunders, 1976.

[2] J. A. Vander, *Human physiology: the mechanisms of body function*, New York: McGraw-Hill, 1990.

[3] A. Vander, J. Sherman and D. Luciano, *Human Physiology: The Mechanisms of Body Function*. 5th Ed. New York: McGraw-Hill, 2001.

[4] Human Physiology: The Mechanisms of Body Function. How does the heart function? http://www.besthearthealth.com/about.html. Retrieved on 14 September 2003.

[5] Myocardial Ischemia, Infarction and Injury. http://216.185.102.50/arrhythmia/professional/mii.html. Retrieved on 1 October 2003.

[6] Instant Access to the minds of medicine: Myocardial Ischemia. http://emedicine.com. Retrieved on 1 October 2003.

[7] A. Taddei, M. Emdin, M. Varanini, G. Nassi, M. Bertinelli, C. Carpeggiani, E. Picano, and C. Marchesi, Computers in Cardiology, vol. 8, pp. 497 – 500, 1996.

[8] E. R. Klabunde, Ph.D. Cardiovascular Physiology Concepts. www.cvphysiology.com/Arrhythmias/A009.htm Retrieved on 14 September 2003.

[9] K. I. Panoulas, L. J. Hadjileontiadis and S. M. Panas, "Engineering in Medicine and Biology Society." *Proceedings of the 23rd Annual International Conference of the IEEE*, vol. 1, pp. 344 – 347, 2001.

[10] What is EECP therapy? www.eecp.com. Retrieved on 16 September 2003.

[11] Vasomedical. What is EECP? http://www.vasomedical.com. Retrieved on 16 September 2003.

[12] History of Enhanced External Counter Pulsation. www.heartcenteronline.com/myheartdr/common/artprn_rev.cfm?filename=&ARTI D=142.htm. Retrieved on 16 September 2003.

[13] Candidates for the EECP treatment. http://naturalbypass.com. Retrieved on 16 September 2003.

[14] American Heart Association. What is enhanced external Counterpulsation? http://www.americanheart.org/presenter.jhtml?identifier=4577.htm. Retrieved on 16 September 2003.

[15] K. Jayaraman, S. S. Reisman and A. M. Petrock, "Preliminary assessment of the effectiveness of enhanced external counter pulsation on heart rate variability for heart failure patients." *IEEE 29th Annual Proceedings of Bioengineering Conference*, pp. 22-23, 2003.

[16] www.strokedoctor.com/eecp1.htm. Retrieved on 16 September 2003.

[17] M. N. Levy and P. J. Martin, "Neural Control of the heart." *Handbook of Physiology. American Phyisology Society*, vol. 1, pp. 581-620, 1979.

[18] M. S. Harald, "Heart rate variability." *American Journal of Physiology and Regulatory Integrative Comp Physiology*, vol. 285, pp. 927 – 931, 2003.

[19] Analysis of heart rate dynamics by methods derived from nonlinear mathematics: Clinical applicability and prognostic significance, History of heart rate variability. http://herkules.oulu.fi/isbn9514250133/html/c210.html. Retrieved on 1 October 2003.

[20] R. E. Kleiger, J. P. Miller, J. T. Bigger, A. J. Moss and the Multicenter Post-Infarction Research Group, "Decreased heart rate variability and its association with increased mortality after acute myocardial infarction." *American Journal of Cardiology*, vol. 59, pp. 256-62, 1989.

[21] Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, "Heart rate variability - Standards of measurement, physiological interpretation, and clinical use," Circ., vol. 93, no 5, pp. 1043-1065, 1996.

[21] G. Nollo, M. D. Greco, F. Ravelli and M. Disertori, "Evidence of low- and high-frequency oscillations in human AV interval variability: evaluation with spectral analysis." *American Journal of Physiology: Heart and Circulatory Physiology*, vol. 267, pp. 1410 – 1418, 1994.

[22] Interest of HRV. http://petrus.upc.es/~wwwdib/biov/heart_rat_var/pruebas/hrv1.html. Retrieved on 13 July 2003.

[23] C. Simon, University of Auckland Medical School, "The Sympathetic Nervous System's Role in Regulating Blood Pressure Variability." *IEEE Engineering in Medicine and Biology*, vol. 1, pp. 17-24, 2001.

[24] K. S. Phyllis, "Assessment of Autonomic Tone Using Frequency Domain HRV."

Journal of the American College of Cardiology, vol. 16, pp. 978-985, 1990.

[25] Fast Fourier Transform, Signal Processing Technique:
http://mathworld.wolfram.com/FastFourierTransform.html. Retrieved on 4 November 2003.

[26] M. Malik and J.A. Camm, *Heart Rate Variability*.1st Ed. Futura Publishing Company.

[27] V. M. Kamath and L.E. Fallen, "Power Spectral Analysis of Heart Rate Variability- A Non Invasive Signature of Cardiac Autonomic Function." *Critical reviews in Biomedical Engineering,* vol. 21(3), pp. 245-311, 1993.

[28] Frequency Domain Analysis
http://petrus.upc.es/~wwwdib/biov/heart_rat_var/pruebas/hrv4.htm. Retrieved on 4 November 2003.

[29] J. P. Saul, Y. Arai, R. D. Berger, L. S. Lilly, W. S. Colucci and R. J. Cohen, "Assessment of autonomic regulation in chronic congestive heart failure by heart rate spectral analysis." *American Journal of Cardiology*, vol. 61, pp. 1292-1299, 1988.

[30] Off-line ECG signal analysis under MATLAB: QRS complex detection.
http://www.gtec.at/products/g.BSanalyse/ gECGtoolbox/gECGtoolbox.html. Retrieved on 4 November 2003.

[31] The royal Windsor Society of Nurse Researchers.
http://www.kelcom.igs.net/~nhodgins/t-test_research_analysis.html. Retrieved on 5 November 2003.

[32] J. T. Bigger, J. L. Fleiss, R. C. Steinman, L. M. Rolnitsky, R. E. Kleiger and J. N. Rottman, "Correlations among time and frequency domain measures of heart period variability two weeks after acute myocardial infarction." *American Journal of* Cardiology, vol. 69, pp. 891-898, 1992.

[33] F. Lombardi, G. Sandrone and S. Pernpruner, "Heart rate variability as an index of sympathovagal interaction after acute myocardial infarction." *American Journal of Cardiology*, vol. 60, pp. 1239-1245, 1987.