EFFECT OF AGE ON AUTONOMIC NEUROCARDIAC FUNCTION IN HEALTHY MALES AND FEMALES

By

ADRIAN M. HARVEY, B.Kin

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AUTHOR: Adrian M. Harvey, B.Kin. (McMaster University)

SUPERVISOR: Dr. Neil McCartney, Ph.D.

SUPERVISORY COMMITTEE: Dr. Markad V. Kamath, Ph.D., P.Eng.
Dr. Ernest L. Fallen, M.D.

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ABSTRACT

Background & Rationale: Heart rate variability analysis has provided scholars and clinicians with a powerful non-invasive tool for the assessment of cardiac autonomic function in health and disease. However, the interpretation of the information provided by this technique would be greatly facilitated by a more precise definition of 'normality'. The purpose of this investigation was to examine the alterations in cardiac autonomic function across a broad spectrum of ages in healthy males and females.

Methods: Heart rate variability data during 20 min supine rest and orthostatic stress (10 min free standing) as well as 24-hour ambulatory Holter ECG recordings were obtained on 123 healthy volunteers (72 female/51 male). Subjects were arbitrarily classified into five categories: pediatric (PED; 5-12 yrs, n=22, 12♀:10♂), adolescent (ADO; 13-17 yrs, n=21, 13♀:8♂), adult (ADU; 18-30 yrs, n=26, 13♀:13♂), middle aged (MDA; 31-60 yrs, n=24, 15♀:9♂) and elderly (ELD; 61+ yrs, n=30, 19♀:11♂) age groups. Power spectral analysis (autoregressive) was determined from supine and standing acute data sets as well as six evenly spaced one hour periods during the Holter recording. Time domain variables (pNN50, R-MSSD, SDNN, SDANN & SDNN index) were derived from the 24-hour data sets.

Results: Heart rate in the supine position declined progressively from age 5 years to 30 years but showed no further changes thereafter. In contrast, power spectral measures remained relatively stable in the younger age groups but subsequently exhibited a significant shift toward a higher LF:HF area signifying sympathetic dominance (or vagal withdrawal) in the MDA and ELD subjects. The heart rate and spectral response to orthostasis was most dramatic in the ADO subjects and exhibited a progressive decline in the three older age groups. With respect to the time domain variables, those parameters characterizing short term variability (pNN50 & R-MSSD) were stable in the PED, ADO and ADU subjects but significantly diminished in the two older age groups. In contrast, time domain variables encompassing long term (SDNN & SDANN) and intermediate (SDNN index) oscillations exhibited age-related increases reaching peak values in the ADU subjects and declining progressively thereafter. Power spectral analysis of the six evenly spaced one hour periods of the 24-hour holter recording revealed a diminished circadian rhythm for the majority of the frequency domain indices in the two oldest age groups.

Conclusions: The present investigation revealed substantial evidence supporting the existence of an age dependent change in cardiac autonomic function. However, this process appeared to act homogeneously across gender. The similarity of these age dependent changes to those previously observed in pathological conditions commonly associated with autonomic neuropathy serves

to emphasize the importance of HRV research aimed at the establishment of reference standards in healthy populations and a more precise definition of 'normal' autonomic neurocardiac function.

Vitality shows not in the ability to persist but the ability to start over...

F. Scott Fitzgerald

Dedication

The thesis is dedicated to.....

the memory of my grandmother, Ethel May Harvey who after a courageous thirteen year battle with Alzheimer's disease succumbed to her illness in May of 1997.

Knowledge advances by steps not by leaps...

Thomas Babington

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GLOSSARY OF TERMS & ABBREVIATIONS

Circadian Rhythm: Oscillations in heart rate with periods of approximately 24 hours.

Ectopic Beat: Heart beat that originates from a pacemaker site other than the SA node.

HF Area: Area under the heart rate spectral curve between 0.15 - 0.50 Hz. A measure of power in the high frequency (HF) band.

HF Peak: Maximum power within the high frequency (HF) band.

HRV: Heart rate variability

LF Area: Area under the heart rate spectral curve between 0.02 - 0.15 Hz. A measure of power in the low frequency (LF) band.

LF CF: Frequency at with the maximum power within the low frequency (LF) band is located.

LF Peak: Maximum power within the low frequency (LF) band.

Neurocardiac: Refers to mechanisms that modulate cardiovascular function via the autonomic nervous system.

Orthostatic Stress: Physiological stress placed on the cardiovascular system to maintain blood flow in the face of gravity induced lower extremity pooling that occurs with the assumption of an upright posture.

pNN50: Percentage of adjacent intervals between normal sinus conducted beats within a given heart rate data set that differ by more than 50 milliseconds.

Power Spectral Analysis: Technique that decomposes the heart rate signal into its component sine waves.

Prognostic Indicator: A measure that predicts the duration, course and outcome of a disease.

R-MSSD: Root mean square of successive differences between normal sinus conducted beats in a given heart rate data set.

Respiratory Sinus Arrhythmia (RSA): Fluctuations in heart rate that occur concurrently with the respiratory cycle. In general, heart rate increases on inspiration and decreases on expiration.

SDANN: Standard deviation of the mean interval between normal sinus conducted beats in adjacent 5 minute segments of a given heart rate data set.

SDNN: Standard deviation of all intervals between normal sinus conducted heart beats within a given heart rate data set.

SDNN Index: Mean of the standard deviations of intervals between normal sinus conducted beats in adjacent 5 minute segments of a given heart rate data set.

1.0 REVIEW OF LITERATURE

1.1 Introduction - Regulation of Heart Rate

Automaticity is a characteristic of all myocardial tissue, particularly the sino-atrial node, atrio-ventricular node and purkinje fibres. The ability to spontaneously generate an action potential is a result of slow ionic currents, (involving sodium, calcium and potassium ions), that gradually push the membrane potential toward threshold during the interval between completion of repolarization and the onset of the subsequent action potential (Berne & Levy 1992). The SA node, a small, flattened ellipsoid strip of myocardium located in the superior lateral wall of the right atrium exhibits this automaticity to the greatest extent (Berne & Levy 1992; Guyton & Hall 1996). If unregulated the heart will beat at the intrinsic rate of the SA node, approximately 100-120 bts/min. The automaticity of the remaining pacemaker sites is held in check by hyperpolarization resulting from the spread of depolarization throughout the myocardium, termed overdrive suppression (Guyton & Hall 1996). Occasionally, under special circumstances the heart beat may originate in a region of the myocardium other than the SA node. These sites are termed ectopic pacemakers and the beats are labeled as ectopic beats. Ectopic beats are often premature and are followed by a compensatory pause (Kamath et al. 1996).

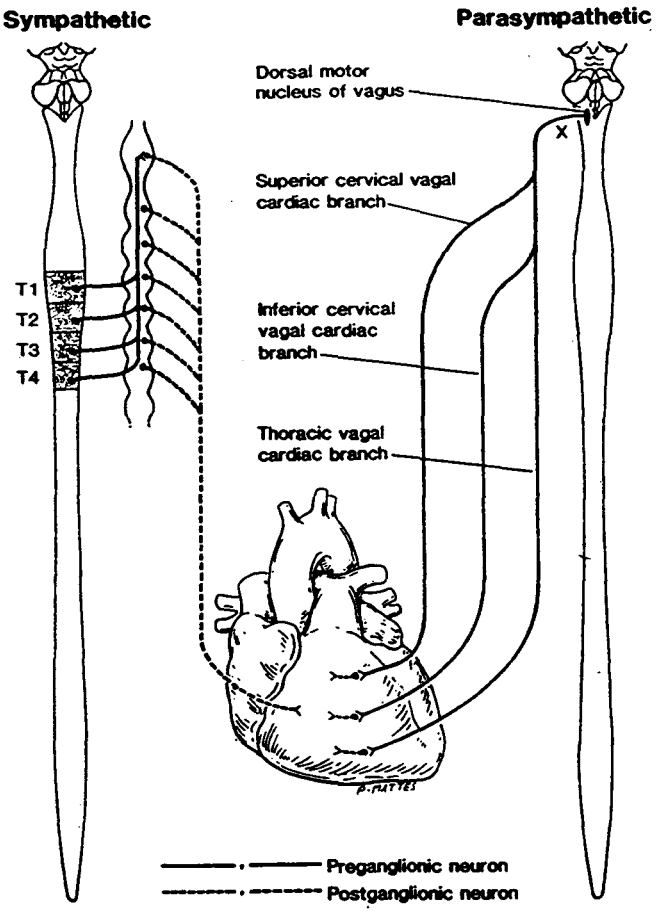


Figure 1. Anatomical origins of the sympathetic and parasympathetic nerve supply to the heart. (From Hockman CH, *Essentials of autonomic function*. Springfield IL, 1987).

Under normal physiological conditions the heart rate is subject to both autonomic neural and humoral regulation. (Braunwald 1992; Guyton & Hall 1996). The anatomical origins of the autonomic innervation of the heart are illustrated in Figure 1. Cardiac sympathetic neurons arise from the interomediolateral columns of the lower one or two cervical and upper five or six thoracic segments of the spinal cord (Berne & Levy 1992). Postsynaptic sympathetic neurons spread throughout the myocardium, innervating the SA and AV nodes as well as the atrial and ventricular myocardium. Parasympathetic

innervation originates from the cells of the dorsal motor nucleus or nucleus ambiguus in the ventrolateral medulla oblongata and travel to the heart via the vagus nerve where they synapse with neurons of the intracardiac ganglia. (Berne & Levy 1992; Hainsworth 1996). Parasympathetic efferent fibres predominantly supply the SA and AV nodes, and to a lesser extent the atrial myocardium. In general, sympathetic cardiac stimulation serves to increase the heart rate while parasympathetic input has the opposite chronotropic effect.

The effects of the sympathetic nervous system are mediated by the neurotransmitter norepinephrine. Although the exact mechanism is unknown it is believed that norepinephrine acts to increase membrane permeability of the myocardial cell to the slow inward calcium and sodium currents which are responsible for the generation of the spontaneous action potential. As a result the membrane potential will reach threshold more rapidly and the heart rate will increase (Guyton & Hall 1996). In addition, norepinephrine acts to increase conduction of depolarization throughout the heart and to augment the contractility of the ventricular myocardium. Sympathetic regulation of the heart is supported by humoral release of norepinephrine and epinephrine by the adrenal medulla. The cells of this structure are actually modified post-synaptic sympathetic neurons and are under direct control by preganglionic sympathetic fibres. Humoral release of these transmitter substances causes more prolonged

alterations in cardicvascular function (Astrand et al. 1986; Berne & Levy 1992; Guyton & Hall 1996).

The negative chronotropic effect of the parasympathetic nervous system is a result of the release of acetylcholine by postsynaptic vagal fibres. Acetylcholine acts to increase the permeability of myocardial cell membranes to the outward flow of potassium ions during repolarization (Berne & Levy 1992; Guyton & Hall 1996). Consequently the cells of the SA node become hyperpolarized and the time required for the slow inward calcium and sodium currents to bring the membrane potential to threshold is increased, thus slowing the heart rate. A strong parasympathetic stimulation can actually stop the heart from beating for short periods of time. Acetylcholine also decreases the excitability of AV junctional fibres resulting in prolonged conduction time (Guyton & Hall 1996).

Sympathetic and parasympathetic actions on pacemaker activity are characterized by distinct response latencies and action duration's. The effect of acetylcholine on nodal activity is swift as the operation of a second messenger system is not required to regulate the opening of the potassium channels (Berne & Levy 1992). As a result the latency time for parasympathetic action is extremely short (≤200 msec, Braunwald 1992). Similarly the duration of the negative chronotropic response is brief as acetylcholine is rapidly hydrolyzed by the rich supply of acetylcholinesterase in the synapses of the SA and AV nodes.

In contrast, the cardiac effects of norepinephrine are mediated predominantly by second messenger systems. As a result the latency time for action of this autonomic limb is significantly longer then the corresponding parasympathetic value (~2 secs, Braunwald 1992). In addition, the duration of the cardiac sympathetic response is prolonged with respect to the time course of parasympathetic action. This phenomenon is the result of a greater dependence on re-uptake of norepinephrine by the neurons themselves to remove the transmitter substance from the SA and AV nodal areas (Berne & Levy 1992). These discrepancies in latency and action time course allow the physiologist to assign modulation of heart rate fluctuations that occur at various frequencies to neural control by separate divisions of the autonomic nervous system.

Autonomic influence on the heart is a reflection not only of the individual activity of the sympathetic and parasympathetic inputs but of the interdependence between the two systems (Talman et al. 1993). Parasympathetic and sympathetic terminal junctions lie in close proximity and may even share a common Schwann cell sheath (Randall et al. 1977). As such, a neurotransmitter released from one nerve may act at both pre and post-junctional membranes of adjacent synapses. Indeed, acetylcholine released from vagal fibres has been shown to exert an inhibitory influence on pre-junctional release of norepinephrine from adjacent sympathetic fibres (Loffelholz et al. 1969). Moreover, post-junctional muscarinic receptor activation of GI

proteins via acetylcholine has been shown to inhibit adenylate cyclase, a second messenger mediating the cardiac β-adrenergic effects of the sympathetic system. Consequently, the chronotropic/inotropic effect for a given level of parasympathetic input will be relatively enhanced against an increased background sympathetic activity (Berne & Levy 1992). In addition, the sympathetic neurotransmitter norepinephrine appears to exert an inhibitory influence on acetylcholine release from vagal fibres through interactions with pre-junctional receptors.

Studies involving autonomic dennervation or pharmacological blockade as well as microneurographic recordings have confirmed that the efferent divisions of both autonomic limbs are constantly active (Kulbertus & Frank 1988). At rest the activity of the parasympathetic system predominates. These basal levels of autonomic traffic are often referred to in the literature as parasympathetic and sympathetic 'tone' , the functional significance of which is to allow either system to independently increase or decrease heart rate (Braunwald 1992). The tone of the parasympathetic system is solely due to the basal firing rates in the vagal cardiac fibres. In contrast, the overall sympathetic tone is a result of complementary neural and humoral mechanisms: from direct sympathetic efferent stimulation and basal levels of epinephrine (0.2ug/kg/min) and norepinephrine (0.05ug/kg/min) secretion from the cells of the adrenal medulla (Braunwald 1992).

1.2 HEART RATE VARIABILITY (HRV)

1.2.1 Historical Perspective

Recognition of beat-to-beat fluctuations in heart rate and arterial blood pressure date back to the 18th century (Hales 1733). Similar variations have since been noted in stroke volume and ECG morphology and are believed to represent the interplay between both exogenous (eg. environmental stress) and endogenous (eg. respiratory related fluctuations of intrathoracic pressure) cardiovascular perturbations and the resultant response of homeodynamic regulatory mechanisms (Appel et al. 1989). This being the case, techniques designed to identify/quantify the individual influences that compose this complex signal would have potential application in both academic and clinical settings. Today HRV analysis in both the time and frequency domains has proven to be a viable measurement tool for the non-invasive assessment of cardiac autonomic function (Kamath et al. 1993; Task Force 1996). Indeed, this tool has been used to assess cardiovascular responses to various pharmacological agents (Vibyral et al 1990; Alcalay et al. 1992; Yeragani et al. 1992; Cowan et al. 1993; Hohnloser et al. 1993) and physiological maneuvers such as orthostasis/postural tilt (Pagani et al. 1986; Weise et al. 1987; Lipsitz et al. 1990) the Valsalva maneuver (Ziegler et al. 1992) and exercise (Kamath et al. 1991; Yamamoto et al. 1991). In addition, HRV techniques have been utilized to investigate autonomic dysfunction in acute myocardial infarction (Kleiger et al. 1987; Malik et

al. 1990; Bigger et al. 1991; Lombardi et al. 1992), congestive heart failure (Saul et al. 1988; Casolo et al. 1991; Panina et al. 1995), heart transplantation (Alexopoulos et al. 1980; Fallen et al. 1988) ,diabetes mellitus (Smith 1982; Murakawa et al. 1993), chronic renal failure (Axelrod et al. 1987) and alterations in autonomic function accompanying the natural aging process (Hellman et al. 1976; Waddington et al. 1979; Finley et al. 1986; 1995, O'Brien et al. 1986; Shannon et al. 1987; Simpson et al. 1988; Lipsitz et al. 1990; Schwartz et al. 1991; Ziegler et al. 1992; Ryan et al. 1994; Tsuji et al. 1994; Liao et al. 1995). Recent work has focused on the development of geometric measures (Scherer et al. 1993; Malik, 1995) which are likely to be more appropriate for dealing with data sets of poor quality (ie. large number of ectopics/anomalous beats) and mathematical indices such as approximate entropy (Pincus et al. 1990) and 1/f scaling (Saul et al. 1988) to quantify the non-linear phenomena involved in the genesis of HRV.

1.2.2 Analysis of HRV

Frequency Domain

Spectral analysis utilizes fast Fourier transform or autoregressive algorithms to decompose the heart rate signal into its component harmonic sine waves of varying amplitudes and frequencies, (Pomeranz et al. 1985; Malliani et al. 1991; Ori et al. 1992; Kamath et al. 1993; Cerutti et al. 1995; Task force

1996). In one of the first efforts to mathematically characterize the mechanisms that generate these fluctuations, Akselrod et al. (1981) identified peaks in three distinct frequency bands of the heart rate power spectrum of unconscious, anesthetized dogs. These same three frequency specific oscillations had earlier been noted by Sayers (1973) in conscious human subjects. A high frequency peak believed to correspond to parasympathetically mediated respiratory sinus arrhythmia was observed between 0.20Hz and 0.35Hz, an assertion supported by pharmacological studies where in this peak was abolished by the administration of atropine and glycopyrrolate (Akselrod et al. 1981 & 1985; Pomeranz et al. 1985; Rimoldi et al. 1990). In addition, two peaks were evident at lower frequencies (0.12Hz and 0.04Hz). The mid-frequency peak (~0.12 Hz) is believed to correspond to baroreceptor modulation of heart rate and contains both a sympathetic and parasympathetic component (Akselrod et al. 1981 & 1985; Pagani et al. 1986; Ori et al 1992; Task force 1996). Power in the frequency band centered around 0.04 Hz was attributed to thermoregulation and local vasomotor control, fluctuations that are minimized by the actions of the renin-angiotensin system. Indeed, blockade of this system with angiotensin converting enzyme inhibitors produces a significant increase in the power located in this region of the spectrum (Akselrod et al. 1981). As the physiological significance of this frequency band is poorly defined and the low signal-to-noise ratio found in the DC region may contaminate the entire signal it is often filtered

out during power spectral analysis (Ori et al 1992; Kamath et al. 1993; Cerutti et al. 1995; Task force 1996). Currently, most investigations focus on power in well defined high frequency (HF) (0.15-0.40 Hz) and low frequency (LF) power bands (0.04-0.15 Hz). The benefit of frequency domain analysis lies in its ability to identify frequency specific oscillations of heart rate which correspond to distinct physiological mechanisms and thereby provide a comprehensive picture of neurocardiac regulation.

Time Domain

Of the various methods available for the investigation of HRV, perhaps the simplest to calculate and interpret are the time domain indices. These measures can be broadly divided into two classes: 1) those derived directly from the intervals between normal sinus conducted beats themselves (N-N intervals); and 2) those derived from the difference between adjacent cycles (Kleiger et al. 1992; Task Force 1996). The first class includes the mean heart rate, standard deviation of all N-N intervals in a given time period, standard deviations of the mean heart rates of short recording segments, (commonly 5 min.) and the mean of the standard deviations of heart rate over similar short recording segments. These indices represent not only short term beat-to-beat variations in heart rate but also oscillations of much longer time course, (such as diurnal variations). Time domain measures derived from the differences between adjacent N-N.

intervals include the percentage of adjacent variables that differ by more than a threshold value (commonly 50ms or 6%) and the root mean square of successive differences. These measures are therefore independent of secular or circadian trends and represent short term variability thought to be modulated solely by the parasympathetic nervous system (Kleiger et al. 1992).

Time domain measures have proven to be highly reproducible over periods of up to 65 days and are therefore suitable for use in clinical intervention research (Huikuri et al. 1990; Kleiger et al. 1991; Kautzner et al. 1995; Dekker et al. 1996). Dekker et al. (1996) assessed the reproducibility of both long and short-term variability measures in 15 healthy young males over three recording periods within a two week period. With the exception of pNN50 all of the 24-hour measures had reliability coefficients of greater than 0.80 and coefficients of variation lower than 0.10. In contrast both frequency and time domain indices calculated from shorter data segments, (5 minutes and 20 seconds) had significantly lower reliability coefficients and higher coefficients of variation. In addition, Kleiger et al. (1991) reported intra-class correlation coefficients of between 0.70-0.90 for 24-hour time domain indices calculated on two separate occasions, (ranging from 3 to 65 days) in 14 healthy men and women, aged 22-55 years. Similar results have been observed in populations of post AMI patients. Kautzner et al. (1995) measured the relative error of standard time domain indices calculated from 24-hour ECG data obtained from 33 survivors of

acute myocardial infarction during days 4 and 5 post infarction. With the exception of the often problematic pNN50, all of the calculated HRV indices had relative error scores ranging from 10-20%. Based on these results it has been recommended that short term variability in these long term recordings be assessed with the R-MSSD measure rather then pNN50 due to its better statistical properties (Kautzner et al. 1995; Task Force 1996).

Despite the high reproducibility of 24-hour time domain indices and their apparent suitability for the assessment of clinical interventions, these measures are subject to some methodological uncertainty (Kleiger et al. 1992; Kamath et al. 1993). These problems stem from the lack of control over the patient's dynamic state throughout the recording period. The subsequent bias on the time domain statistics may make interpretation of these findings problematic. In current practice patients are often asked to keep a brief activity diary during the recording period in an attempt to provide a contextual basis upon which clinical interpretations can be made.

Relationship Between Time and Frequency Domain Measures

In an attempt to establish relationships between time and frequency domain HRV indices Kleiger et al. (1991) found that the short term variability measures, pNN50 and R-MSSD were highly correlated with each other ($r>0.90$) and with HF power spectral values derived from power spectral analysis. In

addition, more broadly based time domain measures such as SDNN were found to be significantly correlated with both HF and LF power spectral values. These correlations exist because of both physiological and mathematical relationships (Task Force 1996). However, investigators have acknowledged the inability of time domain analysis to separate the various autonomic inputs that characterize neurocardiac regulation (Kamath et al. 1993). Therefore these values should not be considered surrogates for the spectral measures with which they are correlated.

1.2.3 Standards and Recommendations

Recent efforts to standardize the measurement and interpretation of HRV indices have provided a number of recommended standards for recording, processing and analyzing heart rate signals (Malliani et al. 1991; Ori et al. 1992; Kamath et al. 1993; Task force 1996). These recommendations are aimed at improving the validity and reproducibility of HRV measures and to allow comparisons between research conducted in various laboratory/hospital settings throughout the world. Standardization in this field is necessary if HRV analysis is to be accepted as a viable diagnostic and prognostic clinical tool. In order to be suitable for HRV spectral analysis, the heart rate signal should meet a number of requirements. The signal must be stationary (i.e. first and second order statistics do not change over the time period selected for the analysis). In addition, the

recording must be of sufficient length as the consistency of power spectral estimates improves with an increase in duration of the signal. The upper limit of signal length is imposed by the increasing likelihood that the assumption of stationarity will be violated as the duration of the heart rate sample is increased. For this reason it is recommended that frequency domain analysis be restricted to short segments of data (~5 min) and analysis of longer segments, including 24-hour Holter recordings be performed with time domain statistics (Kamath et al. 1993).

Heart rate time series must also be as ectopic-free as possible due to the fact that the compensatory pause may be erroneously attributed to vagally mediated high frequency oscillations (Ori et al. 1992; Kamath et al. 1993). The calculation of time domain statistics utilizes various filters to ensure that only normal sinus conducted beats are included in HRV analysis, (Kamath et al. 1995). For spectral analysis of heart rate time series containing occasional ectopics, it is a safe assumption that the autonomic nervous system did not play a significant role in the generation of these anomalous beats. In this case one can interpolate around these ectopics by taking the average of the R-R intervals immediately preceding and following the beat in question. Subsequently, fast Fourier or autoregressive algorithms can be applied to the corrected data segment. When a more significant number of ectopics are present in a given data segment it is generally recommended that the entire section of data be

excluded from further analysis. Based on more then a decade of experience with HRV data Kamath et al. (1995) have set a limit of no more than 2-3 ectopics within a short-term data segment (<5 min) with no more then 2 anomalous beats in succession. For long-term recordings lasting more than an hour, spectral analysis was limited to data containing less then 20 ectopics/hour. Interestingly, the field of HRV has yet to produce a generally accepted set of standards as to the number of ectopics one can interpolate around or accept without adversely affecting the integrity of the data. Currently, investigators are encouraged to quote the number and/or percentage of inter-beat intervals that are omitted or interpolated around (Task Force 1996).

1.2.4 What do HRV Indices Mean?

The meaning and significance of HRV measures is more complex than is generally appreciated often leading to erroneous conclusions/extrapolations in the literature. Often investigators have ascribed LF and HF power measures to levels of sympathetic and parasympathetic tone respectively (Goldsmith et al. 1992; Stein et al. 1994; Panina et al. 1995). However, there exists no physiological basis for this assertion. Indeed the frequency of resting sympathetic & parasympathetic tone are at higher levels than indicated by either the LF or HF power bands of the heart rate power spectrum. In fact, these values are not so much indicative of autonomic tone as they are representative

of modulation of this tone by separate mechanisms (Malik et al. 1993). The value of the LF and HF components of the heart rate power spectrum may therefore lie in their potential to objectively assess the integrity of distinct physiological mechanisms that regulate heart rate through modulation of sympathetic and parasympathetic tones. Such modulated autonomic activity may be significantly reduced in very different physiological situations. Indeed the vagal peak in the power spectrum is dramatically reduced not only in parasympathetic blockade with high dose atropine but also with constant high levels of stimulation resulting from continuous phenylephrine infusion (Malik et al. 1993). Both situations although they produce opposing alterations in heart rate result in levels of autonomic tone that are unresponsive to modulation. Similarly, the LF spectral peak may be substantially reduced by sympathetic pharmacological blockade (Cowen et al. 1993) as well as the saturating levels of stimulation resulting from maximal exercise (Arai et al. 1989). As a result, power spectral analysis does not provide an accurate measure of the absolute level of sympathetic or parasympathetic tone to the SA node. However, a useful measure of relative activity in the two limbs of the autonomic nervous system may be obtained by expressing the aforementioned spectral components in normalized units (ie. as a percentage of the total area in the spectrum) or as a ratio (ie. low frequency area/high frequency area). In addition, the central frequency of the low frequency power band may prove to be a useful measure of

sympathovagal balance (Kamath et al. 1993). This peak is generally considered to represent combined sympathetic and parasymapthetic modulation of heart rate via the baroreceptor reflex arc (Ori et al. 1992; Task Force 1996). As such, an augmentation of sympathetic activity to the SA node may be manifested by a leftward shift of this peak (toward a lower frequency) due to the relatively prolonged time course of action for the sympathetic neurotransmitter. Conversely, an increase in the level of parasympathetic input to the SA node would result in a rightward shift of this peak. This assertion is supported by the work of Weise et al. (1989) in which a rightward shift of the peak was observed with the administration of low dose atropine which is known to be vagomimetic. Moreover, the administration of moderate dose atropine, a known parasympatholytic agent, produced the predicted leftward shift of the LF peak.

Similarly, absolute levels of autonomic tone can not be inferred from mean heart rate alone due to the complexity and number of the mechanisms that determine mean heart rate (Malik et al. 1993; Guyton & Hall 1996). Although heart rate is largely determined by autonomic balance, a decrease in vagal activity can be compensated for by a decrease in sympathetic activity and the two divisions have proven to be independent to a certain extent. Therefore any number of combinations of sympathetic /parasympathetic tone may produce the same heart rate (Guyton & Hall 1996). Currently, a relative indication of parasympathetic tone can be attained by observing the increase in heart rate

following administration of a sufficiently high dose of atropine. Similarly, an indication of sympathetic tone may be gained by observing the heart rate response to adrenergic blockade with propranolol. In future research it is possible that improved methods for simultaneous analysis of both heart rate and heart rate variability may provide a more accurate indicator of autonomic tone.

1.2.5 Respiratory Sinus Arryhthmia - The Importance of Respiration Data

The beat-to-beat variations of heart rate that occur during the breathing cycle, termed respiratory sinus arrhythmia (RSA) have been well documented (Davies et al. 1967; Katona et al. 1975; Hirsch et al. 1981; Eckberg 1983; Schuhmann et al. 1986; Novak et al. 1993; Brown et al. 1993; Sleight et al 1996). These fluctuations in heart period are believed to be mediated predominantly by cardiac vagal efferents although a slight RSA persists after bilateral vagotomy (Levy et al. 1966). Indeed, Levy et al. (1966) observed these fluctuations of heart rate in the absence of respiratory movements or blood pressure variations in dogs with the respiratory muscles paralyzed. Under the same conditions, deepening anesthesia affecting the respiratory centers resulted in the abolition of these heart period oscillations (Jones et al. 1956). In addition, modulation of heart rate with a time course similar to RSA has been observed at the onset or termination of a prolonged breath hold (Hirsch et al. 1981). RSA is thought to be the result of neural connections between a central respiratory

oscillator and cardiac control centers located in the medulla oblongata (Berne & Levy 1993).

However, it appears that RSA is not completely independent of peripheral modulation. Hirsch et al. (1981) examined the relationship between respiratory sinus arrhythmia, tidal volume & breathing frequency. During spontaneous breathing for any given tidal volume the magnitude of respiratory sinus arrhythmia was found to be stable below breathing frequencies of ~7 breaths/min (0.12Hz). Above this cutoff point, termed the 'corner frequency', the amplitude of the RSA decreases linearly with a slope of ~20.4 dB/decade. A considerable amount of inter-individual variation was found in the measurement of the corner frequency. In addition a direct, positive relationship was found between tidal volume and RSA at any given breathing frequency. Tidal volume had no effect on the slope of the RSA roll-off or the value of the corner frequency. In another study Eckberg (1983) quantified RSA in 6 healthy men and women between the ages of 24-26. The amplitude of RSA was observed to progressively increase as the interval between breaths was lengthened from 2.5-10 seconds. A direct positive relationship between tidal volume and RSA was also noted in this study. However, the author concluded that the latter relationship was of minimal importance as a 50% increase in tidal volume (1000ml-1500ml) was found to increase the average peak-valley P-P interval by only 15%. Eckberg (1983) also examined the average timing of P-P interval lengthening and shortening within

the framework of the respiratory cycle. The onset of P-P interval prolongation was consistently observed after the onset of expiration. However, P-P interval shortening was observed to begin progressively earlier in the respiratory cycle as the interval between breaths was increased. At respiratory intervals above 8 seconds, cardio-acceleration actually preceded the onset of inspiration. The dependence of respiratory sinus arrhythmia on parameters of respiration, (breathing frequency and tidal volume), indicates that the oscillator is subject to peripheral control, perhaps from the respiratory afferent neurons of broncho-pulmonary receptors (Sleight et al. 1996; Novak et al. 1993).

Other indirect mechanisms, including the Bainbridge and baroreflex arcs contribute to the respiratory linked oscillations in heart rate, (Novak et al. 1993). On inspiration, the chest cavity enlarges resulting in a decreased intrathoracic pressure and a subsequent augmentation of venous return to the right atrium. This increase in atrial blood volume has a direct effect on receptors located in the wall of the atrium near the veno-atrial junctions and results in activation of the Bainbridge reflex which is manifested by an increase in heart rate (Berne & Levy 1992; Guyton & Hall 1996). The resultant increase in pulmonary arterial and venous pressure leads to a subsequent augmentation in venous return to the left atrium and an increased left ventricular stroke volume. Consequently, increased pressure in the systemic arteries will result in a stimulation of aortic and carotid baroreceptors. However, the paradoxical action of the baroreflex arc appears to

be insufficient to counteract the described cardio-acceleration. On expiration the intrathoracic pressure returns to baseline and the atria are subsequently unloaded. As a result the heart rate returns to pre-inspiration levels.

In summary, three mechanisms are proposed to account for the coupling of heart rate oscillations with the respiratory cycle (Hirsch et al. 1981). The first mechanism involves the interaction of centrally located oscillatory respiration centers with cardiac control centers in the brain stem. In addition, it appears that blood pressure fluctuations secondary to respiratory movements affect heart period via mechanisms mediated by atrial stretch receptors and arterial baroreceptors. Finally, evidence suggests that thoracic stretch receptors (located in the lungs and chest wall) modulate heart rate as a reflex response to pulmonary inflation.

The impetus behind the aforementioned investigations was provided by an increasing clinical use of HRV indices. In order to accept HRV spectral analysis as a viable diagnostic and prognostic tool those influences that affect the interpretation of the spectral bands must be accurately quantified. In the resting state, average respiratory frequency for a normal healthy individual is between 0.17 & 0.33Hz (Berne & Levy 1992). As previously described the HF peak of the HRV power spectrum is correlated with vagally mediated RSA and is therefore centered around this frequency (Sayers et al. 1973; Askelrod et al 1981; Malliani et al. 1991; Ori et al 1992; Kamath et al. 1995; Cerutti et al. 1996).

However, at respiratory frequencies below ~0.15 Hz the HF and LF peaks of the HRV power spectrum may overlap or become fused into a single large peak. When such entrainment occurs it becomes difficult to distinguish the separate influences of RSA and baroreceptor mechanisms on heart period fluctuations. Therefore in this situation the recording of respiratory parameters is crucial to avoid misinterpretation of the power spectrum, in the direction of decreased vagal modulation of heart rate in individuals that tend to breathe more slowly than expected. As respiratory rate appears to be a more important influence on RSA than tidal volume (Eckberg 1983; Brown et al. 1993), the minimum standard for future studies involving HRV spectral analysis should include some measure of breathing frequency. In a review of the literature between 1966 and 1992, Brown et al. (1993) noted that some form of control or measurement of respiratory rate was evident in only 51% of studies.

1.2.6 Circadian Rhythms in HRV

Similar to most physiological phenomena, heart rate and HRV spectral indices exhibit a distinct and reproducible circadian rhythm. Heart rate, as well as spectral indices commonly attributed to modulation of cardiac sympathetic tone are at a minimum at approximately 4:00 a.m. and rise significantly between the hours of 6-11:00 a.m. reaching a maximal value at approximately 4-5:00 p.m., (Fallen et al. 1995). Conversely, spectral indices indicative of the

modulation of cardiac parasympathetic traffic as well as the standard deviation of R-R intervals display an opposing pattern with maximal values reached during the early morning hours and minimal levels achieved in the late afternoon. The focus of attention on these diurnal rhythms is a result of evidence supporting the assertion that major cardiac events do not simply occur at random times throughout the day but exhibit a similar circadian rhythm with an incidence peak between 6-11:00 a.m. (Kamath et al. 1993). Similar rhythms have been revealed in fibrinogen activity (Andreotti et al. 1988), platelet aggregability (Tofler et al. 1987) and plasma catecholamine levels (Linsell et al. 1985). Therefore, an examination of these rhythms may provide information as to which factors contribute to the susceptibility of the heart to major cardiac events including myocardial infarction and sudden cardiac death.

As important as the clinical information provided by these rhythms is the realization that studies using short term recordings for spectral analysis of HRV must ensure that all patients are tested at approximately the same time of the day, if direct comparisons between subjects are to be made. Such information should be quoted in all HRV research literature to facilitate comparisons between independent research studies.

1.2.7 Use of physiological maneuvers.

Prior to the development of HRV analysis autonomic function was assessed by observing the heart rate and blood pressure response to physiological maneuvers such as the Valsalva maneuver, orthostasis, passive tilt and lower body negative pressure. The same techniques along with pharmacological interventions are used in heart rate variability assessment to examine the autonomic response to various stresses. Orthostasis or passive tilt are utilized widely in the literature due the simplicity of the procedure and the reproducibility of the spectral response. The typical response to standing or passive tilt involves a reduction in vagal input to the heart and a significant sympathetic activation which under normal physiological conditions manifests itself as an increase in LF power spectral indices and a significant reduction in the HF power band (Pomeranz et al. 1985; Malliani et al. 1991; Kamath et al. 1993; Pieper et al. 1995; Task Force 1996). Alterations in this pattern are evident in patients with congestive heart failure, diabetic autonomic neuropathy and those susceptible to vaso-vagal syncope (Lipsitz et al. 1990). As pathological conditions are often not apparent until systems are subjected to physiological stress, investigators have acknowledged that tilt may uncover autonomic dysfunction when resting spectral measures show the individual to be normal (Ori et al. 1992).

1.2.8 Clinical applications

Prognostic & Diagnostic Value

The clinical relevance of HRV analysis was first recognized in 1965 when Hon & Lee observed a dramatic reduction in heart rate variability leading to a metronome like rhythm preceding fetal distress. The investigators hypothesized that as these oscillations have their origin in both limbs of the autonomic nervous system the observed loss of variability may be due to depression of the central nervous system secondary to anoxia. Since that time the prognostic significance of various heart rate variability indices has been investigated in patients following a myocardial infarction (Wolf et al. 1977; Kleiger et al. 1987; Odemuyiwa et al. 1992) and in elderly subject cohorts (Algra et al. 1993; Tsuji et al. 1994). Time domain statistics calculated from 24-hour ambulatory ECG recordings provide the most powerful prognostic indicators. Kleiger et al. (1987) examined heart rate variability in 808 survivors of acute myocardial infarction under the age of 70 years. Time domain indices were calculated from 24-hour ambulatory Holter monitoring and mean follow up time was 31 months. An SDNN of <50 ms was associated with a 5.3 fold increased risk of all cause mortality compared to a corresponding value of SDNN >100ms. Heart rate variability remained a significant marker of mortality risk following adjustment for left ventricular ejection fraction, ventricular premature complex frequency, New York Heart Association

class and drug treatment regime. Hull et al. (1990) developed a canine model for post-AMI sudden death risk classification in conscious dogs. Risk was assessed by a protocol of combined exercise and myocardial ischemia at one month post myocardial infarction. Dogs developing ventricular fibrillation during this protocol were considered high risk for sudden death (susceptible), while survivors were classified as low risk/resistant dogs. No significant differences in HRV indices were observed between these two groups prior to myocardial infarction. Those dogs classified as susceptible to life threatening ventricular arrhythmia's during the ischemic protocol were characterized by depressed time domain HRV values post myocardial infarction. In contrast myocardial infarction had no effect on any of the measured HRV variables in resistant dogs.

In a study of 736 elderly (mean age 72±6 years) subjects enrolled in the Framingham heart study, Tsuji et al. (1994) examined the relationship between frequency/time domain indices of HRV and all cause mortality during a four year follow up. The first two hours of the 24-hour ECG recordings were selected for analysis. Following adjustment for major relevant risk factors, (including diabetes and hypertension), total power, very-low-frequency power, low-frequency power, high-frequency power and standard deviation of total normal R-R intervals were all associated with a significant increase in all-cause mortality risk. In another study Algra et al. (1993) examined HRV using time domain indices in 6,693 patients who had 24-hour ECG Holter monitoring at four

participating Rotterdam hospitals between 1980 and 1984. Two hundred and forty-five of these patients died suddenly during a two year follow up period. Low short term variability (mean of per-minute heart rate standard deviations over 24 hours <25 msec) was associated with a 4.1 fold higher risk of sudden death compared to patients in which this index was ≥40msec. In addition, depressed long term variability (standard deviation of per-minute means of R-R intervals <8 msec) was also associated with a significant increase in the risk of sudden death (relative risk: 4.4). The predictive power of both of these prognostic indicators remained significant after adjustment for age, evidence of cardiac dysfunction and a history of myocardial infarction.

Investigation of Autonomic Dysfunction

HRV measures have also proven to be useful in the investigation of autonomic dysfunction associated with a number of disorders and pathological conditions including diabetes mellitus, congestive heart failure and chronic renal failure. Investigations such as these have provided invaluable clues as to the extent and locus of the dysfunction and provide a physiological basis upon which clinical trials may be constructed. Peripheral neuropathy is a frequent complication of diabetes mellitus with observed frequencies ranging between 5-60% (Olefsky 1985). Symptoms such as postural hypotension and bowel dysfunction have long suggested involvement of the autonomic nervous system.

With the advent of HRV power spectral analysis, the extent of autonomic neurocardiac dysfunction can now be quantified in these individuals. Pagani et al. (1988) examined the power spectral density of beat-to-beat heart rate variability in 49 diabetics and 40 age matched healthy controls. In addition to a significant depression of R-R variance at rest, Pagani et al. (1988) observed an altered response of the spectral indices of both sympathetic activation and vagal withdrawal to orthostasis in diabetics compared to controls. Often this spectral alteration with passive tilt in diabetics involves a reduction in the LF:HF area ratio, a response opposite in direction to that commonly observed in normal healthy subjects. In an earlier study, Lishner et al. (1987) noted that depression of power spectral measures, although observed across all frequency bands was most dramatic in the high frequency peak attributed to vagally mediated respiratory sinus arrhythmia.

Investigators in these and similar studies have concluded that power spectral analysis of heart rate variability may be of clinical importance in future screening of diabetics for autonomic dysfunction and serve as a convenient follow up measure. In addition, these findings may provide important clues as to the location and mechanism of autonomic lesions in diabetics. These authors (Lishner et al. 1987; Pagani et al. 1988) cite the more thoroughly quantifiable nature of HRV measures and the lack of required active collaboration on the part of the patient as factors supporting the clinical use of this tool.

Congestive heart failure is a common endpoint in a number of cardiovascular disorders and represents a clinical syndrome in which dysfunction in one or both limbs of the autonomic nervous system are often present (Eckberg et al. 1971; Ferguson et al. 1990). Saul et al. (1988) examined the neuro-humoral regulation of cardiovascular function by power spectral analysis of HRV in 25 patients (ages 24-72) with class III or IV chronic stable congestive heart failure. Comparisons were made with 21 control subjects (ages 28-79) with no significant cardiac or other medical problems. Spectral analysis, performed on 3 to 6 segments of 15 minutes selected at 3 hour intervals from a 24-hour Holter recording revealed a significant depression in power across the entire frequency band (0.01-1.0Hz). This reduction was most marked in the band normally attributed to those fluctuations mediated predominantly by the parasympathetic limb of the autonomic nervous system (>0.04Hz). The authors concluded that these findings were consistent with abnormal baroreflex responsiveness to physiological stimuli and suggest that there is a diminished vagal, yet relatively well preserved sympathetic modulation of heart rate in patients with CHF. In a more recent investigation, Panina et al. (1995) examined the diurnal variation of HRV measures derived from 4 minute 'epochs' selected from each hour of a 24-hour Holter ECG recording in 20 patients with New York Heart Association class II or III congestive heart failure. Despite the observation of an intact and well preserved circadian heart rate rhythm in these individuals, none of the spectral

measures (both sympathetically and parasympathetically mediated) exhibited the 24-hour variations commonly found in healthy control subjects. As such the authors suggested that congestive heart failure at this stage is characterized by a sustained imbalance of sympathetic and parasympathetic tone, a condition that may contribute to the existing proarrhythmic environment and may help determine the progression of symptoms and survival duration of these patients.

Uremic neuropathy, most likely a result of the buildup of metabolic wastes remains a major complication of end-stage kidney failure (Axelrod et al. 1987). Evaluation of its severity has been typically based on tests examining changes in heart rate in response to some cardiovascular perturbation (eg. Valsalva maneuver, orthostatic stress etc.). As HRV measures involve steady state measures and may provide some additional information regarding the autonomic lesions, Axelrod et al. (1987) reasoned that such techniques may provide a more clinically useful tool for the assessment and follow up of these patients. Power spectral analysis of HRV was performed in 25 patients (ages 20-82) with end-stage renal failure. Comparisons were made with 22 healthy controls (ages 38-79). Frequency domain analysis revealed a significant reduction in power across all frequency ranges of the heart rate power spectrum of patients compared to those of controls. Interestingly, this depression appeared to be more severe in those patients undergoing treatment with dialysis. However, this effect may simply represent a more advanced stage of the disease in such patients. The

authors concluded that dysfunction resulting from chronic renal failure was manifest in both limbs of the autonomic nervous system.

Evaluation of Treatment Modalities

Similarly, heart rate variability analysis has demonstrated that the effectiveness of various pharmacological agents and other therapeutic modalities can be evaluated objectively. Bigger et al. (1991) examined the 24-hour heart rate power spectrum in 68 post-AMI patients at 0, 3, 6, 9 and 12 months after enrollment in the study (enrollment was 25±17 days post-MI). Comparisons were made with 95 'normal' individuals of similar age and gender. At baseline, all frequency bands showed a significant power reduction in patients compared to controls. There was a substantial increase in power across the entire spectrum between baseline and 3 months, after which time the measured indices remained relatively stable. However, at twelve months all HRV measures were still only 50-66% of the values observed in the control subjects. It was apparent that the recovery of HRV appears to be completed during approximately the same time period in which post-MI mortality rates drop to a stable value. However, the authors acknowledged that this association may be coincidental rather than causal. In addition, Ori et al. (1992) examined the short-term effects of dialysis on HRV measures in five chronic renal failure patients. Three distinct responses were noted: 1) no change; 2) transient improvement during dialysis

with rapid reversion; and 3) sustained increases in HRV measures over periods of up to 36 hours. These findings illustrate the potential of power spectral analysis as a diagnostic tool to assess the efficacy of dialysis as well as to evaluate the nature and permanence of the existing dysfunction.

Recently a great deal of attention has been paid to the role of exercise rehabilitation in post MI patients. One possible benefit of such an approach to cardiac disease management lies in the potential of aerobic exercise to increase the basal level of vagal activity in these individuals. Indeed, Dixon et al. (1992) noted augmented power in the HF band and diminished LF power during 45 minutes of supine rest in 10 male endurance athletes compared to 14 male sedentary controls. If results similar to these can be achieved in post-MI patients the enhanced vagal activity may provide a protective effect against life threatening dysrhythmias by raising the fibrillation threshold. Protective alterations in this threshold with direct electrical stimulation of the vagus nerve have been previously observed in both dog (Kent et al. 1973) and cat (Zuanetti et al. 1987) models. In situations such as these, HRV analysis would prove to be a useful tool for the assessment and monitoring of such exercise related alterations in autonomic balance.

1.2.9 HRV in Cardiac Transplantation

Cardiac transplantation is a unique situation in that it produces a heart in a completely dennervated state and as such provides the ideal paradigm to test the hypothesis that power spectral analysis of heart rate variability represents a noninvasive signature of autonomic cardiac regulation. Fallen et al. (1988) performed power spectral analysis on heart rate time series in nine cardiac transplant patients. This investigation revealed a broad based power spectra, (resembling white noise) with low intensity peaks in eight subjects. However, frequency domain analysis of a ninth patient revealed a power spectra similar to those typical of healthy control subjects. In addition this patient showed normal HRV responses to metronomic breathing, atropine infusion and orthostatic stress. The investigators concluded that power spectral analysis of heart rate variability provided evidence suggesting functional reinnervation following cardiac transplant in this patient and may therefore prove to be a valuable clinical follow-up tool in these populations.

1.2.10 Summary

In summary, HRV analysis has proven to be a viable research tool for the investigation of autonomic dysfunction in a wide variety of subject populations. Furthermore, the potential of this technique as both a diagnostic tool and prognostic indicator in cardiology and neurology has been demonstrated through

numerous physiological and clinical trials. For these goals to be recognized, collaborative efforts aimed at widespread standardization of the various analysis techniques and more clearly defined quantitative and qualitative interpretations of the individual measures are of the utmost importance. Such an effort has recently been spearheaded by the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (Task Force 1996).

1.3 AGING, GENDER AND HEART RATE VARIABILITY

1.3.1 Development of the Autonomic Nervous System

The initial contractions of the human heart occur at approximately 22 days post-fertilization (Pappano et al. 1977). At this time no neural fibres are evident in the myocardium and the heart beats independent of autonomic modulation. Infiltration by neural fibres is evident at around the 37th day after conception and during the following 7 weeks an epicardial plexus develops (Smith 1970). At the end of the embryonic period (8 weeks) fibres from the right vagosympathetic tract enter the SA node and interventricular septum (Gardner et al. 1976).

Parasympathetic Development

Acetylcholinesterase has been detected in the myocardium of chick and rat embryo's prior to the differentiation of parasympathetic nerve fibres and

production of acetylcholine (Zachs 1954; Gyevai 1969). Consequently, the possibility of an inductive action has been considered for this enzyme (Zachs 1954). The morphological and functional development of parasympathetic cardiac innervation is generally believed to precede that of the sympathetic system (Walker 1975; Pappano et al. 1977). Walker (1975) examined the contractile behavior in response to electrical field stimulation of 21 left atrial preparations from human fetal hearts obtained via therapeutic abortions. Fetuses ranged in size from 7.7 to 18.5 cm, (crown rump length). No response to field stimulation was evident in the seven atrial preparations of less than 13-14 weeks post-conceptional age, suggesting that the neurons are not functionally mature at this stage. In atrial samples of greater than 14 weeks of age the presence of a negative inotropic effect was generally apparent prior to the appearance of a positive effect. Abolition of this phenomena with atropine confirmed that these observation were due to the action of the cholinergic neurotransmitter acetylcholine. Direct administration of low-dose nicotine failed to induce a similar diminution of the contractile response indicating that the ganglia are functionally immature at this time. Thus, vagal transmission from higher centers to the heart is unlikely prior to 20 weeks of age. Existing parasympathetic fibres undergo considerable differentiation beginning at 24 weeks and continuing through the first 10 weeks post-partum. (Porges et al. 1986). During this time substantial gains in fibre diameter and myelination are

achieved. Histological research indicates that by this time vagal tracts morphologically resemble those found in adolescents (Porges et al. 1986). As such, further alterations in parasympathetic function may reflect developmental changes in higher control centers or the various components of cardiovascular reflex arcs.

Sympathetic Development

The capacity to synthesize norepinephrine has been noted in human fetal hearts as early as 13 weeks of age (Genner et al. 1970). However, this finding does not necessarily indicate the presence of adrenergic nerves in the myocardium at this time. Indeed, an excitatory inotropic response to high-dose nicotine in an atrial preparation from a small fetus (7.7 cm) was noted by Walker (1975) prior to the appearance of sensitivity to field stimulation. As this effect was abolished by propranolol it was likely to have been mediated by norepinephrine released from some non-neuronal source. Chromaffin tissue which has been detected prior to development of the adrenergic nerve plexus is thought to serve as the principal site of norepinephrine storage in the early fetal heart (Barry et al. 1950). Histological studies utilizing the Falck-Hillarp formaldehyde method for identification of catecholamine containing cells have revealed either few (in fetal hearts of 10-16 weeks, Partanen et al. 1974) or no (ages 8-18 weeks, Dail et al. 1973) sympathetic neurons at this stage of

development. In addition, the cells that were detected were small, globular and lacked terminal nerve fibres. Thus, it was concluded that cardiac activity in humans is modulated by humoral adrenergic mechanisms during the first half of the gestation period (Partanen et al. 1974; Dail et al. 1973). Development of the sympathetic nervous system continues to lag behind that of the parasympathetic system and in general adrenergic innervation of the mammalian heart at birth is poorly developed (Pappano et al. 1977). As a result, post-partum alteration in cardiac sympathetic function may to a certain extent be due to morphological differentiation of the nerve tracts themselves.

The absence of a contractile effect (positive/positive & negative) in 16 of the 21 atrial samples examined in the previously mentioned study by Walker (1975) did not appear to be due to insensitivity of the myocardial receptors to the transmitter substances. In fact, all fetal preparations responded when acetycholine or norepinephrine were added directly to the organ bath. Thus, both adrenergic and cholinergic cardiac receptors are present and functional at an early stage of fetal development.

1.3.2 Early HRV

Fetal HRV

Until recently the scope of information on fetal heart rate variability has been limited. This lack of knowledge has been in large part due to the

substantial methodological difficulties associated with fetal ECG monitoring. Acquisition of a fetal electrocardiographic waveform via an abdominal lead placement is confounded by background noise due to fetal movement (Widrow et al. 1985). In addition, the amplitude of the mother's ECG waveform is often 2-10 fold greater than that of the fetus and may interfere with the recording (Widrow et al 1985; Akselrod et al. 1987). However, recent times have seen the development of algorithms that facilitate the accurate detection of beat-to-beat fluctuations in fetal heart rate from ECG obtained through the abdominal wall (Widrow et al. 1985; Akeslrod et al. 1987). These techniques typically involve the recording of maternal ECG through four chest leads. The acquired signals subsequently serve as reference inputs to a filter that cancels out the maternal contribution to the combined waveform obtained from the abdominal lead (Widrow et al. 1985).

Since their advent, the aforementioned ECG monitoring techniques have served to shed considerable light on the functional development of the autonomic nervous system in the human fetus (Botts et al. 1978; Wheeler et al. 1980; Dawes et al. 1981; Divon et al. 1984; Karin et al. 1993). Karin et al. (1993) examined fetal heart rate variability in 10 young (gestational age [GA] = 23.5±1 wks) and 22 mature (GA = 39.75±1.5 weeks) pregnancies. In general, young fetuses demonstrated greater spectral power (0.2-1.1 Hz) compared to those in the mature group. This finding is believed to reflect the relatively less organized

patterns of neural activity present at such an early stage of development. In addition, spectral power in both groups was significantly higher during activity periods compared to sleep states. In all fetuses, HRV analysis of 256 second long ECG data segments failed to reveal a high frequency peak attributed to respiration. However, fetal breathing tends to be episodic in nature (Devoe et al. 1989) occurring only 25% of the time for periods of ~30-60 seconds. Consequently, processing of a longer data segment may tend to obscure a respiratory peak if present. Thus, in an attempt to characterize respiratory related fetal HRV Karin et al. (1993) performed fast Fourier transforms on shorter 64 sec sub-segments of the original 256 sec data sets. This analysis revealed respiratory peaks centered around 0.7 Hz in some of the data segments recorded during sleep. The location of the central frequency of this peak is consistent with typical fetal respiratory rates (49±10 breaths/min) reported by Devoe et al. (1989). No such peaks were detected during active periods in any of the fetuses. Compared to adult spectra, power in the respiratory frequency band was not as powerful/ focused and often displayed a bi-modal distribution. Spectral patterns such as these are likely indicative of less organized fetal respiratory movements.

In a similar investigation Divon et al. (1985) examined fetal HRV in 16 pregnant women at term. Electrocardiograms were obtained from fetal scalp electrodes at least two hours after rupture of the membrane and prior to the

onset of uterine contractions. Respiratory movements were monitored via a real time ultrasound scanner with the transducer placed on the maternal abdomen. In this subject group the onset of respiratory activity was associated with a significant increase in both fetal heart rate and HRV. Although the clinical significance of the previously described research has yet to be fully defined it seems reasonable that combined monitoring of heart rate and respiration may prove to be useful indicators of fetal well being.

HRV in Infants

Similar to fetal investigations, assessment of autonomic function by HRV analysis in infants is subject to methodological restrictions. Many established testing protocols (eg. cardiovascular response to orthostasis/Valsalva maneuver) require active subject participation and are therefore of limited use in such a young subject group. As a result, the majority of studies on infant HRV have examined subjects during sleep or active periods in the supine position.

Studies of pre and full-term infants (Richards et al. 1989; Schechtman et al. 1989; Conny et al. 1991; Thompson et al. 1993; Chatow et al. 1994; Snidman et al. 1995) have identified the peri-natal period as a time of rapid alteration in cardiac autonomic function. Chatow et al. (1994) compared heart rate variability power spectra in four groups of pre and full-term infants. The groups (A-D) comprised: group A: 7 one day old pre-term infants (GA=34-35 weeks); group B:

28 pre-term infants (GA=27-34 weeks at delivery) recorded 1-7 weeks post-partum; group C: 7 one day old full-term infants (GA=39-41 weeks); and group D: 6 pre-term infants (GA=27-34 weeks at delivery) recorded at 5-14 weeks post-partum. Groups were organized in this manner such that groups A&B and groups C&D were of comparable chronological age (CA=GA + Post-natal age [PA]) yet differed in GA and PA. Spectral analysis revealed an age-related decline in both HR and LF:HF area and a concurrent progressive increase in HF power. The authors suggested that such alterations indicate a shift in autonomic balance to a predominance of parasympathetic activity during this early stage of development. In addition, it appears that vagal control may develop better in the womb as subjects with similar CA's but greater PA's tended to have higher resting heart rates. Respiratory related HRV in this subject population progressed with age from the bi-modal power distribution seen in fetal spectra (Karin et al. 1993) to a single peak similar to but more dispersed than those seen in adult subjects.

Continuing development of HRV was assessed on a longitudinal basis in infants between the age of 1 week and 6 months by Schechtman et al. (1989). In general, spectral power in all frequency bands was shown to decrease significantly over the first month of life. At this time the trend was reversed and these indices climbed significantly with increasing age over the following 5 months. The time course of these alterations showed significant variation across

frequency bands and sleep/wakefulness states (awake, quiet sleep, REM sleep). Thus, it appears that maturation of autonomic control mechanisms is a process characterized by substantial inter-individual heterogeneity.

Much of the impetus behind HRV investigation in infants lies in the potential of indices of autonomic function to predict future temperament and behavioral reactivity (Richards et al. 1989; Snidman et al 1995). In a longitudinal study, Snidman et al. (1995) examined the relationship between HRV indices and temperament in 112 infants from the end of the fetal period to 21 months of age. The temperamental characteristics of low/high reactivity and fear of the unknown were assessed with a battery of standardized behavioral tests. A general, age-related lengthening of heart period and enhancement of power in both the LF and HF bands were observed in these subjects. These findings were consistent with previous research (Porges et al. 1986; Schechtman et al. 1989) and have been attributed to the augmented effects of parasympathetic influence, increased body size and brain maturation. No significant differences in mean heart period were observed among infants grouped according to temperament scores. However the authors noted that a significantly greater portion of the low reactivity subjects had a fetal heart period above the median compared to those in the high reactivity group. A significant positive relationship between power in the LF band during sleep at 2 weeks of age and fear scores at both 14 and 21 month old infants was observed. In addition, a similar

relationship existed between the LF power response to orthostasis (at 2 weeks) and later fear scores. Interestingly, cardiac data obtained after 4 months of age failed to show any significant relationship to later behavioral status. Based on these observations, the authors suggested that cardiac activity in the first weeks of life may contain unique information pertaining to the autonomic profile and future psychological status of the infant.

In summary, early development is characterized by rapid and heterogeneous alterations in cardiac autonomic function. Despite considerable methodological difficulties HRV analysis has proven to be a viable tool for the assessment of neurocardiac control mechanisms in this population. More importantly, such analyses may provide future clinicians with a powerful technique for the prediction of behavioral and psychological outcome in infants.

1.3.3 HRV in Childhood and Adolescence

The childhood years are a time of continuing refinement in autonomic cardiac control (Finley et al. 1987; Korkushko et al. 1990; Finely et al. 1994). In general the most substantial alterations in HRV indices occur between the ages of 5 and 12 years after which these measures become somewhat stabilized. In a study of 29 healthy individuals ranging in age from 5 to 24 years, Finley et al. (1987) observed a significant age-related diminution of both LF power and the LF:HF area ratio (most substantially between 5-12 years). In addition, the LF

power response to orthostatic stress was most dramatic in 5-7 year olds and declined progressively thereafter. These findings appear to indicate a general decline in autonomic modulation of heart rate (the effects of which are most marked in the sympathetic limb) between the ages of 5-12 years. However, the authors have acknowledged the existence of other, non neural factors that may account for a considerable portion of the observed effect. The volume of the heart increases substantially between the ages of 5-20 years (Henry et al. 1980). A heart of larger volume may be less responsive to fluctuations in venous return that mediate variability of the heart rate through atrial stretch receptors (Melcher 1976). In addition, Finley et al. (1987) have suggested that there may be developmental alterations in physical cardiopulmonary mechanisms involved in respiratory sinus arrhythmia or venous tone (a contributor to variability in the lower frequency range).

Heart rate variability analysis provides evidence supporting the refinement of cardiac autonomic control during childhood. However, the ability to draw conclusions based on this data is limited by the presence of extraneous physical/mechanical factors. Further research involving concurrent echocardiographic monitoring and/or pharmacological interventions may help to clarify the appropriate interpretation of the observed alterations in the HRV power spectra.

1.3.4 Aging Effects on HRV - Adults & the Elderly

A progressive age-related decline in autonomic function (manifested as diminished HRV in both the time and frequency domains) has been well documented (Waddington et al. 1978; Weiling et al. 1982; Pfiefer et al. 1983; O'Brien et al. 1985; Gautschy et al. 1986; Jarisch et al. 1987; Simpson et al. 1988; Lipsitz et al. 1990; Shannon et al. 1990; Korkushko et al. 1991; Schwartz et al. 1991; Zeigler et al. 1992; Ryan et al. 1994; Yo et al. 1994; Craft et al. 1995; Liao et al. 1995; Byne et al. 1996; Yamasaki et al. 1996). However, a global statement such as this does not accurately reflect the considerable heterogeneity inherent in the aging process.

Supine & Standing State

Schwartz et al. (1991) investigated the relationship between age and HRV indices in 56 healthy subjects ranging in age from 20-81 years. Both HF and LF spectral content in the supine and standing positions were progressively diminished with advancing age. Nevertheless as both of these phenomena followed similar time courses the LF:HF area ratio was unaffected in elderly subjects. In a similar investigation Yo et al. (1994) failed to detect any age-related alteration in autonomic balance in 31 normotensive subjects during supine rest. Based on these findings the authors concluded that despite a

general decline in autonomic function in the elderly, the balance between the sympathetic and parasympathetic limbs is maintained throughout adulthood.

A number of studies do not support this viewpoint. Several investigations have noted distinctly different patterns of age-related decline in the HF and LF frequency bands often resulting in a shift of autonomic balance to a state of sympathetic predominance (Shannon et al. 1985; Lipsitz et al. 1990; Korkushko et al. 1991). Shannon et al. (1985) performed spectral analysis on heart rate data recorded during both supine rest and free standing in 33 healthy volunteers between the ages of 9-62 years. High frequency power in both postures decreased linearly between 9-28 years of age showing no significant change thereafter. In contrast, LF spectral content showed a progressive, linear decline throughout the entire age range. Likewise, Korkushko et al. (1991) reported a non-uniform impairment of the sympathetic and parasympathetic influences on heart rate resulting in a predominance of sympathetic activity after the age of 50 years.

Unfortunately, direct comparisons among the studies described above are limited by variations in both the methods used to compute power spectra (fast Fourier transform vs autoregressive algorithm) and the boundaries defined for the LF and HF frequency bands. Such contrary findings illustrate the need for further study to clarify the effects of aging on neurocardiac regulation. In addition, such an endeavor would be invariably aided by the establishment of

standards for the practice of HRV power spectral analysis in academic/clinical research.

Response to Orthostatic Stress

The cardiovascular response to standing/passive tilt is a well established test of autonomic function. As such it has been applied by numerous investigators to the study of interactions between aging and neurocardiac control mechanisms (Jarisch et al. 1987; Simpson et al. 1988; Lipsitz et al. 1990; Schwartz et al. 1991; Yo et al. 1994; Bootsma et al. 1995; Bryne et al. 1996). A summary of the various findings is provided below.

A number of studies report an age-related attenuation of the HRV response to orthostasis in one or both of the spectral bands (LF and HF) resulting in diminished shift of autonomic balance (toward sympathetic predominance) in elderly individuals (Jarisch et al. 1987; Lipsitz et al. 1990; Yo et al. 1994). Lipsitz et al. (1990) compared the HRV response to passive 60° head-up tilt in 12 healthy young (18-35 years) and 10 healthy older (71-94 years) subjects. During the tilt protocol a significant increase in both total and LF power was observed in the young subjects whereas these alterations were absent in older individuals. Spectral content in the HF band remained unchanged in both age groups throughout the procedure. Similarly, Yo et al. (1994) reported that

the percent HF power and LF:HF area response to 60° head-up tilt was diminished with age.

Schwartz et al. (1991) also reported a significantly diminished LF power response to orthostasis in older healthy subjects compared to younger volunteers. However, in contrast to the aforementioned research, when this alteration was normalized by expressing LF power as a percentage of the entire frequency band (%LF) the age-related decline was no longer evident. In a more innovative study Bootsma et al. (1995) noted that both the HR and percent LF responses to orthostatic stress were unaltered by age when a gradual passive tilt protocol was used. In this investigation 80° head-up tilt was achieved via a progression through 13 incremental angles with an adaptation period of 6 minutes at each position. Thus it appears that the limitations in the capacity of the older cardiovascular system to meet the challenges of orthostatic stress are partially determined by how rapidly these adaptations must occur. Interpretation of the existing data is confounded by the same methodological inconsistencies as were previously described in the section on the supine/standing states.

Long Term HRV

Age associated alterations in long term HRV derived from 24-hour Holter monitoring merits further investigation because the most significant prognostic indicators are consistently identified within these data sets (Kleiger et al. 1987;

Algra et al. 1993). Recently, Yamasaki et al. (1996) evaluated the diurnal variation of frequency domain variables in healthy males and females between the ages of 20-78 years. The circadian profiles of the LF and HF spectral measures were found to be prominent in both males and females of the younger age groups. However these rhythms were practically non-existent in the older individuals. Further research is required to more clearly define age-related norms for long term variability in both the frequency and time domains and to establish the physiological and clinical implications of these findings.

Physiological Mechanisms

A number of studies/reviews have evaluated a wide spectrum of tests in an attempt to generate a comprehensive hypothesis regarding the mechanisms that account for the observed age-related alterations in cardiac autonomic function (Pfiefer et al. 1983; Appenzeller 1992). Pfiefer et al. (1983) examined indirect measures of sympathetic cardiovascular (plasma norepinephrine and mean arterial pressure) and adrenomedullary activity (plasma epinephrine) as well as cardiac parasympathetic activity (RSA during β-blockade) in 103 healthy males (19-82 years of age). No significant association between age and plasma epinephrine level was observed. However, parasympathetic influence was diminished with advancing age whereas both measures of sympathetic cardiac activity were enhanced in elderly subjects. Interestingly, measures of general

sympathetic (dark adapted pupil size with administration of atropine) and parasympathetic activity (latency time for pupil response to light stimulus) were both diminished with advanced age. Thus the investigators concluded that the aging process is associated with a general decline in peripheral nerve function. The observed augmentation in cardiovascular sympathetic activity is thought to be a compensatory response to diminished baroreceptor sensitivity. Such an assertion is supported by reports of diminished cardiovascular response to lower body negative pressure (Collins et al. 1980) and phenylephrine infusion (Porges et al. 1986) in elderly subjects.

The mechanism responsible for this age-related decline in baroreceptor function may be at least partially independent of alterations in the autonomic nervous system. Associated with aging is an uncoiling and fracturing of elastic fibres in the walls of blood vessels (Porges et al. 1986). In addition, there is an increase in the collagen matrix and a progressive calcification of the vessel media. The resultant decrease in distensability ensures that baroreceptor sensory endings located in the vessel walls will receive a diminished stretch stimulus at any given level of blood pressure. Consequently, the afferent input from the baroreceptors is representative of a pressure lower than the actual pressure within the vessels and a compensatory elevation of efferent sympathetic activity is evoked by the autonomic control centers.

Additional mechanisms have been proposed to account for the aforementioned decline in neurocardiac function. On the basis of reduced heart rate response to infusion of isoprotenerol, Vestal et al. (1979) reasoned that there was a deficit in β-adrenoreceptor sensitivity in elderly populations. In a more recent study, Stratton et al. (1992) reported similar findings in a sample of 15 older (60-82 years) and 17 young (24-32 years) healthy males. Such an alteration would result in a decreased myocardial response to a given level of sympathetic stimulation. A scenario such as this is consistent with seemingly contrary reports of both reduced low frequency heart rate variation (Shannon et al. 1985; Lipsitz et al. 1990; Korkushko et al. 1991) and increased plasma norepinephrine (Pfiefer et al. 1987) in older individuals compared to their younger counterparts.

Finally, these alterations may also be due to morphological changes in the autonomic nerve fibres/plexuses themselves. Korkushko et al. (1991) suggested that the impairment of heart rate oscillations related to the HF power which was observed in the elderly subset of their sample population may be caused by degenerative changes known to occur in the cholinergic cardiac plexus after the fourth decade of life. Autonomic cell loss in old age may also account for the considerable variability present in the aging and HRV literature. Indeed, Cowen (1993) pointed out that cell loss is not a general feature of the aging autonomic nervous system. In fact age-related cell death is an uneven process that may

lead to losses of ~40% in some groups of neurons while at the same time leaving others relatively unaffected. Cowen (1993) proposed that both the development and maintenance of autonomic neurons is controlled via trophic factors released by the target organ itself. Consequently, cell death may be a result of dwindling production of these factors due to an age-related metabolic down-regulation in the heart. The considerable heterogeneity inherent in this process might stem from competition for these diminished resources by distinct cell groups, the outcome of which may tip a fragile equilibrium in a number of different directions.

A comprehensive understanding of age-related alterations in autonomic neurocardiac function must take into account the broad range of aforementioned experimental findings and physiological mechanisms. Previous research has focussed on the differences between distinct age groups. However, if the non-homogeneous nature of the aging processes affecting autonomic nervous system function are to be fully appreciated, future studies must examine as well the variability present within a given group.

1.3.5 Gender & HRV

Traditionally, human research has been performed on young predominantly male subject groups. More recently however there has been a realization that physiological processes do not necessarily act uniformly across

gender. As such it is crucial that established standards for HRV in healthy individuals take in to account not only age but gender as well if they are to provide a valid comparison group for future research and/or clinical practice.

A limited number of HRV based studies have endeavored to address the issue of gender differences in autonomic function (Gautschy et al. 1986; Ziegler et al. 1992; Ryan et al. 1994; Liao et al. 1995; Bryne et al. 1996; Burke et al 1996; Yamasaki et al. 1996). Liao et al. (1995) examined spectral indices of HRV during supine rest in a large sample of healthy men and women (n=1,984) between the ages of 45-64 years. No gender differences were noted in the HF power band. However, women had a significantly lower LF power and therefore a greater ratio of HF:LF power compared to their male counterparts. In addition Yamasaki et al. (1996) noted substantial gender related discrepancies in the circadian rhythm of HRV measures. The sympathetic activation typically observed during the morning hours was delayed in females with LF power reaching its highest values between 1200-2400 hours (compared to 0800-1200 hours in males).

In addition, it appears that the aging process may act heterogeneously on the autonomic nervous system of men and women. Bryne et al. (1996) noted a more rapid regression of heart rate variations centered around 0.1 Hz in men compared to women. No such difference was noted in the age-related decline of respiratory related oscillations. Similarly, Ryan et al. (1994) reported that the

age-related reduction in approximate entropy (a measure of non-linear heart rate dynamics) was significant in male subjects only.

Not all findings are consistent with the existence of gender differences in neurocardiac control. In fact, Ziegler et al. (1992) failed to demonstrate any significant discrepancy between men and women on an extensive battery of autonomic function tests. Likewise, Gautschy et al. (1986) found no evidence of gender related differences in the age-related decline in HRV (measured as the standard deviation of R-R intervals). If differences in HRV do in fact exist, it is by no means a foregone conclusion that these discrepancies are a result of differential autonomic function. Indeed, Burke et al. (1996) observed significantly longer sinus cycle length in males both before and after double autonomic blockade. Thus the investigators concluded that these heart rate differences were not a result of discrepancies in autonomic tone. Rather, as these effects were also strongly related to exercise capacity, it was suggested that the observed phenomena might be a result of intrinsically mediated effects of exercise on heart rate.

1.4 STATEMENT OF PURPOSE

HRV analysis has the potential to be a useful and viable tool for the investigation of autonomic modulation of cardiac function in both health and disease. However, its effectiveness in both clinical and scholastic arenas would

be enhanced by a more precise definition of 'normality'. Thus, the purpose of this thesis was to examine the alterations in autonomic function related to 'healthy' aging as assessed by HRV methodology. The investigation was carried out with the objective of obtaining reference standards with respect to both age and gender for academic/clinical use. As such, tests of autonomic function which are simple, well established and with previously demonstrated prognostic/diagnostic implications were selected for study. The hypothesis is that advanced age will be associated with: 1) alterations in both sympathetic and parasympathetic modulation of the heart; 2) a reduced baroreceptor sensitivity manifested as an attenuated spectral response to orthostatic stress; 3) a significantly blunted circadian variation in spectral measures; and 4) substantial heterogeneity in the aforementioned processes resulting in differential alterations in those measures mediated by the parasympathetic and sympathetic limbs of the autonomic nervous system respectively. The secondary hypothesis is that the aforementioned age-related alterations in autonomic function will demonstrate considerable non-uniformity across gender.

2.0 METHODS

2.1 Subjects - Recruitment & Screening

A total of 123 healthy subjects (51 males and 72 females) ranging in age between 5 and 78 years volunteered to participate in this investigation. Subjects were from pediatric (PED, 5-12 years, n=22, 10 male, 12 female), adolescent (ADO, 13-17 years, n=21, 8 male, 13 female), adult (ADL, 18-30 years, n=26, 13 male, 13 female), middle aged (MDA, 31-60 years, n=24, 9 male, 15 female) and elderly (ELD, 61+ years, n=30, 11 male, 19 female) age groups. Subjects over the age of 55 years were recruited from the McMaster University Seniors Exercise Program. The remaining participants were recruited from the undergraduate/graduate student populations, faculty and staff at McMaster University or from local public schools. The required sample size was determined by performing a power analysis on data collected from the first 32 subjects (Statistical Power Software, LEA Associates, Hillsdale, NJ, USA). This analysis estimated that a minimum of 100 subjects would be necessary to provide sufficient power ($r>0.80$) for the detection of large and medium effect sizes among the ten groups (age [5] $\times$ gender [2]). The accuracy of this estimate was confirmed by performing a second power analysis on the completed sample ($n=123$, $r=0.97$ for large effect size; $r=0.91$ for medium effect size).

Participants were initially contacted and interviewed by phone to provide information regarding the purpose and procedures involved in the study and to ensure that all general inclusion criteria were met. The investigation required that all subjects were healthy, non-smokers with no history of cardiovascular disease (including hypertension) or diabetes mellitus. The presence of any of the aforementioned conditions, a seated resting blood pressure over 160/95mmHg (Kumar et al. 1992), or a BMI of greater than 25 kg/m^2 resulted in exclusion from the study. In addition, subjects with allergies to adhesives or those who felt they would have difficulty abstaining from heavy physical activity during the 24-hour recording period were withdrawn from the study. Subjects were not on any cardioactive medication at the time of the recordings. Volunteers from the MAC seniors program (those over the age of 55 years) had all been subject to a symptom limited ECG stress test prior to entrance into the program to rule out the presence of silent heart disease.

2.2 Testing Protocol

All subjects were tested in the Clinical Neurocardiology lab at the McMaster University Medical Centre (MUMC). On arrival at the lab subjects were provided with ample time to carefully read the consent form (Appendix A) and ask any questions pertaining to procedures and access to results as well as any risks inherent in the study. Informed consent was obtained from all

participants prior to the commencement of any testing procedures. This investigation was approved by the research ethics committee at the MUMC.

2.2.1 Acute Recordings

All acute recordings were performed between 8:30 a.m. and 10:30 a.m. to minimize the influence of the circadian rhythms that exist in heart rate and HRV indices (Fallen et al. 1995). Electrode sites were shaved if necessary and thoroughly cleaned with isopropyl alcohol to remove dirt and natural oils from the skin. ECG was recorded through Medi Trace 130 Ag/AgCl stress electrodes in a bipolar lead II setup with the reference electrode positioned just inferior to the mid-line of the left clavicle. As a robust R-wave greatly facilitates power spectral analysis of heart rate variability care was taken to avoid DC interference (all nonessential electronic equipment was shut down) and gain settings were adjusted to obtain as clear a signal as possible. The contribution of respiratory sinus arrhythmia to high frequency heart rate variability (Brown et al. 1993) requires that breathing frequency be monitored along with ECG in studies utilizing power spectral analysis. Respiration was monitored through the same electrode placement by the method of impedance plethysmography. In this technique a 1kHz signal is passed through the subjects thoracic cavity and the impedance between two reference electrodes is monitored. Impedance is inversely proportional to the distance between two points. As such, the

expansion of the chest cavity on inspiration would result in a decrease in impedance betweer two electrodes in the thoracic wall. The resultant respiratory related fluctuations in impedance were sampled at 500Hz and displayed concurrently with the ECG signal.

ECG and respiration were initially recorded for 20 minutes in the supine position. The room was maintained in semi-darkness for the duration of the recording and blankets were provided to ensure a comfortable temperature for the participants. Subjects were encouraged to relax with their eyes closed but to attempt to remain awake for the entire 20 minutes. No effort was made to control the rate or depth of breathing at any time during this or the subsequent standing recording. Following the initial supine recording, subjects were instructed to assume a standing position. Cardiovascular adaptation to orthostatic stress typically occurs over a period of less than one minute. As such, an interval of approximately 2-3 minutes was provided to allow the individual to reach a steady state prior to the commencement of the recording in the standing posture. Subjects were instructed to stand quietly for the duration of the recording period but were encouraged to shift their weight when necessary for comfort. This stage of testing lasted 10 minutes.

Both ECG and respiratory signals were fed through analog recorders and amplified with an HP7807C amplifier. These signals were then digitized using a 1kHz (500 Hz/channel), 12 bit analog-to-digital converter. The resultant

waveforms from both channels were displayed simultaneously on a 486/66 MHz personal computer and recorded at 500 samples/sec using a commercially available data acquisition and processing software package (CODAS, DATAQ Instruments Inc. Akron, Ohio, USA). Data files were stored temporarily on the personal computer's hard drive. Following data analysis all files were transferred for long term storage to digital mini-cassette (Verbatim Datalife) using Colorado backup software. File sizes for the supine and standing data were 2400 kb and 1200 kb respectively.

2.2.2 Twenty Four-Hour Recordings

Following the supine/standing testing period subjects were requested to wear an ambulatory ECG 'Holter' monitor (Model Oxford Medilog 4500 by Oxford Medical Ltd. Oxon, UK) for a period of 24 hours duration. Electrode application sites were shaved if needed and cleaned as per the acute recording procedure. The ECG signal was recorded via a two lead pre-cordial setup (V_1 and V_5) with the two reference electrodes placed just inferior to the mid-line of the right and left clavicles. The ground electrode was positioned on the right side of the lower abdomen in the supra-iliac region. A brief signal test of approximately 20 seconds in duration was performed automatically prior to the initiation of the recording to ensure that a waveform of sufficient quality for analysis could be obtained on both channels. In the case of a poor quality signal on one or both

channels, the corresponding electrodes were repositioned and/or the existing sites were more thoroughly cleaned and prepared. Following a positive signal test the monitor performed a short calibration and the recording was subsequently initiated. The ECG electrodes were further secured to the chest with 3M Transpore™ hypoallergenic surgical tape to minimize movement artifact and to reduce the chance of the wires becoming disconnected during the recording. ECG waveforms from both channels were simultaneously recorded at a rate of 125 samples/second and stored on a standard, normal bias 60 minute audio tape for a period of 24 hours. Upon completion of the recording subjects were instructed to remove and dispose of the electrodes, switch the monitor off and return it to the lab or MAC seniors program (for the elderly subjects) at a convenient time arranged during the initial phone interview.

To aid in the analysis of the Holter tapes subjects were required to complete an activity diary concurrently with the 24-hour recording. The reader is referred to Appendix A for a sample completed diary. Start and finish times for sleep, meals, visits to the bathroom and other activities were listed. Participants were encouraged to maintain their normal daily schedules but instructed to abstain from heavy physical activity or showering while wearing the monitor.

2.3 SIGNAL PROCESSING & DATA ANALYSIS

2.3.1 Frequency Domain Analysis

Data files from the acute recordings were visually inspected and the two signals (ECG and respiration) were separated through an advanced post-acquisition processing software. A detection algorithm was utilized to label the individual QRS complexes and compute R-R intervals from the raw ECG signal. An R-R tachogram was then constructed using the interval data.

The compensatory pause following an ectopic beat may be erroneously attributed to high frequency, vagally modulated heart rate variability and thus confound the results of power spectral analysis (Ori et al. 1992; Kamath et al. 1993). As such, the R-R interval tachograms were visually inspected for the presence of ectopic beats prior to the application of autoregressive modeling procedures. In cases in which the data set was corrupted by an inordinate number of ectopics (>3 per 5 min), additional post-acquisition processing was performed to select and remove an uncontaminated segment of data. Files from which appreciable segments (>512 data points/~4-5 minutes) of untainted data could not be extracted were excluded from the analysis. Small numbers of ectopics present in the accepted data segments were corrected for using an interpolation algorithm. Detection of ectopic beats by the algorithm was accomplished by setting a threshold value by which a beat may differ from the one immediately preceding it (eg. 0.90-1.10×previous R-R interval). Beats

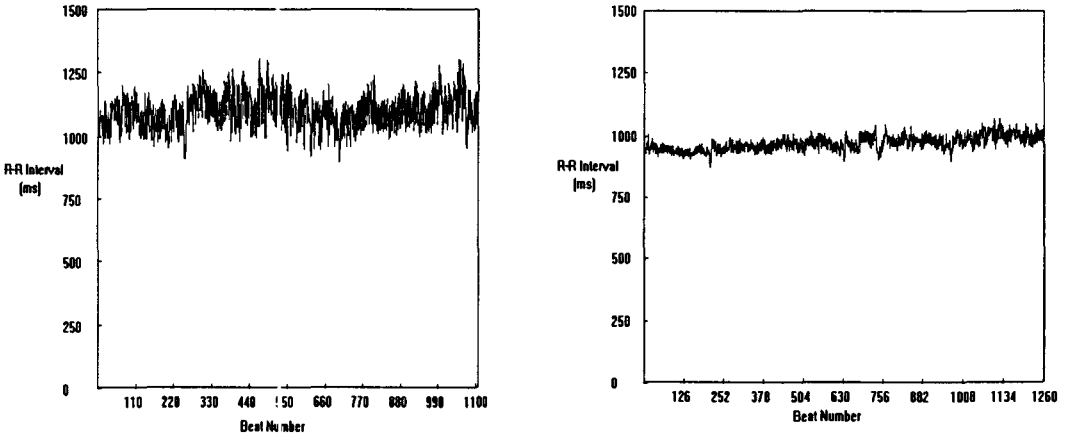


Figure 1. R-R interval tachograms for an 18 year old (left) & 74 year old subject (right). The diminished variability in the older individual necessitates the resetting of the ectopic correction filter for each individual subject.

exceeding this value were labeled as ectopic and subsequently corrected. The amount of 'normal' variability inherent in the heart rate of a young subject can differ substantially from that of an elderly individual (see Figure 2). Therefore the filter was individually adjusted to accommodate these age-related differences following the visual inspection of the data.

An equally sampled data set was obtained by re-sampling the ectopic corrected R-R interval tachogram by linear interpolation at a rate of 2 Hz. Adjacent segments of 256 points (corresponding to 128 seconds of data) were utilized for the calculation of each power spectra. This data time series was then demeaned by subtracting the mean value of the signal and filtered through a second order high band pass Butterworth filter with the cutoff point set at 0.02 Hz. Power spectra were then computed by applying a 9th order autoregressive

model to the modified & filtered signal. The autoregressive (AR) approach previously described by Kay et al. (1981) describes the signal x[n] at any instant 'n' as an output sequence from a black-box in response to an input driving noise u[n],

$$x[n] = -\sum_{k=1}^{p} a[k]\, x[n-k] + u[n]$$

where k={1,2..p} are the autoregressive parameters used to describe the signal.

The AR power spectrum is then calculated using these parameters:

$$P_{AR}(f) = \frac{\sigma^2 \Delta t}{\left[1 + \sum_{k=1}^{p} a[k] \exp(-j2\pi f k \Delta t)\right]^2}$$

where σ^2 is the variance of the input noise sequence and Δt is the sampling interval.

To quantify the available information and facilitate statistical analysis a number of dependent variables were derived from the power spectral plots. The power/area in both the LF (0.02Hz-0.15Hz) and HF (0.15Hz-0.50Hz) bands were computed by taking the numerical integral of the curve in these two regions. These figures were normalized by expressing each measure as a fraction of the total area under the curve between 0.02-0.50 Hz (%HF & %LF Area). In addition, maximum power (HF peak & LF peak) and the central frequency (LF

CF & HF CF) at which these peaks occurred in both power bands were identified. Fluctuations of spectral measures in one power band relative to the other were described by expressing areas and peaks as LF:HF ratios (LF:HF area & LF:HF Peak). Othostatic response was evaluated by comparing the difference scores (standing - supine value) of the various spectral measures. In addition, to control for variation in the baseline values between the age groups these difference scores were normalized by expressing the change as a percentage of the initial supine value. In order to characterize the prevalence of paradoxical HRV responses to orthostasis the change scores were dichotomized by classifying each response as typical or atypical. A typical response to orthostatic stress was defined as a transition from supine to standing accompanied by an alteration in a given spectral measure consistent with that generally reported in the literature (increases in LF area, LF:HF area & LF:HF peak and decreases in HF power & LF CF). Transitions accompanied by change in the opposite direction or no alteration in a spectral measure were thus classified as atypical. Comparisons were made between those individuals under the age of 30 and those beyond 55 years of age.

The respiratory signal was analyzed for data sets containing single peaked spectra (or those with visible entrainment of the LF and HF components). Power spectral analysis using the previously mentioned autoregressive technique was applied to the raw respiration waveform in order to identify the

dominant breathing frequency. Data sets in which this frequency fell within the LF band were excluded from the analysis.

2.3.2 Time Domain Analysis

Holter tapes were scanned on an Oxford Medilog Excel Scanner (Oxford Medical Ltd. Oxon, UK) in the Cardiorespiratory Department at the MUMC. QRS complexes were detected automatically and grouped into templates based on morphological similarity. The ECG strips were then manually inspected and comparable templates were merged. Unclassified complexes were labeled and incorrectly indexed beats were assigned to existing or new templates. Data was then downloaded to a 3.5 inch floppy diskette and transferred to the MUMC Clinical Neurocardiology lab were it was subsequently uploaded onto a 486/66 MHz personal computer (Gateway 2000, N. Sioux City, SD, USA) for HRV analysis.

Time domain measures derived from the beat-to-beat data included the mean R-R interval and heart rate over the entire 24-hour recording as well as a number of long and short term variability indices. The standard deviation of all intervals between normal sinus conducted beats (N-N intervals) for the entire period (SDNN) as well as the standard deviation of the mean of N-N intervals in all adjacent 5 minute segments (SDANN) were calculated to quantify long term variability. Short term measures included the percentage of adjacent N-N

intervals that differed by more than 50 msec (pNN50 [%]) and the root mean square of successive differences (R-MSSD). R-MSSD was computed as follows:

$$\text{R-MSSD} = \frac{\sqrt{\sum_{i=1}^{k} [(R - R_{i+1}) - (R - R_i)]^2}}{n}$$

where $R - R_i$ is the duration of the i^{th} R-R interval and n is the total number of R-R intervals. The mean of the standard deviations of adjacent 5 minute segments throughout the 24-hour period (SDNN index), typically considered an intermediate measure of variability was also available for this analysis. In addition, circadian plots of the hourly means and standard deviations of N-N intervals were provided to allow a qualitative inspection of the results. Time domain printouts also contained information regarding the percentage of intervals accepted for analysis as an added indicator of data quality.

2.3.3 Circadian Analysis of Spectral Measures

In order to evaluate the effect of aging on the circadian rhythms inherent in the frequency domain measures of HRV, power spectral analysis was performed on six evenly spaced one hour periods of the Holter data (4-5 a.m. & p.m., 8-9 a.m. & p.m. and 12-1 a.m. & p.m.). Spectra were computed from

adjacent 128 second segments using the autoregressive modeling algorithm described previously in the section on frequency domain analysis. Dependent measures for this phase of the study were equivalent to those used in the orthostatic analysis.

2.4 Statistics

The nature of existing relationships between age and HRV parameters were examined with multiple regression analyses. The strength of these associations were characterized by R and R^2 measures. Differences between age groups were tested using two and three-way multiple analysis of variance (MANOVA) and evaluated post hoc with analysis of variance (ANOVA) and Tukey HSD techniques. The assumption of homogeneity of variance was violated for a number of the dependent variables in this study. However, non-parametric tests capable of handling a complex design such as this have yet to be developed and the repeated use of a one way testing procedure would serve to inflate alpha to an unacceptable level. As such, this violation is justified and due to the robustness of the MANOVA techniques should not substantially affect the validity of the results. In addition, due to the highly correlated nature of the various dependent variables the MANOVA matrix for the circadian analysis was near singular and could not be inverted. As a result the dependent variables were divided in 2 groups: 1) Heart Rate, LF:HF Area, LF Area and %LF Area &

2) HF Area, % HF Area, LF:HF Peak, LF central frequency and HF central frequency. Consequently, in order to protect alpha the significance level for this test was set at $p<0.025$. Investigations into the prevalence of paradoxical HRV responses to orthostasis were carried out with Chi Square tests with one degree of freedom. The acceptable level of statistical significance for this study was set at $p<0.05$.

3.0 RESULTS

3.1 Acute Phase

Power spectral analysis of HRV demands that the raw ECG from which the heart rate signal is derived be as free of ectopics as possible and of sufficient quality to allow for the accurate detection of R-waves by the automatic algorithm. Individual subject recordings were reviewed and those not meeting these criteria (data quality n=5, ventricular ectopic activity n=2, consistent atrial premature contractions n=1) were excluded from the analysis. Following the application of the autoregressive modeling algorithm to the heart rate signal individual spectra were visually inspected for the presence of entrainment between the LF and HF peaks. Such instances were confirmed via analysis of the respiration trace and subsequently excluded (n=4) from the investigation. In all, complete acute data sets from 111 subjects (49 male, 62 female) were available for the statistical analysis.

3.1.1 Supine & Standing States

Regression analysis revealed significant ($p<0.05$) correlation's of mild to moderate strength ($R=+/-0.35-0.75$) between age and heart rate as well as all HRV measures in the supine position (see Appendix C). Both linear (LF area; $R=0.39$, $R^2=0.16$, LF:HF peak; $R=0.52$, $R^2=0.269$ & LF CF; $R=-0.73$, $R^2=0.53$)

and logarithmic (heart rate (HR); R=-0.35, R^2=0.13, %LF area; R=0.49, R^2=0.24, LF:HF area; R=0.48, R^2=0.23, HF area; R=-0.50, R^2=0.25 & %HF area; R=-0.49, R^2=0.24) associations were observed in this posture. The associations between age and spectral measures during free standing were generally weaker than those seen in the supine state with significant relationships ($p<0.05$) evident only for the LF CF (R=-0.45, R^2=0.20) and total area measures (R=-0.19, R^2=0.04). In contrast, the correlation between age and HR was stronger in the standing position (r=-0.62, R^2=0.39; $p<0.00001$).

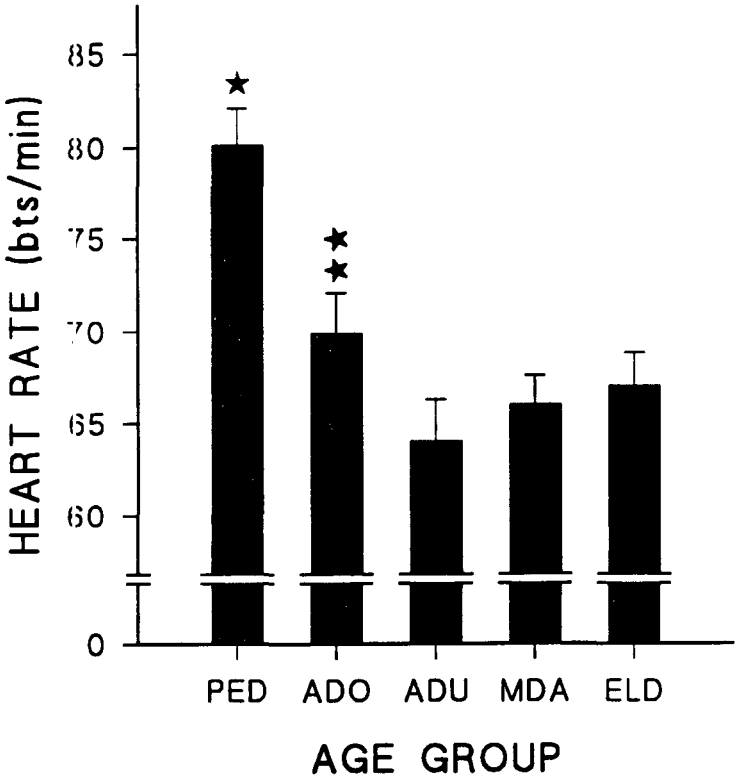
Supine and standing power spectra were compared across age groups and gender within a three-way mixed MANOVA (AGE × GENDER × POSTURE) design that was concurrently utilized to assess the HRV response to orthostasis. This analysis revealed statistically significant main effects for AGE (RaoR (40,350)=5.04; $p<0.0001$), GENDER (RaoR (10,92)=4.05; $p<0.0002$) and POSTURE (RaoR (10,92)=72.8; $p<0.0001$). The AGE × GENDER (RaoR (40,350)=0.84; N.S.) interaction was not statistically significant. However, AGE × POSTURE (RaoR (40,350)=3.67; $p<0.0001$) and GENDER × POSTURE (RaoR (10,92)=2.78; $p<0.005$) interactions were observed. These interactions will be described in the section on spectral response to orthostasis. All results are described as mean ± one standard deviation.

Effect of Age

Heart rate in the supine position declined progressively in the three youngest age groups (PED (80.2 ± 8.5 bts/min) > ADO (69.9 ± 9.5 bts/min) > ADU (64.0 ± 10.7 bts/min); $p<0.001$, see Figure 3). No further alterations in this measure were noted in the MDA and ELD groups. In contrast, HR during free standing showed a more continuous decline encompassing the entire age range under investigation (PED (100.4 ± 6.84 bts/min) & ADO (94.5 ± 13.6 bts/min) > ADU (80.1 ± 11.2 bts/min) & MDA (78.9 ± 9.3 bts/min) > ELD (75.3 ± bts/min); $p<0.05$, see Figure 3).

As described previously, specific indices derived from the heart rate power spectrum may serve as benchmarks of sympatho-vagal balance (Weise et al. 1989; Ori et al. 1992; Task Force 1996). These measures include the LF:HF peak and area ratios as well as the central frequency of the LF band. In general, supine state values for these indices exhibited a shift toward sympathetic dominance manifested as an elevation of the ratios (see Figure 4) & leftward shift of the LF CF (see Figure 5) with advancing age. In all three instances no shift was evident in those subject groups below the age of 30 years (LF:HF Area: PED (0.87 ± 0.3), ADO (0.84 ± 0.3) & ADU (1.03 ± 0.5) < ADU (1.37 ± 0.5) & ELD (1.38 ± 0.4); $p<0.02$, LF:HF Peak: PED (1.02 ± 0.3), ADO (1.05 ± 0.7) & ADU (1.21 ± 0.7) < ELD (1.71 ± 1.1); $p<0.005$, LF CF: PED (0.11 ± 0.01 Hz), ADO (0.10 ± 0.01 Hz) & ADU (0.10 ± 0.01 Hz) > MDA (0.08 ± 0.01 Hz) > ELD

Figure 3. Mean heart rate during 20 min supine rest (top) and 10 min free standing (bottom) for PED, ADO, ADU, MDA and ELD age groups. (Top: ★ PED > ADO, ADU, MDA & ELD; $p<0.001$, ★★ ADO > ADU, MDA & ELD; $p<0.001$, Bottom: ★ PED & ADO > ADU, MDA & ELD; $p<0.05$, ★★ ADU & MDA > ELD; $p<0.05$)


HEART RATE (bts/min)
85
80
75
70
65
60
0
PED
ADO
ADU
MDA
ELD
AGE GROUP

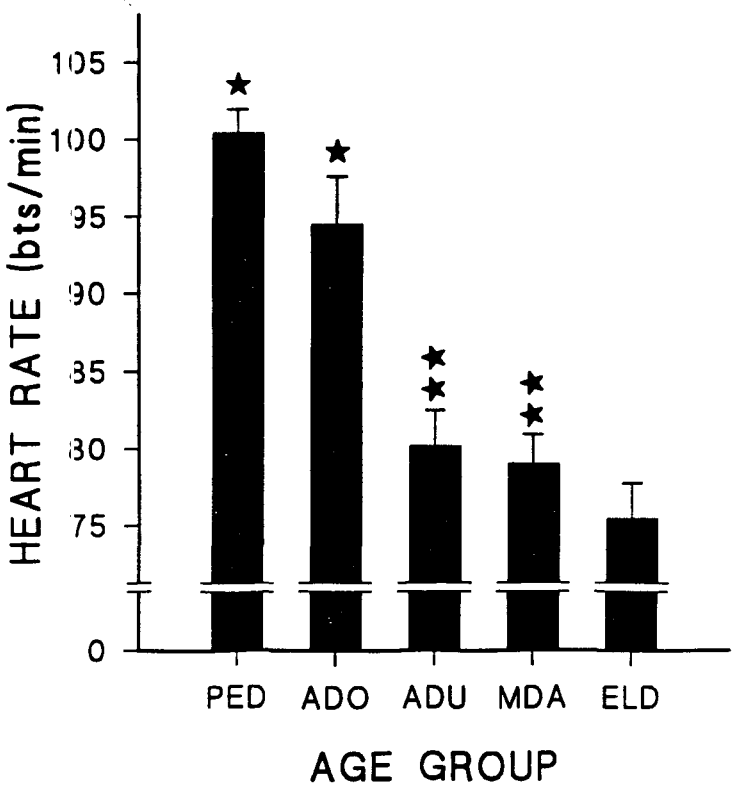
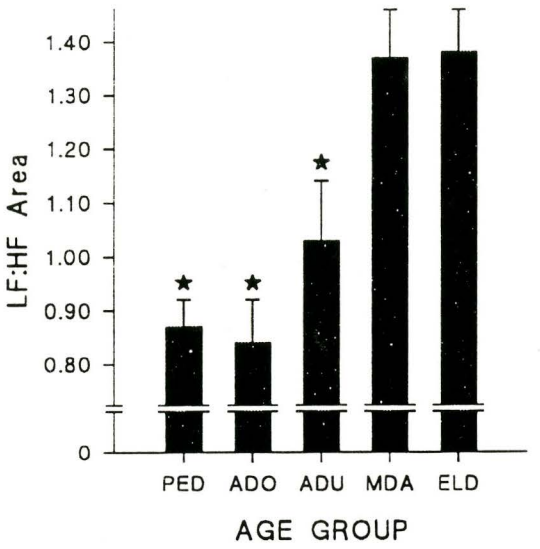

HEART RATE (bts/min)
105
100
95
90
85
80
75
0
PED
ADO
ADU
MDA
ELD
AGE GROUP

Figure 4. Mean LF:HF area (top) and LF:HF peak (bottom) during 20 min supine rest (left side) and 10 min free standing (right side) for the PED, ADO, ADU, MDA and ELD age groups. (Top left: ★ PED, ADO & EDU < MDA & ELD; $p<0.02$, Top right: ★ MDA > PED & ELD; $p<0.0003$, ★★ ADO > PED; $p<0.003$, Bottom left: ★ PED, ADO & ADU > ELD; $p<0.005$, Bottom right: ★ ADO > PED, ADU & ELD; $p<0.02$, ★★ MDA > PED; $p<0.004$)


LF:HF Area
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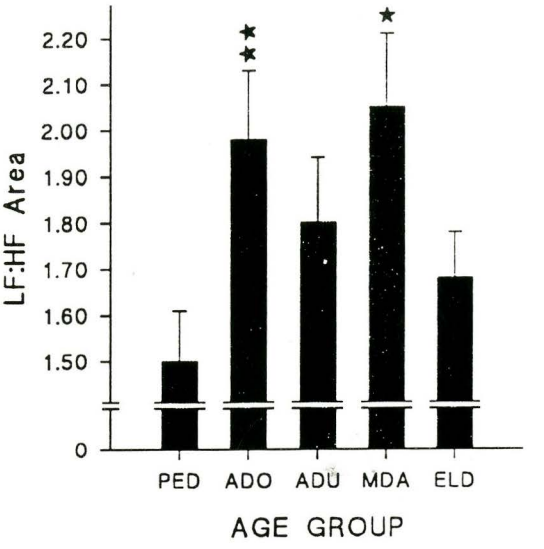
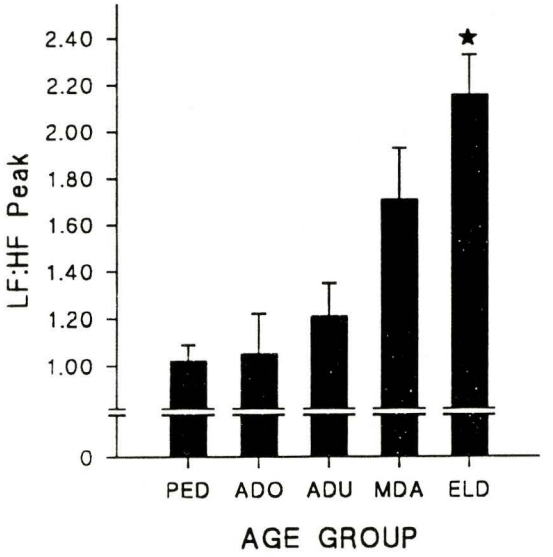
1.30
1.20
1.10
1.00
0.90
0.80
0
PED ADO ADU MDA ELD
AGE GROUP


LF:HF Area
2.20
2.10
2.00
1.90
1.80
1.70
1.60
1.50
0
PED ADO ADU MDA ELD
AGE GROUP


LF:HF Peak
2.40
2.20
2.00
1.80
1.60
1.40
1.20
1.00
0
PED ADO ADU MDA ELD
AGE GROUP

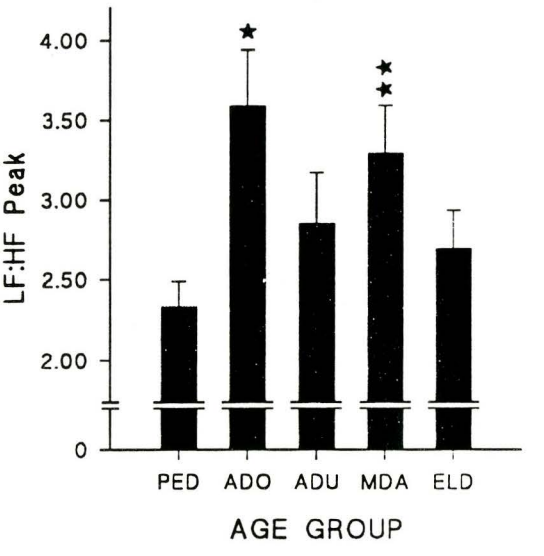
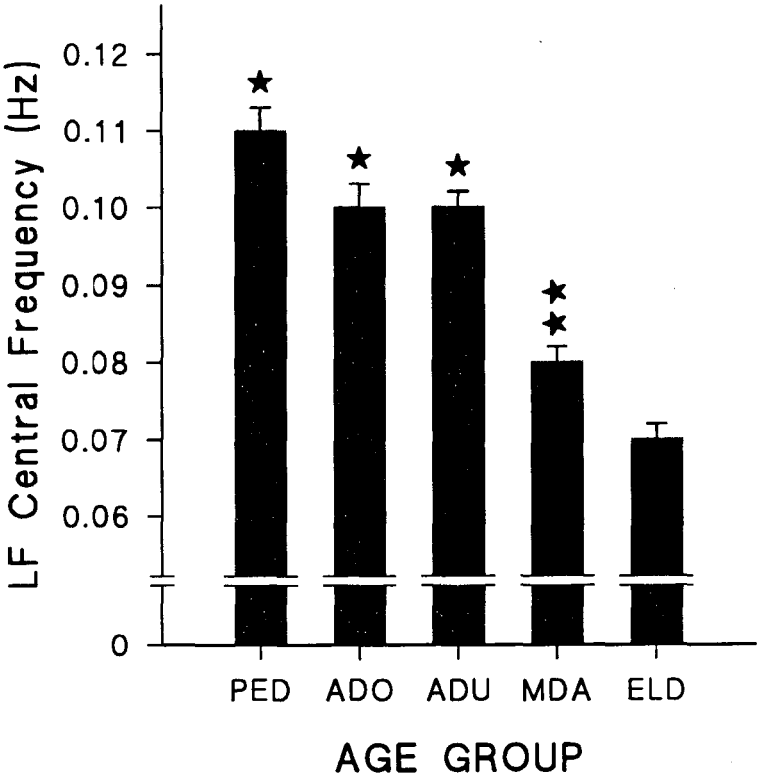
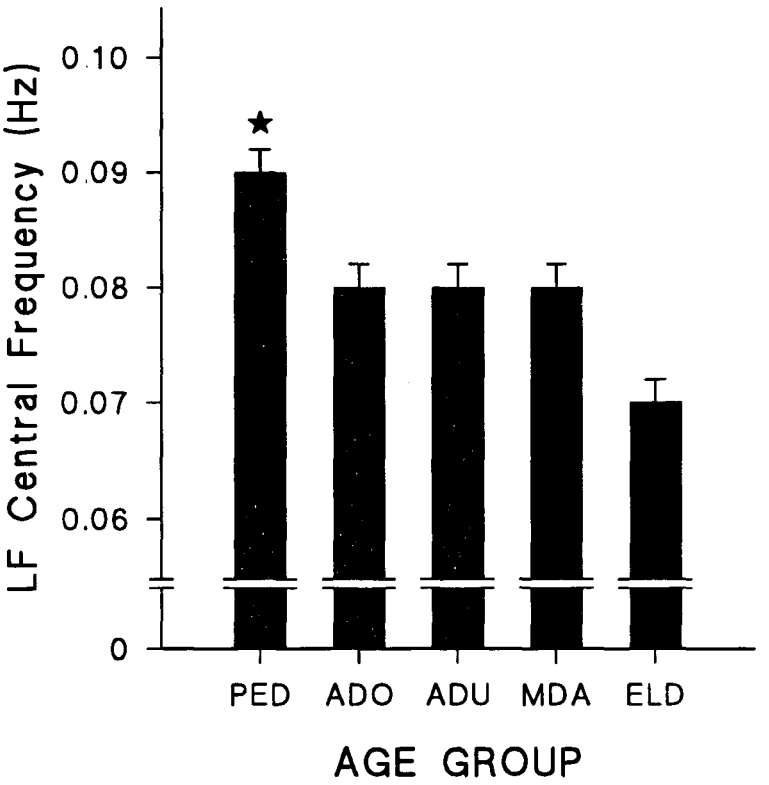

LF:HF Peak
4.00
3.50
3.00
2.50
2.00
0
PED ADO ADU MDA ELD
AGE GROUP

Figure 5. Mean LF CF during 20 min supine rest (top) and 10 min free standing (bottom) for the PED, ADO, ADU, MDA and ELD age groups. (Top: ★ PED, ADO & ADU > MDA & ELD; $p<0.02$, ★★ MDA > ELD; $p<0.02$, Bottom: ★ PED > MDA & ELD; $p<0.003$)

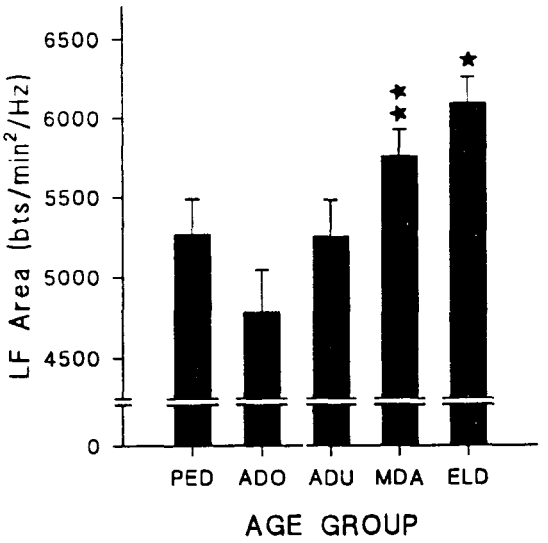

LF Central Frequency (Hz)
0.12
0.11
0.10
0.09
0.08
0.07
0.06
0
PED
ADO
ADU
MDA
ELD
AGE GROUP

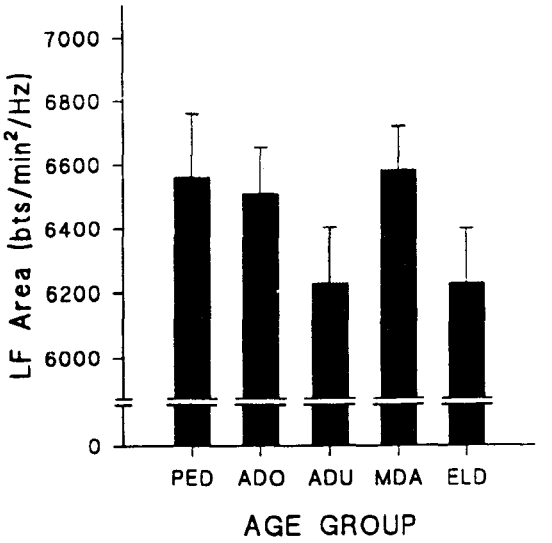

LF Central Frequency (Hz)
0.10
0.09
0.08
0.07
0.06
0
PED
ADO
ADU
MDA
ELD
AGE GROUP

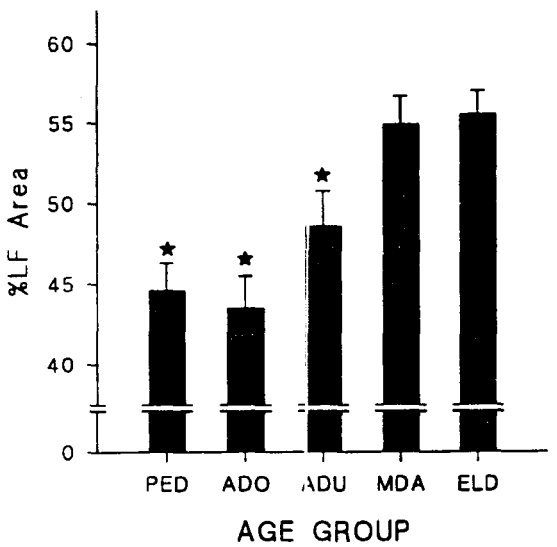
(0.07 ± 0.01 Hz); p<0.02). Inspection of the free standing data revealed a more complex pattern for these important measures. The area and peak ratios exhibited an irregular M-shaped pattern across age groups (see Figure 4). In this posture LF:HF area was significantly elevated in the ADO group (1.98 ± 0.7) compared to the PED subjects (1.5 ± 0.5; p<0.003) and in the MDA group (2.05 ± 0.8) compared to those in both the PED (p<0.0003) & ELD groups (1.68 ± 0.6; p<0.02). Similarly the standing LF:HF peak was significantly elevated in the ADO group (3.59 ± 1.5) compared to PED (2.33 ± 0.7; p<0.0002), ADU (2.85 ± 1.5; p<0.02) and ELD age groups (2.69 ± 1.3; p<0.02) and in the MDA (3.29 ± 1.5) group compared to the PED subjects (P<0.004). In contrast, the standing LF CF measure showed an age-related leftward shift (see Figure 5) similar to that seen in the supine posture (MDA (0.08 ± 0.01 Hz) & ELD groups (0.07 ± 0.01 Hz) < PED subjects (0.09 ± 0.01 Hz; p<0.003)).

The aforementioned phenomena were contributed to by statistically significant alterations in both the LF and HF regions of the HRV spectra (see Figures 6 & 7). In the supine position both the LF area and %LF area were augmented in the older age groups (LF Area: ELD (6088 ± 849.0 bts·min^{-2}/Hz) > ADU (5249 ± 1389 bts·min^{-2}/Hz), ADO (4784 ± 1128 bts·min^{-2}/Hz) & PED (5267 ± 973.0 bts·min^{-2}/Hz); p<0.0005, and MDA (5756 ± 805.8 bts·min^{-2}/Hz) > ADO; p<0.002, %LF Area: ELD (55.5 ± 7.8%) & MDA (54.9 ± 8.5 %) > ADU (48.5 ± 10.6 %), ADO (43.51 ± 8.7 %) & PED (44.6 ± 7.5 %); p<0.0003). In the standing

Figure 6. Mean LF area (top) and %LF area (bottom) during 20 min supine rest (left side) and 10 min free standing (right side) for the PED, ADO, ADU, MDA and ELD age groups. (Top left: ★ ELD > ADU, ADO & PED; $p<0.0005$, ★★ MDA > ADO; $p<0.002$, Bottom left: ★ PED, ADO & ADU < MDA & ELD; $p<0.0003$, Bottom right: ★ PED < MDA & ADO; $p<0.0002$)


LF Area (bts/min²/Hz)
6500
6000
5500
5000
4500
0
PED ADO ADU MDA ELD
AGE GROUP


LF Area (bts/min²/Hz)
7000
6800
6600
6400
6200
6000
0
PED ADO ADU MDA ELD
AGE GROUP


%LF Area
60
55
50
45
40
0
PED ADO ADU MDA ELD
AGE GROUP

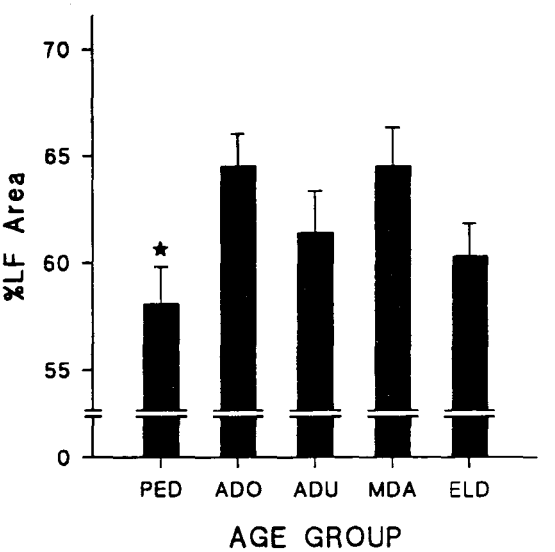
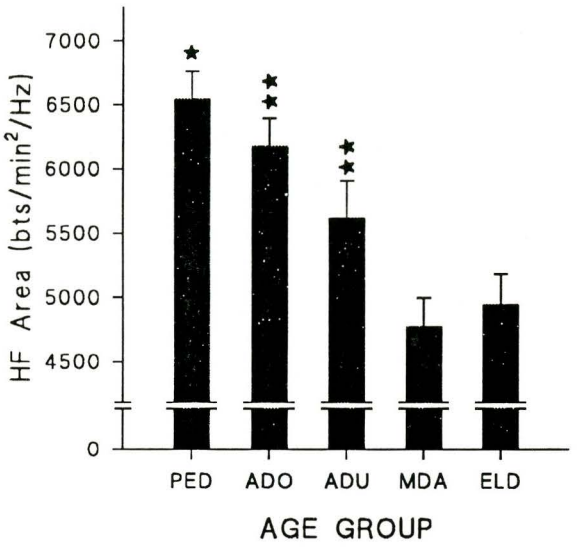
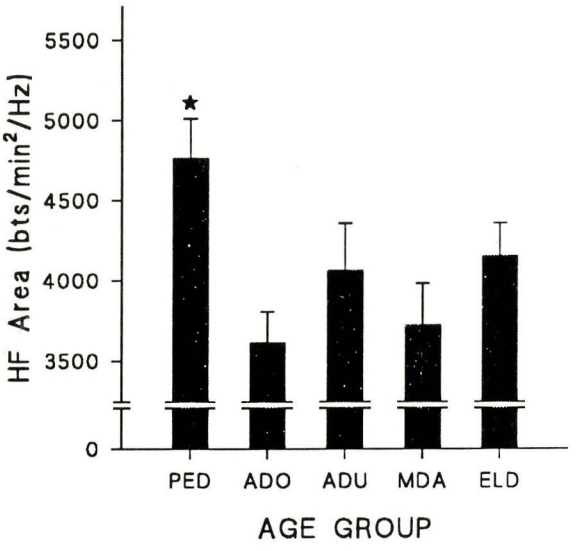
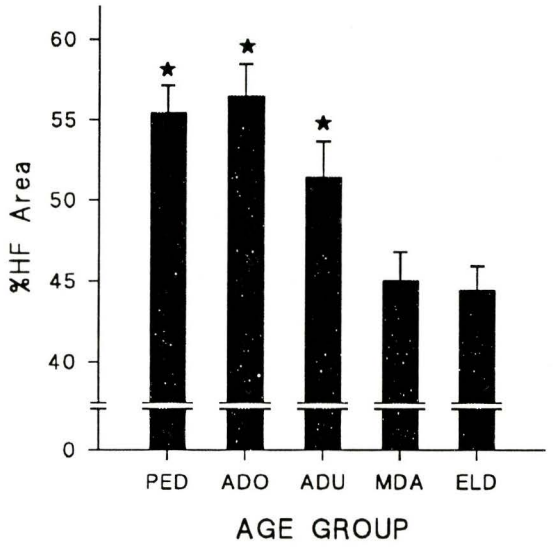
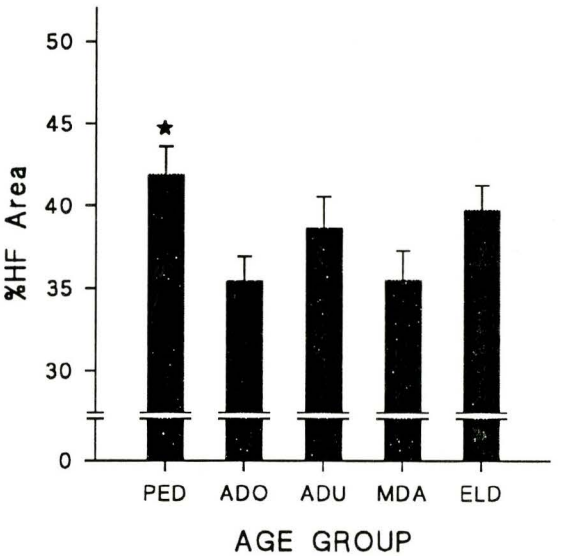

%LF Area
70
65
60
55
0
PED ADO ADU MDA ELD
AGE GROUP

Figure 7. Mean HF area (top) and %HF area (bottom) during 20 min supine rest (left side) and 10 min free standing (right side) for the PED, ADO, ADU, MDA and ELD age groups. (Top left: ★ PED > ADU, MDA & ELD; $p<0.0002$, ★★ ADO & ADU > MDA & ELD; $p<0.02$, Top right: ★ PED > ADO & MDA; $p<0.002$, Bottom left: ★ PED, ADO & ADU > MDA & ELD; $p<0.0002$, Bottom right: ★ PED > ADO & MDA; $p<0.002$)


HF Area (bts/min²/Hz)
7000
6500
6000
5500
5000
4500
0
PED
ADO
ADU
MDA
ELD
AGE GROUP


HF Area (bts/min²/Hz)
5500
5000
4500
4000
3500
0
PED
ADO
ADU
MDA
ELD
AGE GROUP


%HF Area
60
55
50
45
40
0
PED
ADO
ADU
MDA
ELD
AGE GROUP


%HF Area
50
45
40
35
30
0
PED
ADO
ADU
MDA
ELD
AGE GROUP

posture no effect of age was observed for the LF area measure whereas the %LF area index showed the more complex M-shaped pattern observed with the previously described peak and area ratios (PED (58.1 ± 7.5%) < ADO (64.5 ± 6.5%) & MDA (64.5 ± 8.6%); $p<0.0002$). In addition, the observed shifts in the supine measures of autonomic balance were contributed to by a statistically significant age-related diminution of power in the HF band in this position (HF area: PED (6543 ± 948.4 bts·min^{-2}/Hz) > ADU (5620 ± 1389 bts·min^{-2}/Hz), MDA (4770 ± 1087 bts·min^{-2}/Hz) & ELD (4944 ± 1232 bts·min^{-2}/Hz); $p<0.0002$ and ADO (6176 ± 969.7 bts·min^{-2}/Hz) & ADU > MDA & ELD; $p<0.02$, %HF area: PED (55.4 ± 7.5%), ADO (55.5 ± 8.7%) & ADU (51.5 ± 10.7%) > MDA (45.0 ± 8.5%) & ELD (44.4 ± 7.8); $p<0.0002$). Both of these measures demonstrated an inverted M-shape in the standing position with the PED group exhibiting greater absolute (HF area: 4763 ± 1076 bts·min^{-2}/Hz) and fractional power (%HF area: 41.9 ± 7.5%) compared to both the ADO (HF area: 3616 ± 833.9 bts·min^{-2}/Hz & %HF area: 35.4 ± 6.4%; $p<0.0002$) and MDA subjects (HF area: 3722 ± 1239 bts·min^{-2}/Hz & %HF area: 35.5 ± 8.6%; $p<0.002$).

Effect of Gender

Although gender differences were not as readily apparent as those observed in the age group analysis some notable discrepancies were evident when males and females were compared irrespective of age. Heart rate,

although statistically similar in the supine position was greater during standing in males (86.4 ± 15.0 bts/min) compared to females (83.2 ± 13.7 bts/min; $p<0.03$). In addition, the LF:HF peak index was significantly higher in male subjects (supine: 1.73 ± 0.9 & standing: 3.53 ± 1.5) compared to females in both postures (supine: 1.30 ± 0.9; $p<0.02$ & standing: 2.48 ± 1.0; $p<0.0002$).

3.1.2 Spectral Response to Orthostasis

The application of multiple regression analysis to the change scores revealed significant mild to moderate strength associations (R=+/-0.32-0.58) between age and the magnitude of the heart rate and power spectral responses to orthostatic stress (see Appendix C). The observed trend toward a diminished response magnitude was generally linear (ΔHR: R=-0.58, R^2=0.34, ΔLF CF: R=0.47, R^2=0.22, ΔLF area: R=-0.41, R^2=0.17, Δ%LF area: R=-0.48, R^2=0.231, ΔHF area: R=0.399, R^2=0.160 & Δ%HF area: R=0.481, R^2=0.231; $p<0.05$). However, the LF:HF peak and area ratios exhibited 2nd order polynomial associations with age (ΔLF:HF area: R=-0.327, R^2=0.107, ΔLF:HF peak: R=-0.338, R^2=0.114; $p<0.05$).

As previously described a three-way mixed MANOVA (AGE × GENDER × POSTURE) revealed significant AGE × POSTURE and GENDER × POSTURE interactions. In addition, the magnitude of the spectral response to orthostatic stress was assessed by taking the difference between standing and supine

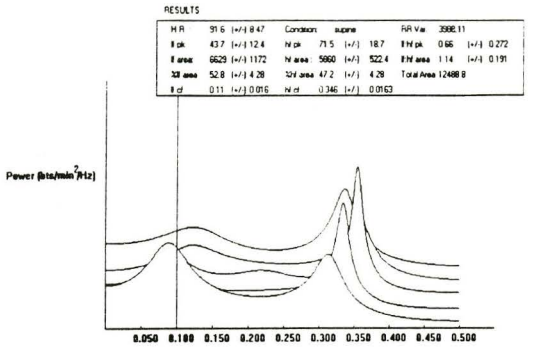
values. To control for the variability in baseline values between age groups, these scores were normalized by expressing them as percentages of the original supine value. A two-way between subjects MANOVA design (AGE × GENDER) was applied to these scores and revealed significant main effects for both AGE (RaoR (80,325)=3.07; $p<0.00001$) and GENDER (RaoR (20,82)=3.06; $p<0.0002$). However, the AGE × GENDER interaction (RaoR (80,325)=1.07; N.S.) was not statistically significant.

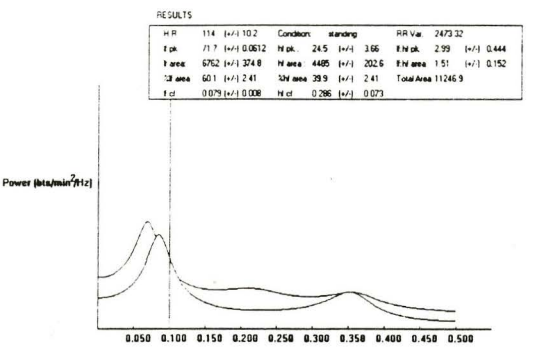
Effect of Age

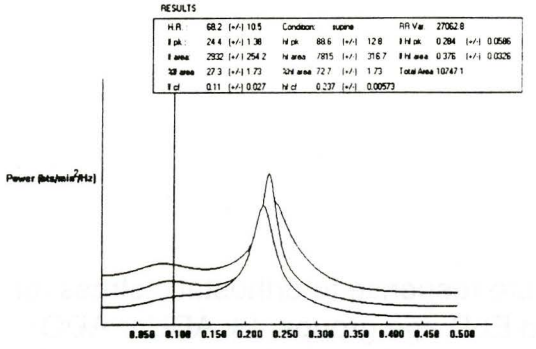
Sample power spectra for subjects of various ages are illustrated in Figure 8. Heart rate increased significantly on standing in all five age groups ($p<0.0002$, see Figure 9). However, the magnitude of this response was greatest in the ADO group and declined progressively thereafter (ΔHR: PED (20.2 ± 5.8 bts/min) < ADO (24.6 ± 8.6 bts/min; N.S.) > ADU (16.1 ± 7.8 bts/min; $p<0.0003$) > MDA (12.9 ± 9.0 bts/min; $p<0.0002$) > ELD (7.6 ±5.9 bts/min; $p<0.0002$). Statistical significance in this effect was retained when these scores were expressed in relative terms.

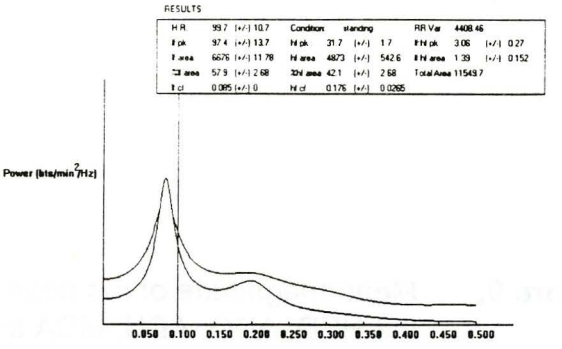
With respect to those measures believed to be indicative of sympathovagal balance, statistically significant increases in both the LF:HF area ($p<0.0005$) and peak ratios ($p<0.0009$) on standing were noted in all but the ELD group whereas the observed significant leftward shift of the LF CF ($p<0.0006$)

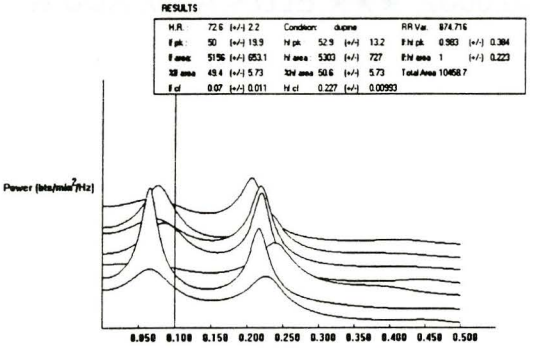
Figure 8. Power spectral profiles during 20 min supine rest (left side) and 10 min free standing (right side): (top) 6 year old female subject; (2nd from top) 13 year old male subject; (2nd from bottom) 37 year old female subject; (bottom) 69 year old male subject.

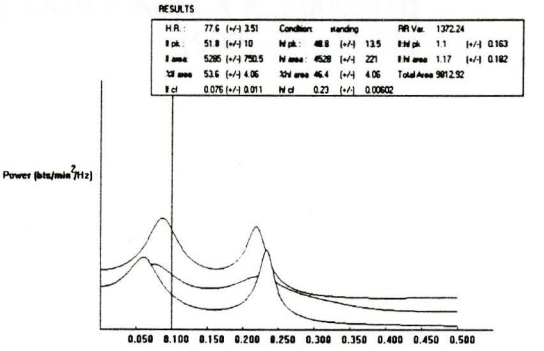


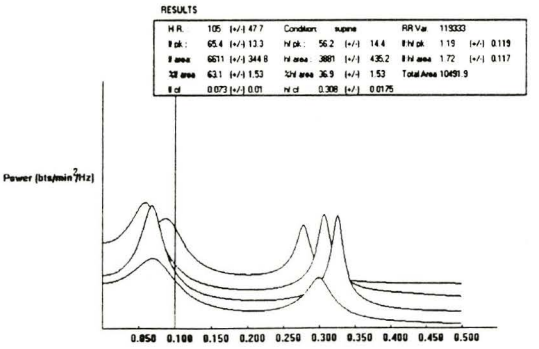


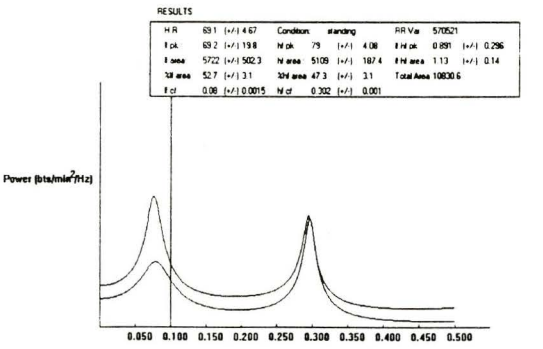








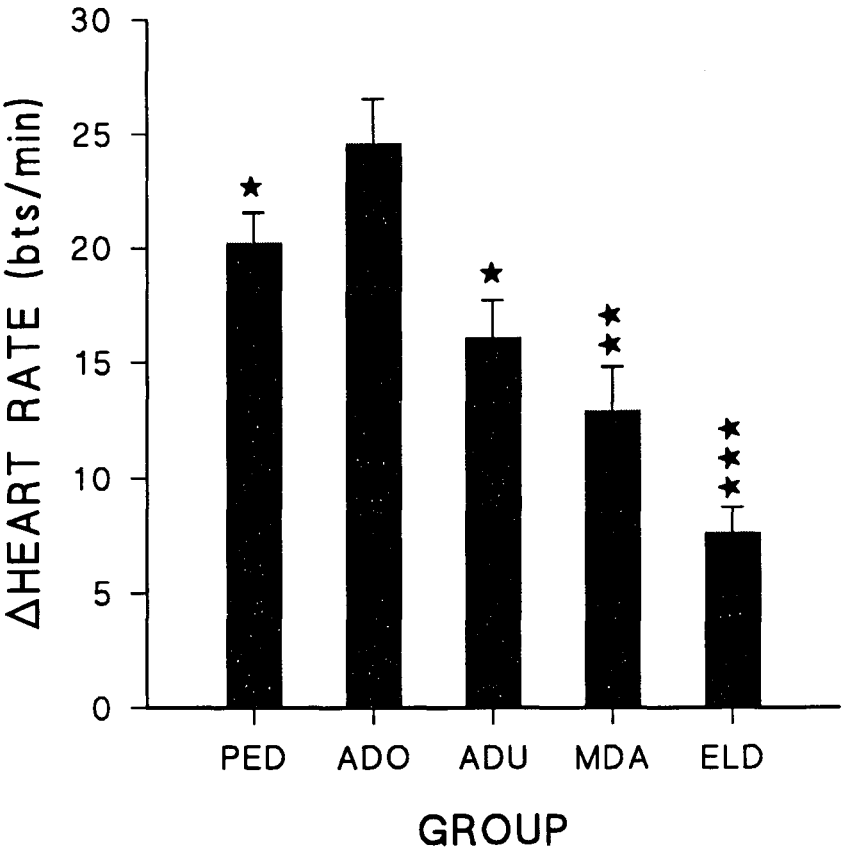




Frequency (Hz)

Frequency (Hz)

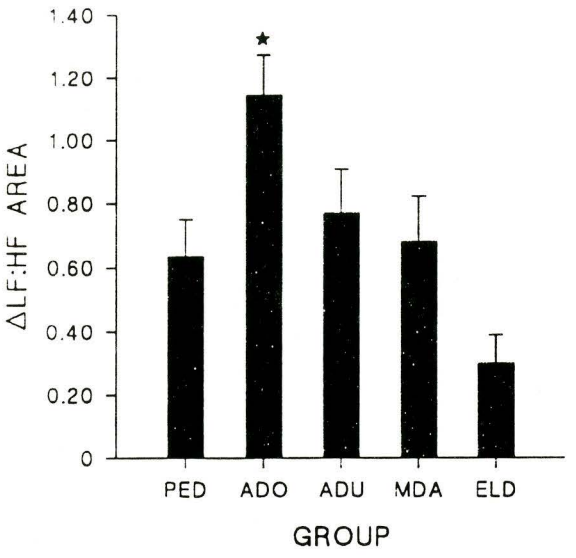
Figure 9. Mean magnitude of the heart rate response to orthostatic stress for the PED, ADO, ADU, MDA and ELD age groups. (★ ADU < ADO; $p<0.0003$, ★★ MDA < ADO; $p<0.0002$, ★★★ ELD < ADO, ADU & MDA; $p<0.0002$)

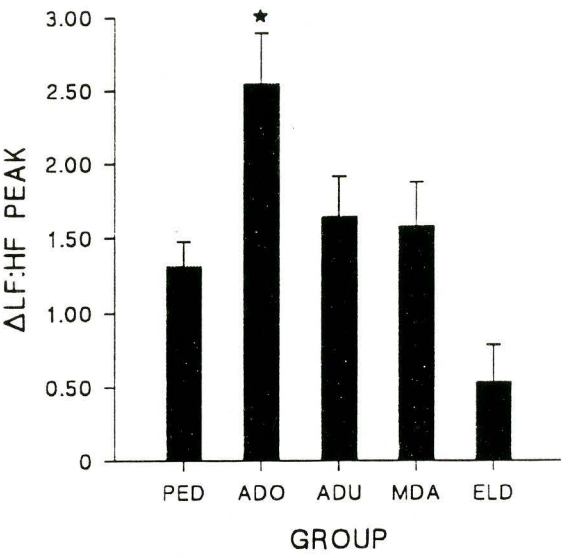

30
25
20
15
10
5
0
ΔHEART RATE (bts/min)
PED
ADO
ADU
MDA
ELD
GROUP

was absent in both the ELD and MDA subjects (see Figure 10). Among those groups exhibiting a significant increase in the peak ratio on standing, the magnitude of this response was greater in the ADO subjects (ΔLF:HF peak: 2.54 ± 1.5) compared to the PED (Δ LF:HF peak: 1.31 ± 0.7), ADU (ΔLF:HF peak: 1.64 ± 1.3) and MDA groups (ΔLF:HF peak: 1.58 ± 1.4; $p<0.05$). Similarly, the increase in the area ratio on standing was significantly greater in the ADO group (ΔLF:HF area: 1.14 ± 0.6) compared to PED subjects (ΔLF:HF area: 0.63 ± 0.5; $p<0.04$). No statistically significant differences were noted among the three groups exhibiting a posture related leftward shift of the LF CF (PED (-0.019 ± 0.017 Hz), ADO (-0.021 ± 0.018 Hz) & ADU (0.016 ± 0.016 Hz; N.S.). Similar to the heart rate data, the normalization of these scores did not alter the observed age effect.

Both the LF and HF power responses to orthostatic stress were blunted with advanced age (see Figure 11). Assumption of the standing posture was accompanied by a significant augmentation of both absolute (LF area; $p<0.02$) and fractional power (%LF area; $p<0.0002$) in the LF frequency band in all but the ELD subject group. Within those groups showing the typical LF power response to orthostasis the magnitude of the fractional LF power increase was significantly greater in ADO subjects (21.0 ± 8.0%) compared to those in the PED (13.5 ± 8.3%; $p<0.05$), ADU (12.8 ± 8.8%;$p<0.02$) and MDA (9.5 ± 7.4%;$p<0.0003$) groups. Concurrently, absolute (HF area; $p<0.02$) and fractional

Figure 10. Mean magnitude of the LF:HF area (top left), LF:HF peak (top right) and LF CF (bottom) responses to orthostatic stress for the PED, ADO, ADU, MDA and ELD age groups. (Top right: ★ADO > PED; $p<0.04$, Top left: ★ ADO > PED & MDA $p<0.05$, Bottom: ELD > PED, ADO & ADU; $p<0.005$)


ΔLF:HF AREA
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0
PED
ADO
ADU
MDA
ELD
GROUP


ΔLF:HF PEAK
3.00
2.50
2.00
1.50
1.00
0.50
0
PED
ADO
ADU
MDA
ELD
GROUP

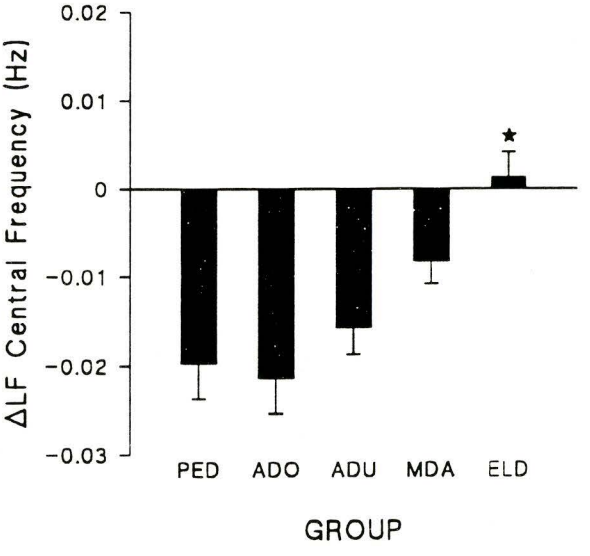
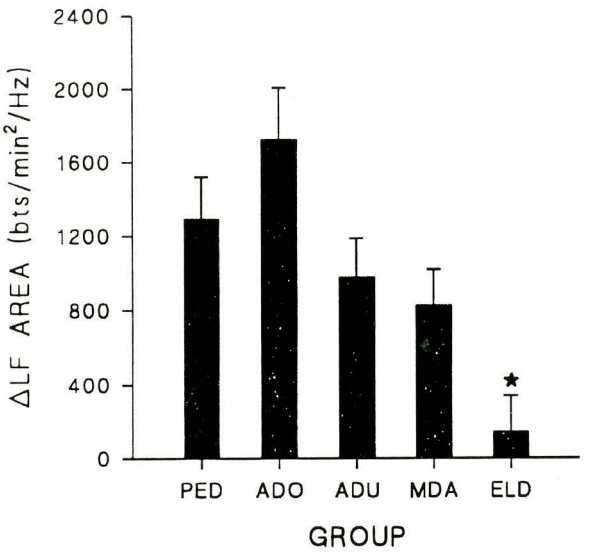
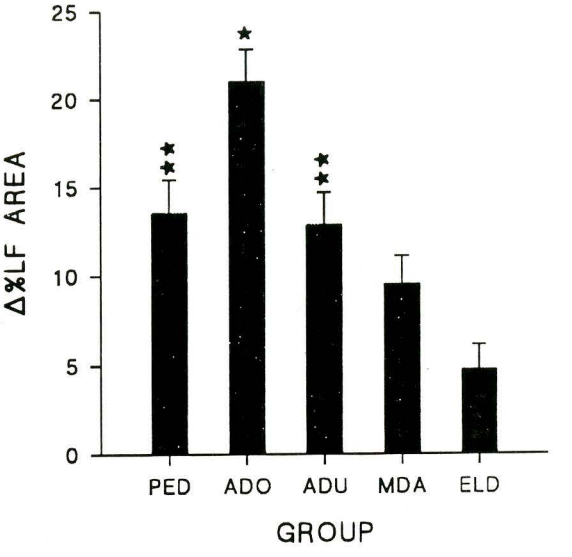
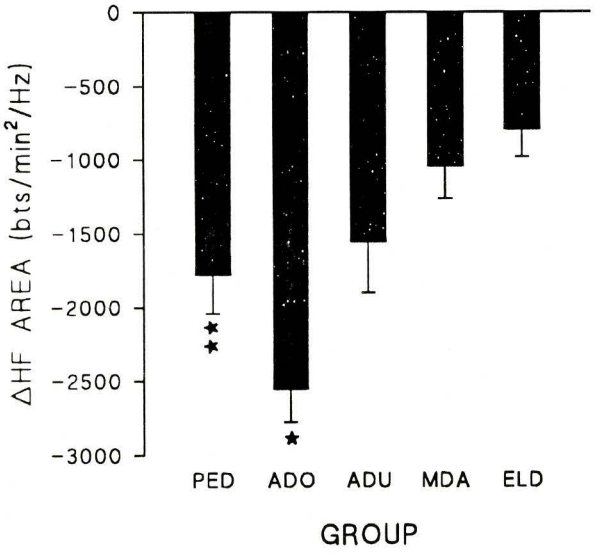
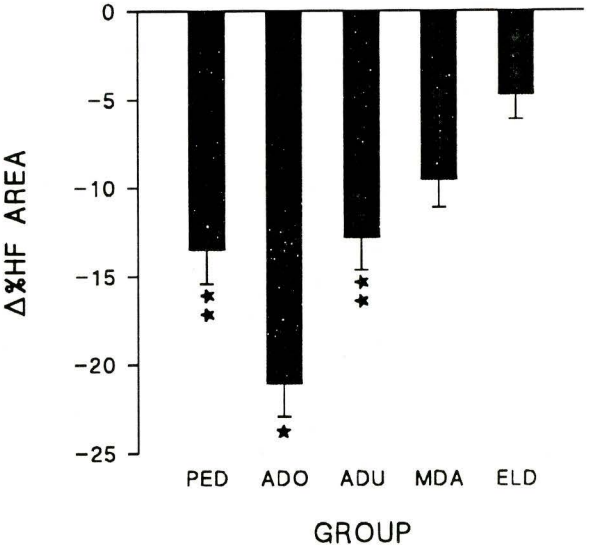

ΔLF Central Frequency (Hz)
0.02
0.01
0
−0.01
−0.02
−0.03
PED
ADO
ADU
MDA
ELD
GROUP

Figure 11. Mean magnitude of the LF area (top left), %LF area (top right), HF area (bottom left) and %HF area (bottom right) responses to orthostatic stress for the PED, ADO, ADU, MDA and ELD age groups. (Top left: ★ Change not significant in ELD, Top right: ★ ADO > PED, ADU, MDA & ELD; $p<0.02$, ★★ PED & ADU > ELD; $p<0.004$, Bottom left: ★ ADO > ADU, MDA & ELD; $p<0.05$, ★★ PED > ELD; $p<0.04$, Bottom right: ★ ADO > PED, ADU, MDA & ELD; $p<0.05$, ★★ PED & ADU > ELD; $p<0.004$)


2400
2000
1600
1200
800
400
0
ΔLF AREA (bts/min²/Hz)
PED
ADO
ADU
MDA
ELD
GROUP


25
20
15
10
5
0
Δ%LF AREA
PED
ADO
ADU
MDA
ELD
GROUP


0
-500
-1000
-1500
-2000
-2500
-3000
ΔHF AREA (bts/min²/Hz)
PED
ADO
ADU
MDA
ELD
GROUP


0
-5
-10
-15
-20
-25
Δ%HF AREA
PED
ADO
ADU
MDA
ELD
GROUP

(%HF area; $p<0.0002$) HF power measures showed statistically significant reductions in response to orthostasis in all groups and in all but the ELD subjects, respectively. Similar to that seen in the LF frequency band, the magnitude of the fractional HF power response was significantly greater in the ADO subjects (-21.1 ± 8.0%) compared to those in the PED (13.5 ± 8.3%;$p<0.05$), ADU (12.8 ± 8.8%;$p<0.02$) and MDA groups (9.6 ± 7.4%;$p<0.0003$). Again the aforementioned effects were retained when expressed relative to their original supine values.

A non-parametric 2×2 Chi-square analysis revealed no significant difference in the prevalence of atypical heart rate responses to orthostasis in subjects over 55 year of age compared to those under the age of 30 ($\chi^2=0.838$; N.S., see Appendix D). In contrast, a significantly elevated proportion of this elderly cohort exhibited paradoxical responses in a number of spectral measures (LF:HF area: $\chi^2=7.06$; $p<0.01$, LF area: $\chi^2=4.88$; $p<0.05$, LF:HF peak: $\chi^2=11.11$; $p<0.001$ & LF CF: $\chi^2=10.16$; $p<0.01$) compared to their younger counterparts.

Effect of Gender

Post hoc analysis of the gender main effect revealed a more dramatic heart rate response to standing in males compared to females. This effect was evident when the increase in heart rate was expressed in both absolute terms (♂: 17.5 ± 10.8 bts/min, ♀: 13.9 ± 7.9 bts/min; $p<0.02$) and as a percentage of

the resting supine value (♂: 26.0 ± 16.5%, ♀: 20.6 ± 12.1%; $p<0.04$). In addition, males exhibited a larger increase in the LF:HF peak on standing (1.80 ± 1.4) compared to female subjects (1.18 ± 1.35; $p<0.02$).

3.2 Twenty Four Hour Data

Twenty four hour ambulatory ECG monitoring was performed on all subjects following the acute phase of the study. However, complete data sets for 6 subjects (4 male, 2 female) were unavailable for statistical analysis due to recorder malfunction (n=3) and low data quality (accepted intervals < 80%, n=3). Consequently, time domain analysis was performed on data from 117 subjects (47 male, 70 female). An additional subject (male) was excluded from the circadian analysis due to an isolated segment of poor quality data within one of the periods selected for power spectral computations.

3.2.1 Time Domain Variables

Three distinct patterns of age-related alterations in the time domain variables were demonstrated through the regression analysis (see Appendix C). The mean 24-hour heart rate and N-N interval measures exhibited a significant logarithmic decline and lengthening with age respectively (HR: $R=-0.51$, $R^2=0.26$, N-N interval: $R=0.50$, $R^2=0.246$; $p<0.05$). Additionally, a negative linear correlation was demonstrated between age and those parameters characterizing

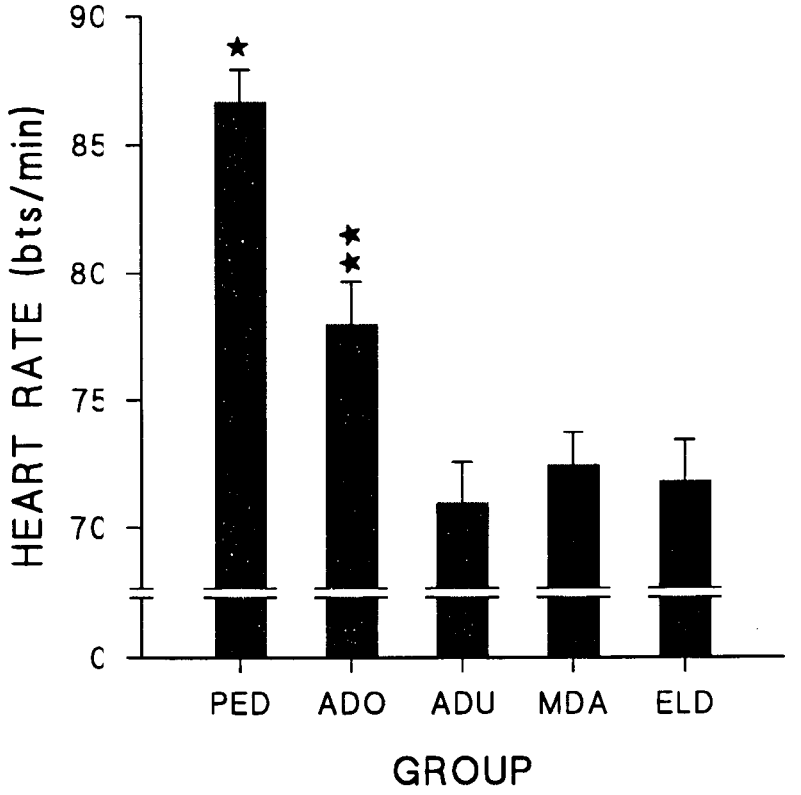
short term, beat-to-beat variability (pNN50: R=-0.67, R^2=0.45 & R-MSSD: R=-0.56, R^2=0.31; p<0.05). Finally, a significant 2nd order polynomial relationship was observed with respect to the more broad base time domain measures (SDNN: R=-0.40, R^2=0.16, SDNN Index: R=-0.66, R^2=0.25 & SDANN: R=-0.27, R^2=0.08; p<0.05).

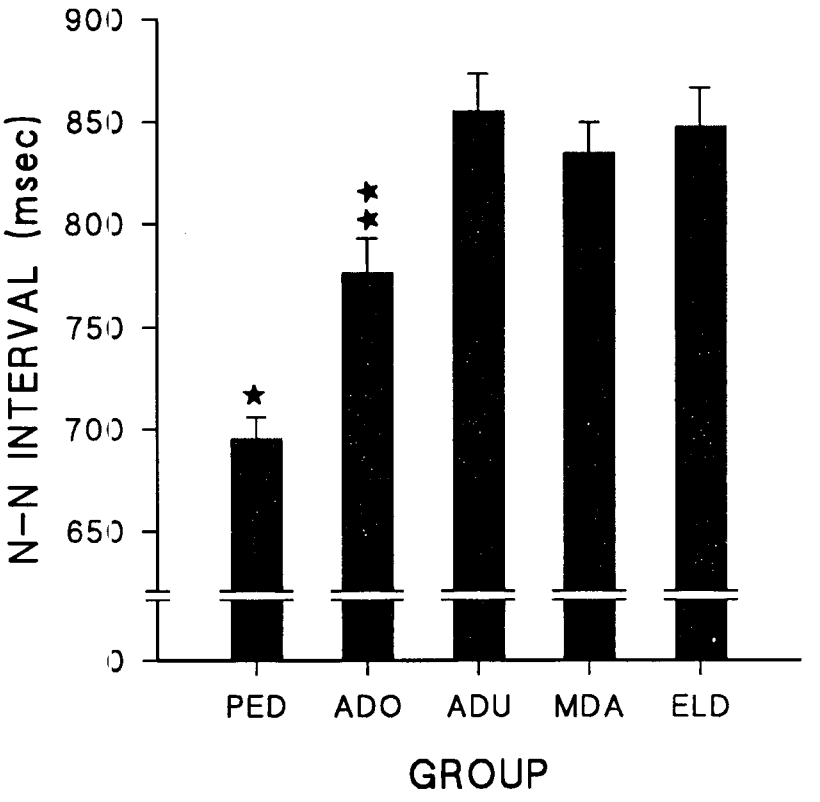
Age group and gender related alterations in time domain HRV indices derived from 24-hour ECG Holter recordings were evaluated with a two-way between subjects MANOVA (AGE × GENDER). Significant main effects were observed for both AGE (RaoR (28,365)=10.8; p<0.00001) and GENDER (RaoR (7,101)=5.36; p<0.00003). However the AGE × GENDER interaction did not reach statistical significance (RaoR (28,365)=1.46; N.S.).

Effect of Age

Average heart rate and N-N interval over the 24-hour recording period exhibited a progressive age-related slowing and lengthening respectively over the youngest three age groups (HR: PED (86.7 ± 5.7 bts/min) > ADO (77.9 ± 7.7 bts/min; p<0.002) > ADU (70.5 ± 7.6 bts/min; p<0.0002) and N-N interval: PED (695.4 ± 47.1 msec) < ADO (776.6 ± 74.2 msec; p<0.008) < ADU (860.3 ± 86.4 msec; p<0.0002)). However, no further alterations were observed in the older two age groups (see Figure 12).

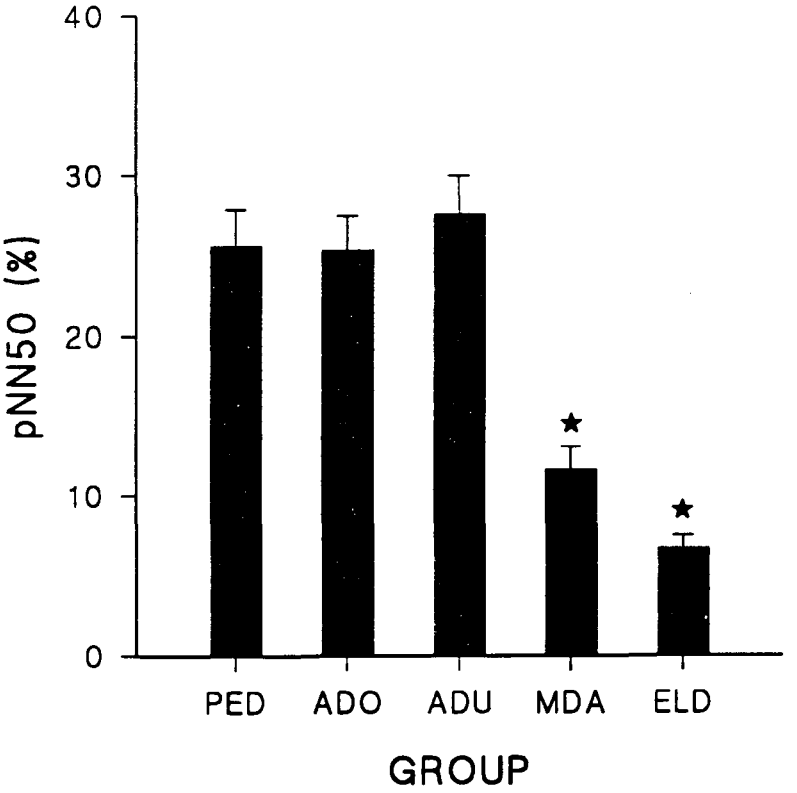
Figure 12. Mean 24-hour heart rate (top) and N-N interval (bottom) derived from the holter ECG recordings for the PED, ADO, ADU, MDA and ELD age groups. (Top: ★ PED > ADO, ADU, MDA & ELD; $p<0.008$, ★★ ADO > ADU, MDA & PED; $p<0.008$, Bottom: ★ PED < ADO, ADU, MDA & ELD; $p<0.008$, ★★ ADO < ADU, MDA & ELD; $p<0.008$)


HEART RATE (bts/min)
90
85
80
75
70
0
PED
ADO
ADU
MDA
ELD
GROUP


N-N INTERVAL (msec)
900
850
800
750
700
650
0
PED
ADO
ADU
MDA
ELD
GROUP

The variability measures computed from these recordings showed two distinct patterns of age-related change. Those indices characterizing short term variability (pNN50 & R-MSSD) were significantly diminished only in the oldest two age groups (pNN50: PED (25.6 ± 10.2%), ADO (25.4 ± 9.6%) & ADU (27.6 ± 11.2%) > MDA (11.6 ± 7.1%) & ELD (6.7 ± 4.2%: $p<0.0002$) and R-MSSD: PED (61.5 ± 20.6 msec), ADO (57.6 ± 17.5 msec) & ADU (59.5 ± 19.8 msec) > MDA (34.6 ± 10.5 msec) & ELD (30.1 ± 11.0 msec; $p<0.0005$, see Figure 13)). In contrast, those measures encompassing longer term oscillations showed a distinct pattern generally reaching their peak value in the ADU age group (see Figure 14). The SDNN was significantly higher in the ADU subjects (173.8 ± 30.0 msec) compared to those in the PED (144.6 ± 27.2msec; $p<0.005$), MDA (150.9 ± 27.9 msec; $p<0.02$) and ELD groups (126.0 ± 26.7 msec; $p<0.0002$). In addition, SDNN in ADO (168.9 ± 37.6 msec) subjects was significantly greater than those in the ELD group ($p<0.0004$). Similarly the SDANN was significantly enhanced in the ADU subjects (150.3 ± 31.9 msec) compared to those in the PED (122.6 ± 27.2 msec; $p<0.02$) and ELD groups (113.9 ± 26.7 msec; $p<0..0005$) and in the ADO group (145.0 ± 37.8 msec) compared to the ELD subjects ($p<0.03$). SDNN index, generally considered to be a measure of intermediate variability exhibited a similar pattern with significantly higher values in the ADO (85.1 ± 19.4 msec) and ADU (87.5 ± 19.0 msec) groups compared to

Figure 13. Mean pNN50 (top) and R-MSSD (bottom) time domain parameters derived from the 24-hour holter ECG recordings for the PED, ADO, ADU, MDA and ELD age groups. (Top: ★ MDA & ELD < PED, ADO & ADU; $p<0.0002$, Bottom: ★ MDA & ELD < PED, ADO & ADU; $p<0.0005$)


40
30
20
10
0
pNN50 (%)
PED
ADO
ADU
MDA
ELD
GROUP

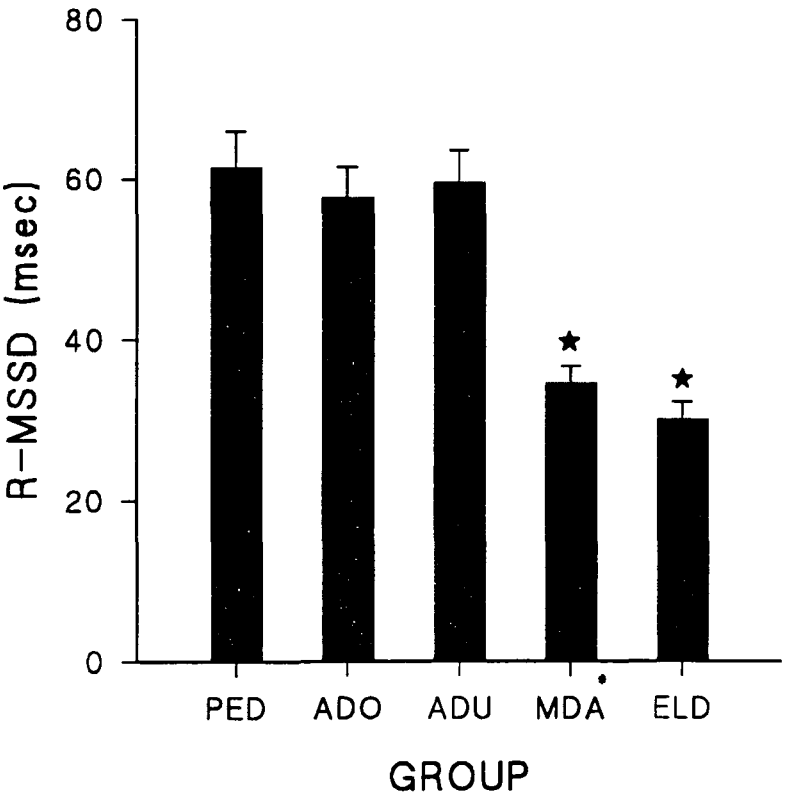
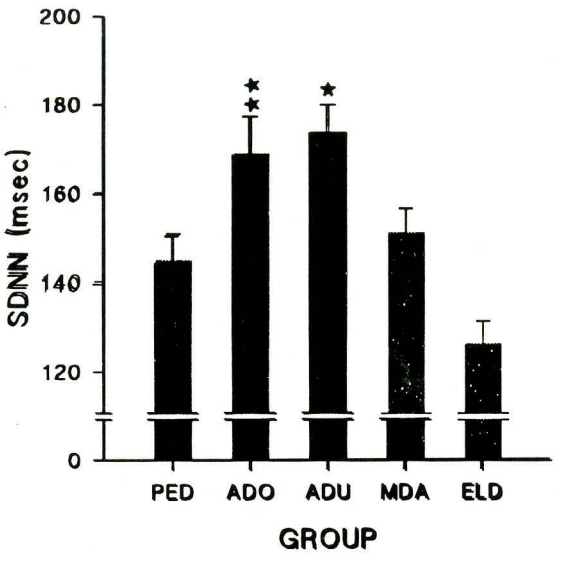
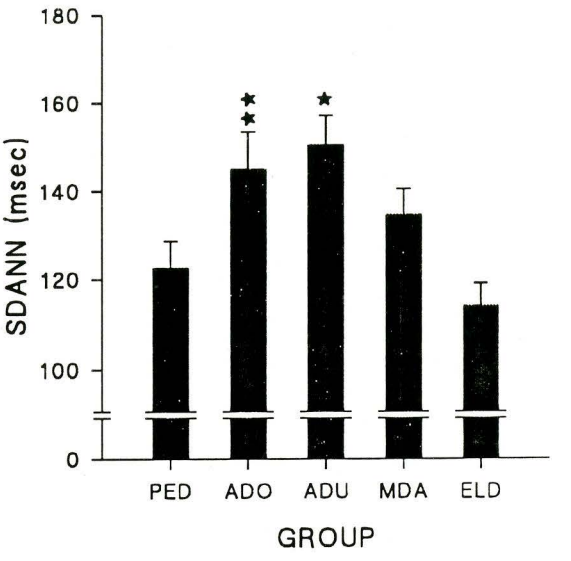
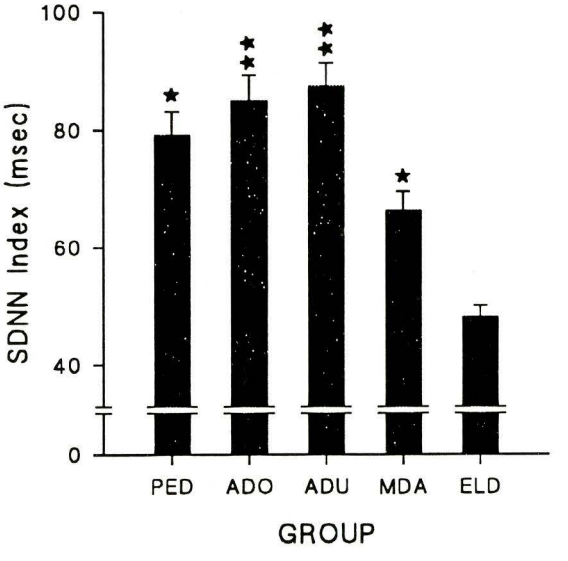

80
60
40
20
0
R-MSSD (msec)
PED
ADO
ADU
MDA
ELD
GROUP

Figure 14. Mean SDNN (top left), SDANN (top right) and SDNN index (bottom) time domain parameters derived from 24-hour holter ECG recordings for PED, ADO, ADU, MDA and ELD age groups. (Top left: ★ ADU > PED, MDA & ELD; $p<0.02$, ★★ ADO > ELD; $p<0.0004$, Top right: ★ ADU > PED & ELD; $p<0.02$, ★★ ADO > ELD; $p<0.03$, Bottom ★ ADO & ADU > MDA & ELD; $p<0.004$, ★★ PED & MDA > ELD; $p<0.003$)


200
180
160
140
120
0
SDNN (msec)
PED
ADO
ADU
MDA
ELD
GROUP


180
160
140
120
100
0
SDANN (msec)
PED
ADO
ADU
MDA
ELD
GROUP


100
80
60
40
0
SDNN Index (msec)
PED
ADO
ADU
MDA
ELD
GROUP

the MDA (66.4 ± 15.7 msec; p<0.004) and ELD subjects (48.2 ± 10.2 msec; p<0.0002).

Effect of Gender

Post hoc analysis of the gender main effect revealed no significant differences between males and females with respect to the time domain variability indices. However, the average heart rate and N-N interval over the entire 24-hour period were significantly slower and longer respectively in males compared to females (HR: ♀ (76.7 ± 9.0 bts/min) > ♂ (72.9 ± 9.3 bts/min; p<0.002) & N-N interval: ♀ (792.1 ± 89.2 msec) < ♂ (836.2 ± 104.6 msec; p<0.001)).

3.2.2 Circadian Rhythms of Spectral Parameters

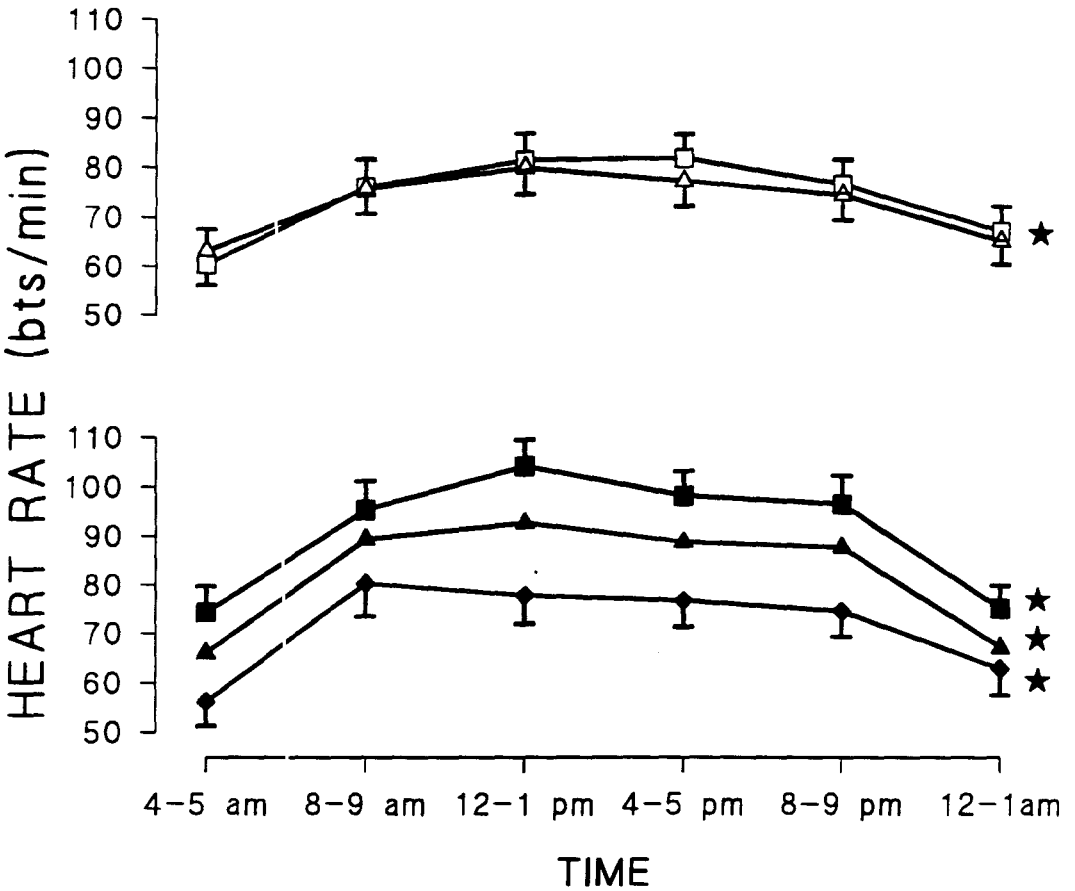
Diurnal variations in frequency domain HRV indices were examined through spectral analysis of heart rate data for the six previously identified one hour intervals during the Holter ECG recording. A three-way mixed MANOVA (AGE × GENDER × TIME) test was applied to this data and revealed significant main effects for AGE (RaoR (16,315)=11.5; p<0.000001), GENDER (RaoR (4,103)=8.5; p<0.000005) and TIME (RaoR (20,87)=48.9; p<0.000001) as well as a significant AGE × TIME interaction (RaoR (80,345)=1.71; p<0.0006). In general, heart rate as well those measures believed to reflect cardiac

sympathetic modulation (LF area & % LF area) increased between the 4-5 a.m. and 8-9 a.m. time periods and remained consistently elevated throughout the day. Between the 8-9 p.m. and 12-1 a.m. time periods these measures returned to levels comparable to those seen during the 4-5 a.m. epoch. Parasympathetically mediated measures (HF area & %HF area) exhibited an opposing pattern with peak levels reached during the 12-1 a.m. and 4-5 a.m. time slots. As would be expected the peak and area ratios traced a diurnal path similar to that seen in the LF area and %LF area indices. However, the LF CF followed a distinct pattern with no significant increase in the measure until after the 8-9 a.m. time period. This index then remained elevated into the early morning hours falling to its baseline level between 12-1 a.m. and 4-5 a.m.

Effect of Age

Significant variation in heart rate throughout the day was evident in all age groups (see Figure 15). However, the diurnal variations of several HRV indices were significantly attenuated in the older subject groups. The peak and area ratios followed the typical circadian pattern previously described in the ADO ($p<0.003$) and ADU ($p<0.001$) subjects but failed to show any significant variation throughout the day in ELD subjects (see Figure 16). The PED subjects exhibited somewhat diminished patterns with both the LF:HF area and LF:HF peak significantly elevated between 12-1 p.m. only (with respect to the 12-1 a.m.

Figure 15. Circadian variations in mean heart rate derived from the 24-hour holter ECG recordings for the PED, ADO, ADU (bottom), MDA and ELD (top) age groups: ■ PED, ▲ ADO, ◆ ADU, □ MDA, Δ ELD. (★ significant circadian variation in all groups; $p<0.025$)


HEART RATE (bts/min)
110
100
90
80
70
60
50
110
100
90
80
70
60
50
4–5 am
8–9 am
12–1 pm
4–5 pm
8–9 pm
12–1am
TIME

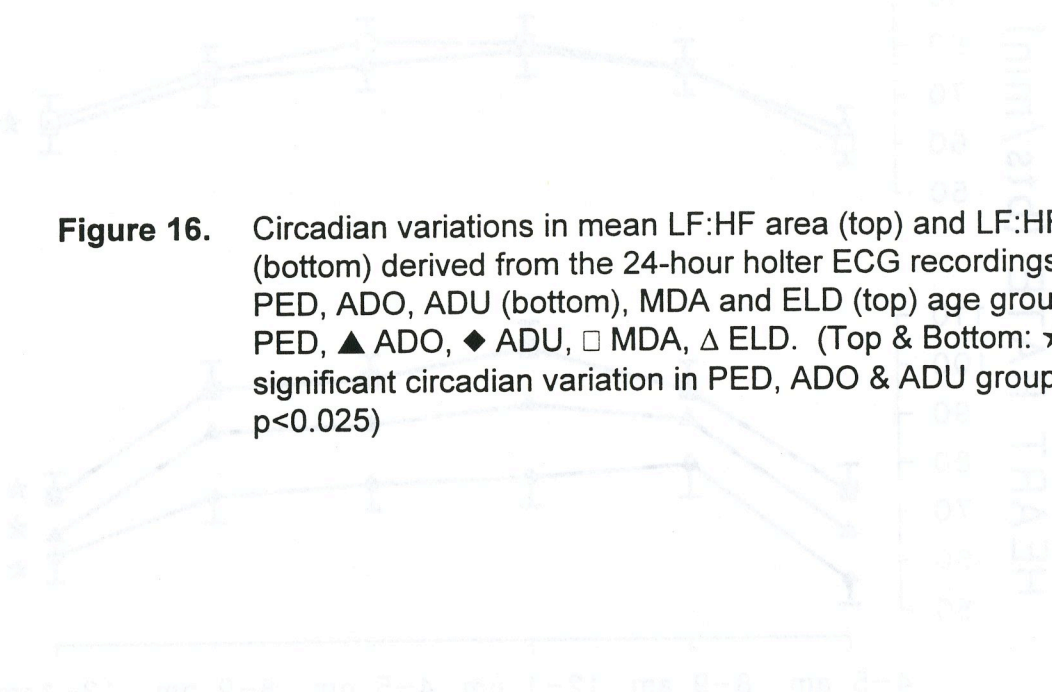
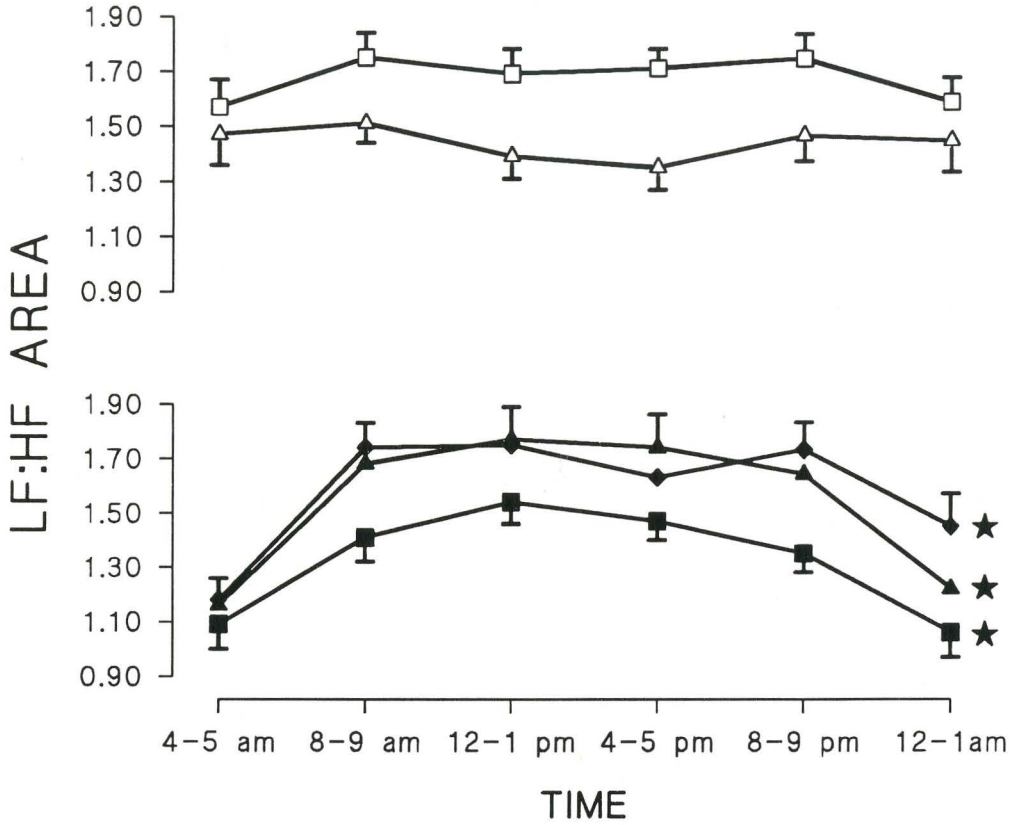
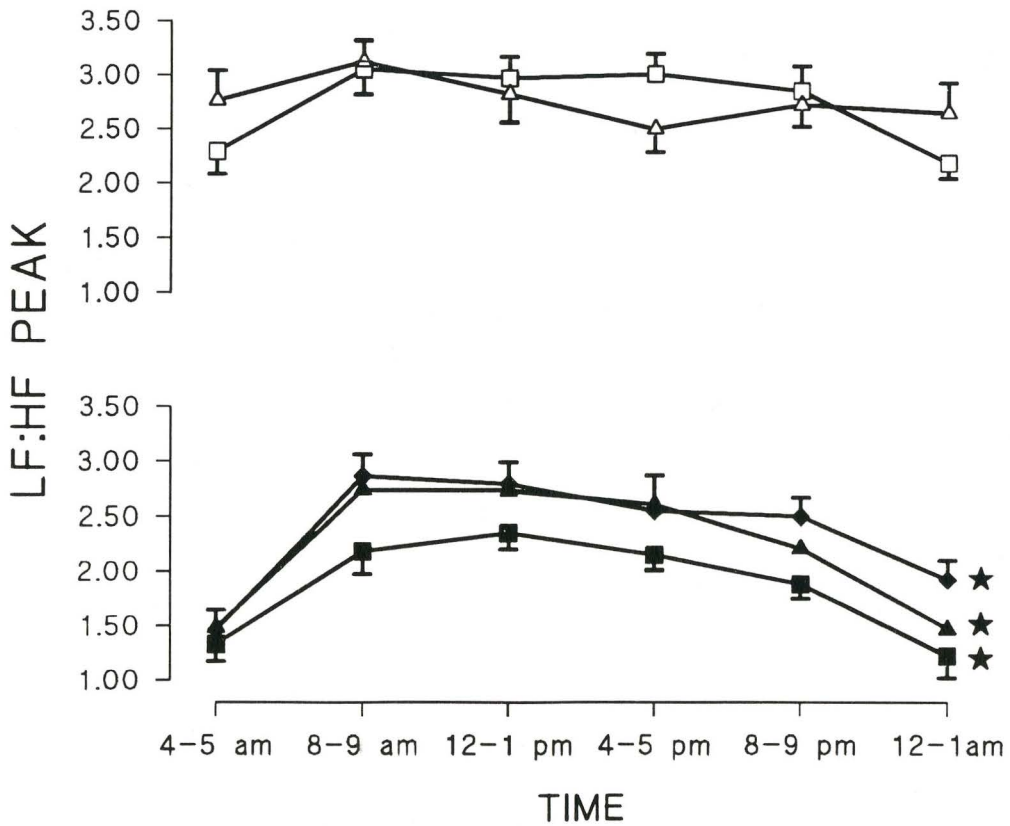


Figure 16. Circadian variations in mean LF:HF area (top) and LF:HF peak (bottom) derived from the 24-hour holter ECG recordings for the PED, ADO, ADU (bottom), MDA and ELD (top) age groups: ■ PED, ▲ ADO, ◆ ADU, □ MDA, Δ ELD. (Top & Bottom: ★ significant circadian variation in PED, ADO & ADU groups; $p<0.025$)


LF:HF AREA
1.90
1.70
1.50
1.30
1.10
0.90
4-5 am
8-9 am
12-1 pm
4-5 pm
8-9 pm
12-1am
TIME


LF:HF PEAK
3.50
3.00
2.50
2.00
1.50
1.00
4-5 am
8-9 am
12-1 pm
4-5 pm
8-9 pm
12-1am
TIME

& 4-5 a.m. time periods; $p<0.009$). No significant variation in the area ratio was observed in the MDA subjects. However, the LF:HF peak was significantly elevated during the 8-9 a.m., 12-1 p.m. and 4-5 p.m. time slots compared to the period between 12-1 a.m. ($p<0.02$). Interestingly the LF CF exhibited no significant variation throughout the day in the youngest two groups of subjects (PED & ADO, see Figure 17). A diurnal pattern was observed in the ADU and MDA subjects with the LF CF significantly elevated during the 12-1 p.m., 4-5 p.m. and 8-9 p.m. time periods compared to the 4-5 a.m. epoch ($p<0.04$). The extent of this rhythm was somewhat attenuated in the ELD subjects with the LF CF significantly elevated only during the 4-5 p.m. time slot compared to the period between 4-5 a.m. ($p<0.02$).

Variations in both the absolute (LF area) and fractional (%LF area) power in the LF band followed the previously mentioned pattern with significantly elevated levels during the 8-9 a.m., 12-1 p.m., 4-5 p.m. and 8-9 p.m. time periods in the three youngest age groups (PED; $p<0.005$, ADO; $p<0.004$ & ADU; $p<0.001$, see Figure 18). However, no significant diurnal variations were observed for either of these measures in the MDA and ELD subjects. Similarly, the fractional power of the HF band was significantly diminished during the 8-9 a.m., 12-1 p.m., 4-5 p.m. & 8-9 p.m. time periods in the PED ($p<0.03$), ADO ($p<0.00007$) and ADU groups ($p<0.00004$) only (see Figure 19). Diurnal

Figure 17. Circadian variations in mean LF CF derived from the 24-hour holter ECG recordings for the PED, ADO, ADU (bottom), MDA and ELD (top) age groups: ■ PED, ▲ ADO, ◆ ADU, □ MDA, Δ ELD. (★ significant circadian variation in ADU, MDA & ELD groups; $p<0.025$)

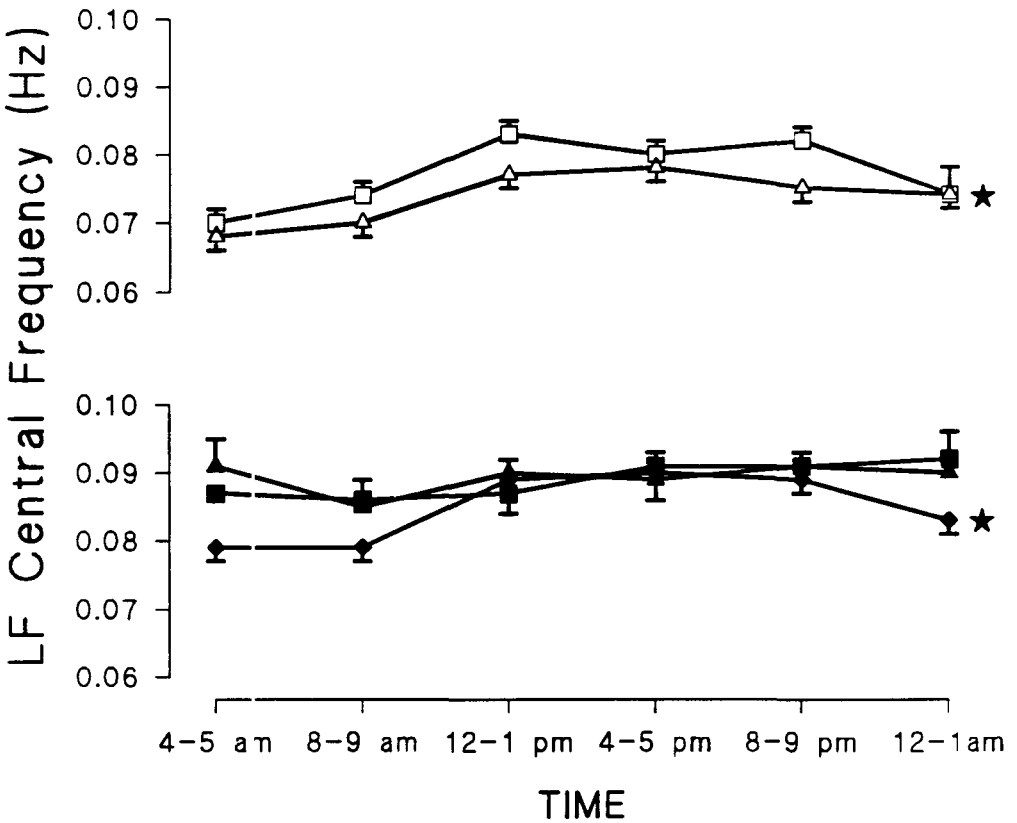
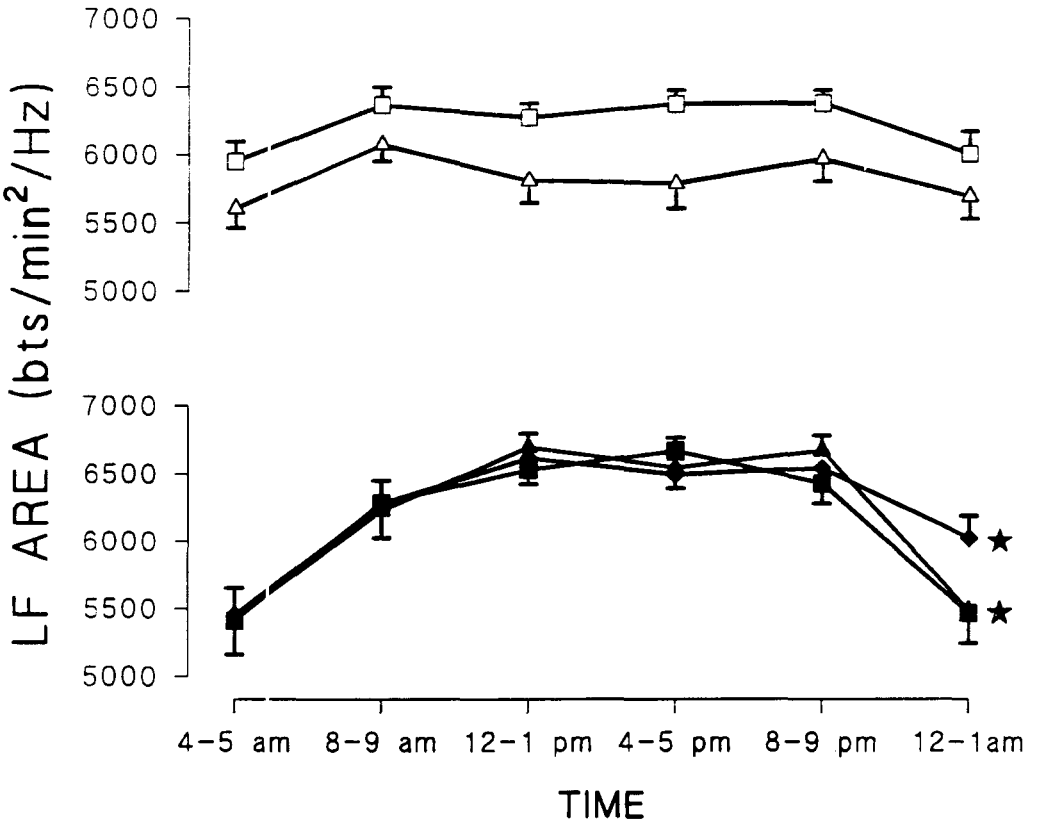

LF Central Frequency (Hz)
0.10
0.09
0.08
0.07
0.06
0.10
0.09
0.08
0.07
0.06
4-5 am
8-9 am
12-1 pm
4-5 pm
8-9 pm
12-1am
TIME

Figure 18. Circadian variations in mean LF area (top) and %LF area (bottom) derived from the 24-hour holter ECG recordings for the PED, ADO, ADU (bottom), MDA & ELD (top) age groups: ■ PED, ▲ ADO, ◆ ADU, □ MDA, Δ ELD. (Top & bottom: ★ significant circadian variation in PED, ADO & ADU groups; $p<0.025$)


LF AREA (bts/min²/Hz)
7000
6500
6000
5500
5000
7000
6500
6000
5500
5000
4-5 am
8-9 am
12-1 pm
4-5 pm
8-9 pm
12-1am
TIME

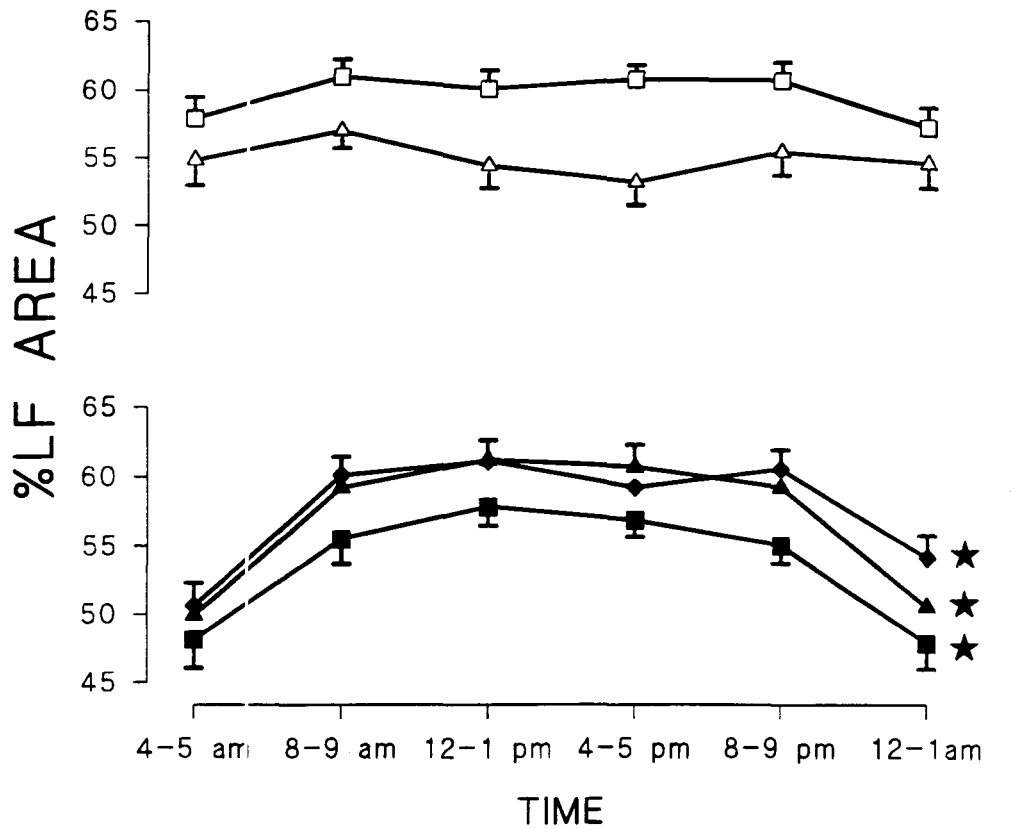
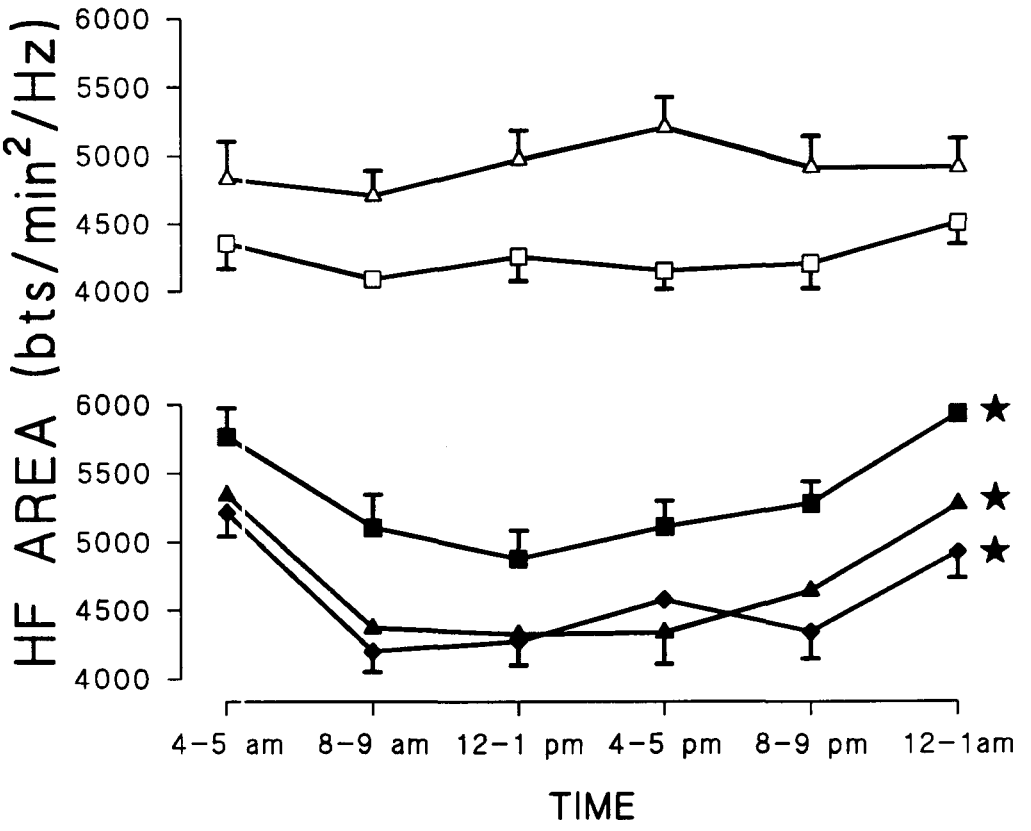
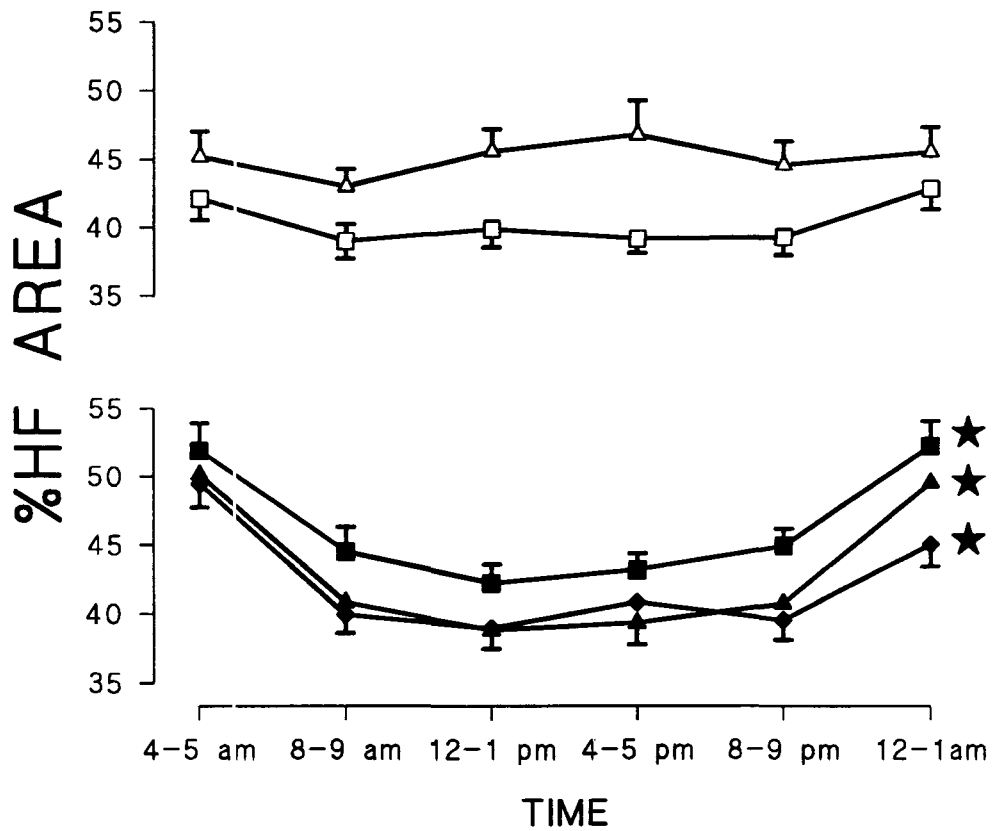

%LF AREA
65
60
55
50
45
65
60
55
50
45
4-5 am
8-9 am
12-1 pm
4-5 pm
8-9 pm
12-1am
TIME

Figure 19. Circadian variations in mean HF area (top) and %HF area (bottom) derived from the 24-hour holter ECG recordings for the PED, ADO, ADU (bottom), MDA and ELD (top) age groups: ■ PED, ▲ ADO, ◆ ADU, □ MDA, Δ ELD. (Top & bottom: ★ Significant circadian variation in PED, ADO & ADU goups; $p<0.025$)


HF AREA (bts/min²/Hz)
6000
5500
5000
4500
4000
6000
5500
5000
4500
4000
4-5 am
8-9 am
12-1 pm
4-5 pm
8-9 pm
12-1am
TIME


%HF AREA
55
50
45
40
35
55
50
45
40
35
4-5 am
8-9 am
12-1 pm
4-5 pm
8-9 pm
12-1am
TIME

variations of both the HF area and %HF area were absent in the MDA and ELD subjects.

Effect of Gender

No differences were noted with respect to the diurnal variation of HRV indices in males compared to females. However, when these measures were averaged across the six time periods a significant gender effect was observed. Heart rate was significantly elevated in females compared to males during these time periods (♀: 78.9 ± 13.2 bts/min > ♂: 74.3 ± 12.9 bts/min; $p<0.001$). However, the LF:HF peak and area ratios as well as the LF and %LF area indices were elevated in males compared to females during these times (LF:HF peak: ♂ (2.66 ± 1.1) > ♀ (2.26 ± 0.99; $p<0.0009$), LF:HF area: ♂ (1.62 ± 0.45) > ♀ (1.44 ± 0.48; $p<0.005$), LF area: ♂ (6276.1 ± 605 bts·min^{-2}/Hz) > ♀ (5982.5 ± 838 bts·min^{-2}/Hz; $p<0.005$) & %LF area: ♂ (58.8 ± 7.0%) > ♀ (55.1 ± 8.3%; $p<0.001$). In addition, females exhibited higher levels of HF area and % HF area throughout these one hour epochs (HF area: ♀ (4930.0 ± 1052 bts·min^{-2}/Hz) > ♂ (4460.5 ± 940 bts·min^{-2}/Hz; $p<0.001$) & %HF area: ♀ (44.9 ± 8.3%) > ♂ (41.2 ± 7.0%; $p<0.0009$)).

3.3 Summary of Findings

Heart rate in the supine position declined progressively in the three youngest groups and exhibited no age-related alterations in the older subjects. In contrast, supine spectral measures were relatively stable below 30 years of age and demonstrated a shift towards relative sympathetic dominance (increased LF:HF ratios and LF power as well as decreased HF power and LF CF) in the two older groups. In the standing posture both heart rate and LF CF exhibited a successive decline across all five groups. The remaining spectral measures followed a more complex M-shaped pattern in this posture. Both the heart rate and spectral responses to orthostasis were greater in magnitude in the ADU group compared to the PED subjects and exhibited a progressive age-related decline in the three oldest groups of individuals.

The mean 24-hour heart rate derived from the 'Holter' recordings declined significantly in the three youngest groups but demonstrated no further alterations in the MDA and ELD subjects. Conversely, the short-term time domain indices (pNN50 & R-MSSD) were relatively stable in the PED, ADO and ADU subjects and significantly diminished in the two older age groups. Time domain parameters encompassing both long and short-term oscillations (SDNN, SDANN & SDNN index) increased progressively in the three youngest groups, reaching peak values in the ADU subjects. These measures were then significantly diminished in the MDA and ELD groups.

Heart rate exhibited a significant circadian rhythm in each of the five age groups. However, significant diurnal variations in the majority of the spectral parameters were evident in the PED, ADO and ADU subjects only. In addition, the LF CF measure exhibited significant circadian variations in the ADU, MDA and ELD subjects. These LF CF variations were somewhat attenuated in the ELD age group.

Heart rate, although similar between males and females in the supine position was significantly elevated in males during the 10 minutes of free standing. In addition, the LF:HF peak measure was significantly elevated in males compared to females in both postures. Consequently, both the heart rate and LF:HF peak responses to orthostasis were greater in magnitude in males compared to females.

Mean heart rate derived from the 24-hour 'Holter' recordings was significantly elevated in females compared to males. Similarly, heart rate averaged over the six, one hour periods examined in the circadian analysis was greater in females. Power spectral analysis of the same six hours of data revealed significantly diminished LF power and LF:HF peak and areas ratios and elevated HF power in females compared to males.

4.0 DISCUSSION

4.1 Overview

Historically, investigations into autonomic function have been limited by the relative inaccessibility of the system for direct study. More recently, the recognition that the heart rate signal contains information pertaining to autonomic regulatory mechanisms (Hon & Lee, 1965) and the development of techniques such as power spectral analysis (Sayers et al. 1973; Akselrod et al. 1981) have provided a non-invasive means of assessing neurocardiac regulation in humans. It has been demonstrated in the literature that numerous pathological conditions including diabetes (Pagani et al. 1988; Murkawa et al. 1993), end-stage renal failure (Axelrod et al. 1987) and myocardial infarction (Kleiger et al. 1987; Malik et al. 1990; Lombardi et al. 1992; Odemuyiwa et al. 1992) are associated with depressed autonomic function manifested as an overall reduction in heart rate variability, attenuated spectral responses to physiological stress and alterations in sympathovagal balance. Unfortunately, investigations in elderly populations are somewhat complicated by the existence of age dependent alterations in HRV parameters which appear to be similar in nature to those commonly associated with these conditions. Consequently, if HRV is to reach its full potential as a valuable tool for researchers and clinicians alike considerable effort must be directed toward the establishment of standards

characterizing 'normal' autonomic function for healthy individuals in all age groups and across gender.

The purpose of the present investigation was to examine the alterations in cardiac autonomic function associated with 'normal' healthy aging in males and females. Additionally, this study was undertaken to supplement the existing literature in this area and to establish a data base for traditionally neglected yet potentially useful areas of HRV investigation.

4.2 Regression Data

In general, mild to moderate correlation's were detected between age and the majority of the computed HRV measures. As expected HRV parameters that are considered to characterize oscillations generated through separate autonomic mechanisms demonstrated distinct developmental patterns. However, what is most immediately apparent from this analysis is that considerable variability exists in both time domain and power spectral measures of HRV in a normal healthy population at all stages of life. As a result of this broad range of scores, the regression analysis appeared to obscure some of the phenomena evident when statistical comparisons were made between the selected age groupings. Consequently, interpretations regarding the possible physiological mechanisms underlying the observed age-related trends made in this discussion will be based primarily on the findings of the MANOVA's. That is

not to say that all of the information provided by the regression analysis is redundant. In fact, as the ultimate goal of this investigation was to provide a more precise definition of 'normality' with respect to cardiac autonomic function an indication of the typical range of HRV scores found within the 'normal' healthy population would be invaluable to future scholastic endeavors in this field. Thus, for future reference scatterplots with 'best fit' lines and 95% confidence ellipses were generated for the significant age × HRV parameter relationships. This data is displayed along with the reported R and R^2 values in Appendix C. It is interesting to note that the lower 95% confidence band for both the pNN50 and R-MSSD measures intersects the X-axis at approximately 40 years. As such, pathologically low values for these parameters can not be identified in elderly individuals and any diagnostic or prognostic information provided by these measures is therefore lost in the older populations.

4.3 Acute Phase

4.3.1 Supine & Standing States

Mean heart rate in the supine state exhibited an age-related decrease that was restricted to the three youngest groups. However, in contrast to the findings of Finley et al. (1987) there was no evidence of concurrent alterations in the LF:HF peak and area ratios or the LF CF during this period. As such, this phenomenon can not be attributed to an age-related alteration in autonomic

input to the SA node. More likely this diminution in resting heart rate is a result of intrinsic alterations in SA node function (secondary to structural changes). Indeed, Lev (1954) noted an increase in the size of the human SA node up until the age of 20 years. What is of particular interest in this analysis is the absence of further heart rate alterations in the MDA and ELD subjects despite a significant shift towards sympathetic dominance in those indices believed to reflect autonomic balance (LF:HF area, LF:HF peak & LF CF). These spectral alterations are consistent with findings reported by Korkushko et al. (1991). Previous studies (Vestal et al. 1979; Stratton et al. 1992) have reported evidence of reduced β-adrenoreceptor sensitivity in elderly subjects. In addition, Jose et al. (1970) documented a steady decline in intrinsic heart rate determined in the resting state under dual blockade (propranolol and atropine) in subjects between the ages of 16 and 70 years. Consequently, the apparent age-related augmentation of sympathetic influence on the SA node appears to be offset by the aforementioned alterations in intrinsic heart rate and receptor sensitivity resulting in a stable resting heart rate over this period of the life-span.

The age-related decline in free standing heart rate was continuous across the entire age range under investigation (5-78 years). It is likely that this effect is due to alterations in the mechanisms responsible for the augmentation of cardiac function in response to physiological stress. This possibility will be explored in more detail in the discussion of the HRV response to orthostatic stress.

Power spectral indices in the standing posture demonstrated a more complex M-shaped pattern across the 5 age groups. Such a trend is difficult to explain on a physiological level. However the difference between the ADU and MDA groups failed to reach statistical significance on any of the spectral measures. As such, it is possible that this second peak in the LF power and ratio measures may represent a chance aberration of the data and these measures are actually stable at this time (a pattern similar to that observed with the orthostatic changes scores).

4.3.2 Orthostatic Stress

In general, the magnitude of the heart rate and power spectral responses to orthostasis followed a characteristic pattern, reaching peak values in the ADO group and declining progressively thereafter. Physiologically, the initial age-related enhancement of the cardiovascular response may be due to functional maturation in the baroreceptor arc. However, an additional explanation may lie in the dramatic increase in body size seen during the childhood and adolescent years. The resultant increase in the volume of the lower limb vascular tree would allow for greater pooling of blood in the feet and legs on standing. Subsequently, the baroreceptors would be unloaded to a greater extent and the resultant increase in sympathetic drive to the SA node would be more dramatic. Such an assertion is supported by the previous observation that the inflation of

blood pressure cuffs around the thighs, effectively removing the vascular tree of the lower limbs from the circulation results in an attenuated heart rate response to orthostasis (Astrand & Rodahl 1986).

The aforementioned age-related phenomena is inconsistent with the findings of Finley et al. (1987) who reported that the most dramatic spectral adaptation to orthostasis is seen in 5-7 year old subjects. In the aforementioned investigation these young children exhibited a significantly higher baseline level of LF:HF area compared to those individuals 10-12 and 20-24 years of age, a trend not observed in the present investigation. Consequently, if the observed change is expressed relative to the original supine value, the magnitude of this spectral response to orthostasis in the 5-7 year olds is substantially smaller than that of the two older groups. Thus, it may be that these young children were more affected by the unfamiliarity of the research setting than their older counterparts and the observation of a greater absolute response may simply be a laboratory artifact.

The magnitude of the spectral response to orthostasis declined progressively in the three oldest groups of subjects. Likewise, diminished cardiovascular responses to orthostatic stress in aging populations have been reported by numerous investigators (Jarisch et al. 1987; Lipsitz et al. 1990; Yo et al. 1994). This phenomenon is generally attributed to a decrease in the sensitivity of the baroreflex mechanisms that mediates the response. Associated

with aging are a number of degenerative changes in the walls of blood vessels that act to produce a stiffer, less compliant vasculature (Porges et al. 1986). Elastic fibres uncoil and fracture while the stiff collagen matrix increases and progressive calcification occurs in the vessel media. As a result the baroreceptors located in the walls of the carotid arteries receive a diminished stimulus from a given change in blood pressure. Consequently, this reflex will trigger an attenuated response to a cardiovascular challenge such as that presented by the gravitational stress resulting from the change in posture from supine to standing. This hypothesis is supported by reports of a diminished heart rate response to phenylephrine infusion (Porges et al. 1986) in elderly individuals.

Additional mechanisms may contribute to this age-related deficit in cardiovascular responsiveness. Particularly, the established decline in β-adrenoreceptor sensitivity (Stratton et al. 1992) mentioned in the previous section would ensure that any alteration in the sympathetic drive to the SA node induced by the baroreflex arc would have a significantly diminished chronotropic effect. In addition, degenerative changes affecting the autonomic neurons themselves might be involved in the functional deficits evident in elderly populations (Korkushko et al. 1991).

What is off particular concern in this analysis is the significant percentage of elderly individuals exhibiting an 'atypical' spectral response to othostatic

stress. In fact, paradoxical shifts in the LF area and LF CF were observed in 31% and 38% of subjects over the age of 55 respectively. In addition, the prevalence of these same 'atypical' phenomena in subjects under the age of 30 years was only 13% and 11%, which is by no means a negligible subset of the study population. Previous studies have utilized response patterns such as these as benchmarks of autonomic neuropathy (Kamath et al. 1987). However, this study clearly demonstrates that these responses are also present in populations of apparently healthy individuals. As such, these findings underscore the need to establish standards of normality in HRV analysis and re-examine the definition of pathology with respect to autonomic function.

4.4 Long Term Variability - 24-Hour Data

4.4.1 Time Domain

The observed age-related decline in 24-hour heart rate and lengthening of the mean N-N interval in the three youngest age groups are consistent with the findings of the acute phase of the study and thus support the conclusions made in the preceding sections.

Inspection of the time domain data revealed two distinct patterns of age-related alteration in 24-hour heart rate variability. Parameters characterizing short term variability were greatest in the PED subjects, remained stable throughout the ADO and ADU groups and dropped off dramatically in the older

two groups. In contrast, those indices incorporating intermediate and long term heart rate oscillations (SDNN, SDANN & SDNN Index) increased steadily in the younger age groups reaching their maximum values in the ADU sample and declining progressively thereafter. In the human embryo and fetus, structural and functional development of the parasympathetic system precedes that of the sympathetic limb (Dail et al. 1973; Walker et al. 1975; Pappano et al. 1977). The findings of this investigation appear to indicate that a similar pattern exists with respect to the continuing maturation of these mechanisms throughout childhood and adolescence. The decline of the SDNN in the older age groups appears to contradict the increase in the LF:HF peak and area ratios seen in these subjects in the supine state during the acute phase of the study. However, time domain methods are relatively imprecise compared to power spectral analysis in their ability to separate the influence of the two autonomic limbs. As such, a considerable amount of the variability accounted for in these long term measures is generated by parasympathetically mediated mechanisms. Thus, the decrease in these measures may be due in large part to a decline in the parasympathetic contribution, an assertion consistent with the trend observed in the pNN50 and R-MSSD measures and the supine frequency domain parameters.

Hon & Lee in 1965 noted a dramatic reduction in the variability of the heart rate immediately preceding the onset of fetal distress. Since this time much research has been done to develop HRV analysis as a prognostic tool in

various populations including post-MI patients (Wolf et al. 1977; Kleiger et al. 1987; Odemuyiwa et al. 1992) and elderly subject cohorts (Algra et al. 1993; Tsuji et al. 1994); It is consistently within the time domain measures that the most powerful prognostic indicators are found. As such, the neglect of time domain analysis in the aging literature is surprising. The data contained in this thesis indicate that advanced age is associated with a loss of heart rate variability as assessed through time domain parameters. Of particular interest is the fact that such an alteration is also frequently associated with pathology and an elevated risk of sudden death (Kleiger et al. 1987). A similar pattern was encountered in the acute phase of this study with respect to the spectral response to orthostasis. Consequently, the potential inability of these tools to distinguish between natural aging processes and pathological states may limit their future usefulness in a clinical setting.

4.4.2 Diurnal Variations in Heart Rate & HRV

Heart rate and spectral indices exhibit a distinct and reproducible circadian rhythm (Fallen et al. 1995). In general those parameters attributed to sympathetic modulation are at their peak levels at approximately 4-5 p.m. in the afternoon and fall steadily to a minimal level at sometime around four o'clock in the morning. Parasympathetically mediated variability measures tend to follow an opposing/reciprocal pattern. Recent studies have indicated that these

rhythms may be disturbed in certain pathological states including congestive heart failure (Casolo et al. 1991; Panina et al. 1995) and myocardial infarction (Lombardi et al. 1992). Similarly, aging appears to be associated with a diminished diurnal variation in both heart rate and power spectral measures. As the physiological mechanisms underlying the circadian rhythm evident in autonomic cardiac control are not yet fully understood it is impossible at this time to propose a comprehensive hypothesis as to the direct cause of this phenomenon. However, it is conceivable that this effect may be due to some alteration or degeneration in a central oscillatory center.

As previously mentioned the low frequency peak in the heart rate spectrum is thought to represent baroreceptor related oscillations jointly mediated by both limbs of the autonomic nervous system. The location of the central frequency in this band may therefore provide an indication of autonomic balance, an assertion supported by previous pharmacological study (Weise et al. 1989). As such, the observation that the circadian rhythm of this variable differed substantially from that of other measures also believed to reflect sympathovagal balance was unexpected. In fact the diurnal pattern exhibited by the LF CF suggests an augmentation of parasympathetic influence at the SA node during the daytime hours. This assertion is in direct opposition to the information provided by more traditional measures of autonomic balance such as the LF:HF peak and area ratios. Additionally, the age-related alterations in the

extent of LF CF circadian variations followed a pattern that was clearly distinct from that of both the LF:HF peak and LF:HF area ratios. There are two possible explanations for this apparent discrepancy. At first, it was thought that these puzzling findings were a manifestation of an unidentified problem with the power spectral analysis software. Indeed the acute phase of the study which utilizes a separate software package provided results in which the alterations in the LF CF on standing paralleled those of both the LF:HF peak and area ratios. However, this explanation seems unlikely given that these programs are rigorously tested using sinusoidal waveforms prior to their utilization in a research setting. A second more plausible hypothesis is that the physiological determinants of the central ferquency of the LF peak are more complex than is generally appreciated. Therefore, further study is necessary before definitive conclusions regarding the interpretation of this largely neglected spectral parameter can be made.

4.5 Gender & the Aging Process

In contrast to the findings reported by Ryan et al. (1996), the results of this investigation have provided no evidence supporting the hypothesis that the observed age dependent patterns of decline in cardiac autonomic function are dissimilar in males compared to females. It is possible that these dissimilarities are small relative to the observed age effects and the study therefore may have

lacked sufficient power to detect phenomena of this nature. However, significant discrepancies in both time domain and power spectral parameters were noted when males and females were compared irrespective of age. Males exhibited a significantly greater LF:HF peak in both the supine and standing postures compared to females. This finding is consistent with those of studies conducted by Ryan et al. (1994) and Liao et al. (1996) both of which reported an elevated high/low frequency power ratio in women compared to men. Nonetheless, this finding is somewhat puzzling given the observation that heart rate in the supine position was similar in the two groups. It is important to realize that the determinants of heart rate are more complex than just the sum of the autonomic inputs. Therefore, our observation may reflect a difference in intrinsic SA node function. Indeed, Burke et al. (1996) reported a significantly higher heart rate after total autonomic blockade in females compared to males. However, this discrepancy was attributed to differences in exercise capacity rather than an intrinsic effect of gender itself. In addition, there may be a discrepancy in the sensitivity of the pacemaker regions to the neurotransmitter substances or perhaps some inconsistency in humoral regulatory factors between males and females.

The magnitude of the heart rate and LF:HF peak responses to the change in posture from supine to standing were more dramatic in male subjects compared to females. This observation may indicate that the 'gain' of the

baroreflex arc is set considerably higher in males. However the possibility that this phenomena is the result of a physical size difference between the two genders (the mechanism of which was discussed previously in regards to the enhanced orthostatic response in ADO vs PED subjects) cannot be ruled out.

Similar to the acute phase of the study, analysis of the 24-hour heart rate data revealed a number of significant discrepancies in the heart rate and N-N interval measures as well as the spectral profiles of males and females. Females exhibited a significantly faster mean heart rate and shorter mean N-N interval over the 24-hour recording compared to males. A comparable trend was observed when heart rate values from the circadian analysis were averaged over the six selected one hour periods of the Holter recording. However, as was the case with the supine recordings LF:HF peak and area ratios as well as the LF and %LF area measures were significantly elevated in males. Possible explanations for a discrepancy between heart rate and sympathovagal balance at the SA node were outlined in the previous sections.

5.0 SUMMARY & LIMITATIONS

5.1 Summary

Historically, HRV analysis has been applied to the investigation of autonomic function in specific pathological conditions known to be associated with significant neuropathy. However, the extent to which definitive conclusions could be drawn was limited by the lack of an accurate description of 'normality' defined with respect to age and gender. Thus, the purpose of this study was to characterize the age dependent alterations in cardiac autonomic function observed in both males and females and to address some commonly neglected areas (ie. 24-hour time domain parameters) in the existing literature pertaining to HRV in healthy populations.

The current findings support the existence of an age dependent decline in autonomic neurocardiac function. As assessed by HRV analysis this decline is manifest as an attenuation of both circadian variation in frequency domain parameters and spectral response to orthostasis as well as differential alterations in the LF and HF bands of the baseline power spectrum resulting in a shift toward relative sympathetic dominance in advanced age. In the context of the existing literature, the acute phase of the current investigation adds to a data base containing numerous seemingly contrary findings. The results of any single study are therefore insufficient to provide final clarification on the issues in

question. Consequently, it may prove necessary to apply meta-analysis techniques to the existing literature in order obtain a more accurate understanding of the alterations in neurocardiac function related to the aging process.

Additionally, the current investigation is the first to document the age-related changes in 24-hour time domain parameters of heart rate variability. The present study has failed to produce evidence supporting the hypothesis that the aging process acts differentially on the autonomic nervous systems of men and women. However, the existence of significant gender differences in both heart rate and power spectral measures across all age groups demands that separate standards of 'normality' be utilized for comparisons in future studies.

The results of the current investigation have raised two pertinent questions: 1) is autonomic function in elderly people comparable to that seen in various pathological disorders?; and 2) if there is a difference in the observed decline of autonomic function associated with aging and disease, are these techniques sensitive enough to distinguish between the two?

5.2 Study Limitations & Future Recommendations

There are a number of factors present in this study that if improved upon might increase the effectiveness of future investigations. The ability of any test to detect a significant difference between two groups is characterized by the

statistical property of power (Cohen 1992). Power in a statistical test is ultimately a function of sample size, the assigned alpha level and the magnitude of the actual difference between the populations under investigation (termed the effect size). A power analysis performed at the conclusion of the data collection period indicated that a sufficient sample size existed for the detection of both medium and large effect sizes among the ten groups (age groups (5) × gender (2)) examined (power >0.80). Thus, it is conceivable that the failure to detect differential effects of the aging process in males compared to females if they do in fact exist may be due to the relatively small magnitude of these differences. Consequently, future investigators may wish to perform an additional power analysis on the raw data contained within this thesis to determine an adequate sample size for the possible detection of these effects. The major drawback of this suggestion is that an investigation involving such an extensive sample would require the investment of a great deal of time and resources. Thus, before such an endeavor is undertaken one should first answer the question of whether or not such a small effect is of theoretical interest or practical importance in a clinical/academic setting.

The second weakness of the study lies in the artificial and for many subjects unfamiliar nature of the laboratory setting in which the acute recordings were performed. It is not unreasonable to question whether or not one subset of the sample population (e.g. perhaps the pediatric subjects) may be more

emotionally reactive to the novelty of the situation. Consequently, as the autonomic nervous system is known to be extremely sensitive to external stimuli this uncontrolled variable may serve to confound the interpretation of the findings. One possible solution for this limitation is for future studies to perform one or two additional follow-up visits for acute recordings to allow the subjects to become familiarized/habituated with the laboratory environment.

Finally, the non-invasive nature of HRV analysis necessitates that much of the physiological conclusions made in a study of this nature be based on computation and inference. The purpose of this study was to establish reference standards that will form the basis of future inferences pertaining to HRV parameters in special populations. As such, it may be desirable to confirm these findings with a traditional yet somewhat more invasive measure of autonomic function such as bolus injection of pharmacological agents or muscle sympathetic nerve activity recordings.

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SECTION 6.0

APPENDICES

APPENDIX A:

Forms

Aging & Gender Standards for Heart Rate Variability
Subject Consent Form

Researchers

- Adrian Harvey, B.Kin, Graduate Student, Dept. Human Biodynamics
- Dr. Mark Kamath, Ph.D, Assistant Professor, Dept. Medicine
- Dr. Neil McCartney, Ph.D, Professor, Dept. Kinesiology

Purpose

To establish heart rate variability standards for normal healthy subjects with respect to age & gender for future use in research & clinical practice.

Outline

Procedure will involve both short term, (approx. 45 minutes), and 24 hour ECG recordings. Prior to the recording subjects will be asked to complete a short subject information sheet & a medical questionnaire, (including a standard noninvasive blood pressure measurement). The purpose of these measures is to ensure that the subject population consists of normal, healthy individuals. The short term procedure will involve a single lead setup, (requiring 3 chest electrodes), capable of recording both ECG, (electrical activity of the heart) & respiration. Recording will involve 20 minutes in the supine position & 10 minutes in the standing position, to assess the acute autonomic cardiovascular response to orthostatic stress, (drop in blood pressure on standing), in healthy subjects. The 24 hour recording will be performed with an Oxford Medilog 4500 holter monitor which can be worn with relative comfort on a belt or a shoulder strap. The monitor utilizes a 2 lead setup, (requiring 5 chest electrodes), and records only ECG. Subjects will also be required to fill out an activity diary listing start and finish times for significant activities, (e.g. meals, sleep, work…), during the recording period. The purpose of this long term recording is to identify any age or gender related differences in the normal circadian rhythm known to exist in heart rate variability. The procedure is noninvasive and has no associated risks, (unless the subject has allergies to adhesives used to hold the electrodes and wires to the skin). Subjects that have difficulty tolerating adhesive bandages or any form of athletic or medical tape should not take part in this study.

All collected data will be stored in the Heart Rate Variability Lab, (3E25), at the McMaster University Medical Center under the supervision of Dr. Mark Kamath, (Assistant Professor, Medicine). Data will be published or submitted for thesis credit in such a manner as to maintain the anonymity of those involved. No names or other information that might be used to identify any subjects will be included in these reports. Research findings will be available to the subjects upon completion of the project. Subjects who wish to review their data should contact Adrian Harvey at 525-9140 xt27390.

Participation in the study is on a volunteer basis and subjects are free to withdraw themselves as well as any of their previously collected data at any time during the study. Subjects may also choose not to answer any questions, (on the subject information sheet or medical questionnaire), or list any activity, (in the activity diary), if they do not feel comfortable doing so.

I have read & understand the procedures & risks involved in this research & I am aware that I may withdraw myself or any of my data at any time during the study:

Name:_____Signature:_____Date:_____

Witness:_____Signature:_____

Aging & Gender Standards for Heart Rate Variability

Medical Questionnaire

Name:_____Subject #:_____ Date of Birth:_____Age:___

1. Do you smoke, (currently or on a regular basis at any time during the past two years)?

Yes:____ No:____

2. Do you have diabetes?

Yes:____ No:____

3. Is there a history of heart disease in your family?

Yes:____ No:____

If so, Who?, At what age did it start?:

4. Have you ever been diagnosed with, or taken medication for hypertension, (high blood pressure)?

Yes:____ No:____

5. Do you suffer from any form of heart disease, (angina, previous heart attack, valvular disease, congestive heart failure, congenital defects etc.)?

Yes:____ No:____

If so what type?:

6. Have you ever fainted in the last 5 years?

Yes:____ No:____

7. Have you ever had a problem with high blood cholesterol?

Yes:____ No:____

8. Please list all medications currently being taken:

9. Blood Pressure, (SBP/DBP):

Date of recording:_____ Signature:_____

Patient Information Profile
Aging and Gender Standards in Heart Rate Variability

Name: Subject#:
Date of Birth(age): ( )
Date of Recording: Time of Recording:
Gender:M( ) F( ) Height: Weight:

Medications?:

Medical Questionnaire?:Yes( ) No( )

Other Tests:

Acute-supine:Yes( ) No( ) Backup?:Yes( ) No( ) Tape#:
Analyzed?:Yes( ) No( )

Acute-standing:Yes( ) No( ) Backup?:Yes( ) No( ) Tape#:
Analyzed?:Yes( ) No( )

Holter:Yes( ) No( ) Backup?:Yes( ) No( ) Tape#:
Diary?:Yes( ) No( )
Time Domain Analyzed?:Yes( ) No( )
Circadian Analyzed?:Yes( ) No( )

Age And Gender Standards in Heart Rate Variability
Holter Diary

Name: John Doe Subject#: N2696B

Record start and finish times for the 24 hours during the holter recording, (ie. Sleep, Meals, Medications, Work, Visits to the bathroom...)

Time (Start-Finish)	Description
10:45-11:00	**Walk to Car**
11:20-11:35	**Coffee**
12:00-12:05	**Bathroom**
1:15-1:55	**Lunch**
3:00-3:45	**Walk to and from store**
4:00-4:05	**Bathroom**
6:10-7:00	**Dinner**
7:15-7:25	**Dishes**
8:25-8:30	**Bathroom**
9:12-9:17	**Bathroom**
10:00-10:15	**Snack**
11:30	**Go to Bed**
3:10-3:15	**Bathroom**
8:25	**Wake up, Get out of bed**
9:30-9:50	**Drive to McMaster, Walk from car to Lab**

APPENDIX B:

RAW DATA

i) Raw Spectral Data During 20 minutes of supine rest (top) and 10 minutes of quiet standing (bottom) in pediatric (5-12 years) subjects.

Subject Number	Age	Gender	Heart Rate (bts/min)	LF:HF Area	LF Area	LF Area (%)	HF Area	HF Area (%)	Total Area	LF:HF Peak	LF cf (Hz)	HF cf (Hz)
X13061	5	F	91.5	0.687	4620	40.71	6728	59.28	11347.7	0.731	0.12	0.326
X10072	6	M	76.5	0.802	5030	42.33	6852	57.66	11881.8	0.523	0.11	0.371
D2196B	6	F	87.2	1.15	6646	50.01	6643	49.98	13289.1	1.64	0.078	0.276
X12061	6	F	91.6	1.19	7171	54.15	6070	45.84	13241.4	0.983	0.091	0.333
X14121	7	F										
X23082	7	M	84.6	1.15	5561	52.91	4948	47.08	10508.4	1.63	0.099	0.261
X05072	7	F	78.2	0.638	4739	38.93	7434	61.07	12172.9	1.16	0.11	0.24
X19061	8	F	90.4	0.807	5215	43.72	6712	56.27	11927.2	1.3	0.12	0.187
X13091	9	M	77.5	1.03	6011	49.89	6036	50.10	12047.1	0.818	0.11	0.307
X14081	9	M	74.5	0.696	5023	40.72	7312	59.27	12335.1	0.746	0.099	0.253
X05062	9	M										
D2296A	9	F	80.4	0.769	5496	42.87	7324	57.13	12819.8	1.01	0.11	0.225
X10071	9	F	69	0.384	3192	27.66	8346	72.33	11537.2	0.441	0.12	0.267
X31052	10	M	77.8	1.11	5822	50.80	5637	49.19	11459.3	1.02	0.099	0.262
X23081	10	M	95.1	1.25	5372	54.89	4415	45.11	9786.18	1.19	0.15	0.17
X16082	10	M	76.3	0.828	5215	42.02	7194	57.97	12408.2	1.32	0.1	0.221
D2196A	10	F	72.4	1.18	6447	52.33	5870	47.65	12317.9	0.888	0.12	0.331
X07062	11	M	72.3	0.901	5269	46.37	6092	53.62	11361.2	0.956	0.1	0.293
X09081	12	M	87	0.742	4871	42.09	6700	57.90	11570.9	1.09	0.14	0.155
X16081	12	F	63.5	0.433	3321	30.22	7667	68.77	10988.5	1.04	.13	.15
X27061	12	M										
X23083	12	M	77.4	0.808	5054	44.31	6351	55.68	11405.2	1.04	0.12	0.213

Mean	80.16	0.87	5267	44.57	6543	55.42	11810.7	1.02	0.11	0.25
STD	8.53	0.25	973.0	7.46	948.4	7.46	870.92	0.31	0.01	0.06
SEM	1.95	0.05	223.2	1.71	217.5	1.71	199.80	0.07	0.003	0.01

Subject Number	Age	Gender	Heart Rate (bts/min)	LF:HF Area	LF Area	LF Area (%)	HF Area	HF Area (%)	Total Area	LF:HF Peak	LF cf	HF cf
X13061	5	F	110	0.936	4777	47.74	5228	52.25	10004.7	1.61	0.11	0.15
X10072	6	M	91.6	0.822	6078	45.09	7401	54.90	13478.8	1.75	0.098	0.225
D2196B	6	F	103	1.11	6469	52.47	5858	47.52	12327.1	2.07	0.086	0.15
X12061	6	F	114	2.23	7921	69.06	3549	30.94	11469.1	2.5	0.08	0.346
X14121	7	F										
X23082	7	M	102	1.67	7470	62.35	4511	37.65	11980.5	2.04	0.1	0.176
X05072	7	F	102	1.44	6542	57.09	4916	42.90	11457.9	3.26	0.087	0.15
X19061	8	F	103	1.21	5667	54.41	4748	45.58	10414.8	1.3	0.11	0.15
X13091	9	M	95.8	1.37	6695	57.91	4864	42.07	11559.2	2.17	0.083	0.215
X05062	9	M										
X14081	9	M	103	1.29	6021	56.00	4730	43.99	10750.9	2.23	0.08	0.177
D2296A	9	F	100	1.6	7725	60.82	4976	39.17	12700.4	2.47	0.076	0.181
X10071	9	F	88.8	0.897	5891	46.37	6813	53.62	12703.9	1.14	0.1	0.266
X31052	10	M	96.5	1.28	6006	55.90	4737	44.09	10743	1.95	0.086	0.165
X23081	10	M	100	1.39	5661	56.36	4383	43.63	10044.1	2.03	0.08	0.198
X16082	10	M	97.7	2.4	8116	70.57	3384	29.42	11499.4	3.14	0.096	0.15
D2196A	10	F	96.2	1.81	7063	64.37	3909	35.62	10972.4	2.97	0.095	0.296
X07062	11	M	96.3	1.64	6399	60.77	4130	39.22	10528.9	3.65	0.091	0.15
X09081	12	M	114	2.7	7449	71.69	2940	28.29	10389.8	3.53	0.093	0.19
X27061	12	M										
X23083	12	M	101	1.58	6483	59.38	4433	40.60	10916.2	1.98	0.09	0.189
X16081	12	F	92.4	1.24	6199	55.37	4996	44.62	11194.7	2.63	0.11	0.15

Mean	100.38	1.50	6559	58.09	4763	41.90	11322.9	2.33	0.09	0.19
STD	6.84	0.50	874.1	7.50	1076	7.50	962.83	0.71	0.01	0.05
SEM	1.56	0.11	200.5	1.72	246.9	1.72	220.88	0.16	0.002	0.01

ii) Raw Spectral Data During 20 minutes of supine rest (top) and 10 minutes of quiet standing (bottom) in adolescent (~ 3-17 years) subjects.

Subject Number	Age	Gender	Heart Rate (bts/min)	LF:HF Area	LF Area	LF Area (%)	HF Area	HF Area (%)	Total Area	LF:HF Peak	LF cf (Hz)	HF cf (Hz)
X310595	13	M	65.1	0.785	4315	44.20	5446	55.79	9761.12	0.942	0.096	0.268
X28082	13	M	62.3	0.908	4781	46.59	5479	53.40	10260.1	1.7	0.1	0.196
D2296B	13	F	68.2	0.422	3266	29.60	7766	70.39	11031.9	0.61	0.12	0.216
J1897A	13	F	87.6	1.29	6648	54.77	5489	45.22	12137.1	1.76	0.084	0.246
X05071	13	F	58.2	0.773	4143	43.57	5365	56.42	9508.71	0.933	0.13	0.213
X31071	14	F	60.7	0.713	4398	41.33	6243	58.66	10641	0.797	0.1	0.281
X51072	14	F	73.3	1.31	5386	54.78	4446	45.22	9831.88	3.41	0.085	0.174
X28061	15	M	75	0.596	4071	37.22	6865	62.77	10936	0.636	0.13	0.263
X11111	15	M										
X28062	15	M	86.8	0.858	5201	45.26	6288	54.72	11489.3	0.769	0.1	0.285
J2597B	15	M	79.8	0.932	5269	48.03	5699	51.95	10968.1	1.05	0.1	0.217
X28081	15	F	81.4	0.636	4396	38.94	6892	61.05	11287.8	0.689	0.11	0.25
X02081	16	M	62.4	1.77	7540	60.26	4972	39.73	12511.5	2	0.11	0.295
X24073	16	M	69.5	1.12	5944	50.98	5715	49.01	11658.9	1.28	0.088	0.263
X24072	16	F	63.8	0.702	5153	48.84	5397	51.15	10549.9	0.702	0.11	0.333
X240595	16	F	56.8	0.494	3581	32.63	7390	67.35	10971.5	0.362	0.099	0.269
X21081	17	F	67.2	0.483	3667	32.39	7654	67.60	11321.3	0.447	0.09	0.219
Y240595	17	F	80	0.859	5245	43.01	6948	56.98	12192.3	1.17	0.13	0.207
X26071	17	F	61.6	0.857	4829	44.93	5917	55.05	10746.5	0.454	0.086	0.325
J2597A	17	F	68.2	0.423	3065	29.36	7373	70.63	10438.6	0.295	0.11	0.242
X26072	17	F										

Mean	69.89	0.84	4784	43.51	6176	56.48	10960	1.05	0.10	0.25
STD	9.48	0.34	1128	8.68	969.7	8.67	823.8	0.74	0.01	0.04
SEM	2.18	0.08	258.9	1.99	222.4	1.99	189.0	0.17	0.003	0.01

Subject Number	Age	Gender	Heart Rate (bts/min)	LF:HF Area	LF Area	LF Area (%)	HF Area	HF Area (%)	Total Area	LF:HF Peak	LF cf (Hz)	HF cf (Hz)
X310595	13	M	99.2	2.24	7151	68.90	3227	31.09	10378.2	3.86	0.086	0.15
X28082	13	M	91.4	3.27	7211	76.75	2184	23.24	9394.96	6.21	0.082	0.17
D2296B	13	F	99.9	1.3	6154	56.07	4721	43.01	10974.7	2.66	0.085	0.2
J1897A	13	F	102	1.42	6213	57.97	4503	42.02	10716.2	2.43	0.094	0.15
X05071	13	F	85.5	1.86	6755	64.62	3697	35.37	10451.9	2.68	0.084	0.161
X31071	14	F	74.3	1.42	6833	58.63	4820	41.36	11653.6	3.3	0.083	0.197
X51072	14	F	85.7	2.01	7161	66.40	3623	33.59	10784.6	2.68	0.096	0.15
X28061	15	M	111	1.84	6152	64.12	3442	35.87	9594.08	3.26	0.079	0.15
X11111	15	M										
X28062	15	M	126	2.78	5722	72.37	2184	27.62	7906.43	4.29	0.062	0.191
J2597B	15	M	103	1.23	4904	55.06	4002	44.93	8905.34	2.04	0.087	0.193
X28081	15	F	102	1.88	7415	64.39	4100	35.60	11515.1	2.91	0.093	0.17
X02081	16	M	93.9	3.44	6834	75.88	2172	24.11	9005.88	8.36	0.073	0.191
X24073	16	M	94.2	1.67	6369	61.82	3932	38.17	10301	3.59	0.079	0.18
X24072	16	F	80.9	2.37	7052	68.06	3309	31.93	10360.3	3.56	0.075	0.294
X240595	16	F	79.5	1.41	6521	58.47	4630	41.51	11151.4	3.07	0.077	0.183
X21081	17	F	82.2	1.52	6010	59.99	4008	40.00	10018.1	2.02	0.078	0.174
Y240595	17	F	116	1.95	6570	65.19	3507	34.80	10076.7	3.74	0.086	0.15
X26071	17	F	85.2	2.58	6927	71.84	2715	28.15	9642.23	4.79	0.082	0.15
J2597A	17	F	83.2	1.49	5716	59.22	3936	40.78	9652.01	2.85	0.09	0.245
X26072	17	F										

Mean	94.48	1.98	6508	64.52	3616	35.43	10130	3.59	0.08	0.18
STD	13.57	0.65	638.1	6.50	833.9	6.44	943.7	1.52	0.01	0.03
SEM	3.11	0.15	146.3	1.49	191.3	1.48	216.5	0.35	0.002	0.008

iii) Raw Spectral Data During 20 minutes of supine rest (top) and 10 minutes of quiet standing (bottom) in adult (18-30 years) subjects.

Subject Number	Age	Gender	Heart Rate (bts/min)	LF:HF Area	LF Area	LF Area (%)	HF Area	HF Area (%)	Total Area	LF:HF Peak	LF cf (Hz)	HF cf (Hz)
N0596A	18	M	62.1	1.02	4751	49.38	4869	50.61	9619.54	1.06	0.11	0.24
O2896A	18	F	54.5	0.465	3573	31.64	7718	68.35	11291.2	0.432	0.098	0.23
N0296A	18	F	55.5	0.949	5796	47.94	6294	52.06	12089.8	1.01	0.088	0.304
N2796A	18	F	70.6	0.848	4721	45.67	5615	54.32	10336.5	0.774	0.1	0.24
F0397A	18	F	70.1	0.752	5056	42.56	6824	57.44	11879.5	0.844	0.12	0.193
O1896A	19	F	62.4	0.377	3125	26.51	8660	73.47	11785.7	0.404	0.094	0.241
N2696A	19	F	76.5	0.527	3003	34.38	5731	65.61	8733.74	0.339	0.1	0.196
X12072	20	M	62.2	0.69	4241	39.80	6412	60.18	10653.6	0.732	0.083	0.233
X17071	20	F	60.1	0.744	4883	42.63	6570	57.36	11453	1	0.11	0.22
X29051	21	F										
X30051	21	F										
S1196A	21	F	43.4	0.0811	5708	43.99	7265	56.00	12973	0.674	0.096	0.272
N0796A	22	M	60.8	0.924	5266	47.25	5878	52.74	11143.4	0.811	0.1	0.28
N1896A	22	F										
N1196A	23	M	72.6	1.58	6211	60.65	4030	39.35	10240.6	1.55	0.11	0.289
MA1597A	24	F	90.8	0.816	4576	44.53	5698	55.46	10274	0.983	0.1	0.189
M1297A	24	M	79.6	1.91	6081	62.36	3670	37.63	9751.21	1.98	0.084	0.319
O1596A	24	M	55.4	1.26	5018	55.75	3981	44.23	8999.32	2.75	0.096	0.19
N1296A	24	M	59.2	1.06	5847	50.94	5630	49.05	11477.4	1.68	0.12	0.189
J1597A	25	M	65.7	1.16	5717	53.43	4983	46.57	10699.7	1.18	0.11	0.236
MA1697A	25	M	60.6	1.19	6539	54.41	5478	45.58	12017	1.76	0.11	0.198
D0396A	26	M	57.2	0.799	4510	43.14	5942	56.84	10452.4	0.657	0.085	0.231
J2897A	26	F	64.8	1.28	6829	55.50	5473	44.48	12302.5	1.01	0.11	0.317
N0996A	27	M	62.8	2.34	6981	69.83	3016	30.16	9997.01	2.76	0.095	0.226
N1496A	27	M	78.4	2.07	6778	67.18	3310	32.81	10088.2	2.39	0.081	0.272
D0296A	28	M	47.2	0.879	5519	46.99	6224	53.00	11742.9	1.14	0.12	0.235

Mean	64.02	1.03	5249	48.54	5620	51.45	10869	1.21	0.10	0.24
STD	10.75	0.53	1116	10.66	1389	10.66	1095.3	0.70	0.01	0.04
SEM	2.24	0.11	232.7	2.22	289.7	2.22	228.4	0.14	0.002	0.008

Subject Number	Age	Gender	Heart Rate (bts/min)	LF:HF Area	LF Area	LF Area (%)	HF Area	HF Area (%)	Total Area	LF:HF Peak	LF cf (Hz)	HF cf (Hz)
N0596A	18	M	79.8	1.09	6026	51.98	5566	48.01	11592.2	1.81	0.075	0.202
O2896A	18	F	65.5	1.23	6279	54.18	5310	45.82	11588	1.82	0.078	0.218
N0296A	18	F	69.4	2.4	5783	70.33	2439	29.66	8221.67	2.88	0.086	0.312
N2796A	18	F	89.2	1.82	5369	58.78	3765	41.22	9133.44	2.93	0.086	0.165
F0397A	18	F	87.4	1.4	6329	57.56	4666	42.44	10994	1.69	0.065	0.185
O1896A	19	F	65.8	1.05	6618	49.81	6668	50.18	13285.7	1.64	0.099	0.221
N2696A	19	F	92.8	0.665	4017	37.09	6812	62.90	10829	1.27	0.078	0.245
X12072	20	M	85.9	1.52	5601	58.82	3921	41.17	9522.09	1.59	0.081	0.179
X17071	20	F	76.9	1.58	5070	60.49	3312	39.51	8381.19	1.56	0.1	0.174
X29051	21	F										
X30051	21	F										
S1196A	21	F	59	1.94	6995	65.99	3604	34.00	10599.1	2.71	0.082	0.178
N1896A	22	F										
N0796A	22	M	92.6	1.62	6124	61.72	3798	38.28	9921.17	3.49	0.082	0.186
N1196A	23	M	72.2	1.85	7899	64.45	4356	35.54	12255.8	2.48	0.062	0.19
MA1597A	24	F	102	0.875	5441	46.79	6186	53.20	11627.6	0.996	0.092	0.194
M1297A	24	M	86.6	1.52	6494	60.10	4311	39.90	10804.4	2.92	0.087	0.15
O1596A	24	M	71.7	3.68	6321	77.91	1792	22.08	8112.53	5.3	0.08	0.15
N1296A	24	M	80.8	1.67	6538	61.78	4044	38.21	10582.1	2.79	0.082	0.205
MA1697A	25	M	73.6	2.38	6385	70.42	2681	29.57	9066.06	3.3	0.097	0.15
D0396A	26	M	92.7	2.81	6280	72.67	2361	27.32	8641.26	6.65	0.083	0.15
J1597A	26	M	85.2	2.13	6018	68.10	2818	31.89	8836.19	3.41	0.086	0.15
J2897A	26	F	77.2	1.71	7068	62.04	4324	37.95	11391.4	3.15	0.097	0.181
N0996A	27	M	79.7	2.75	6160	72.58	2328	27.43	8487	6.54	0.088	0.157
N1496A	27	M	92.3	2.28	6589	69.47	2895	30.52	9484.43	3.58	0.083	0.201
D0296A	28	M	63.8	1.43	7829	58.93	5456	41.07	13284	1.15	0.11	0.212

Mean	80.09	1.8	6227	61.39	4061	38.60	10288	2.85	0.08	0.18
STD	11.24	0.69	837.3	9.39	1424	9.39	1572	1.54	0.01	0.03
SEM	2.34	0.14	174.6	1.95	297.1	1.95	327.8	0.32	0.002	0.007

iv) Raw Spectral Data During 20 minutes of supine rest (top) and 10 minutes of quiet standing (bottom) in middle aged (31-60 years) subjects.

Subject Number	Age	Gender	Heart Rate (bts/min)	LF:HF Area	LF Area	LF Area (%)	HF Area	HF Area (%)	Total Area	LF:HF Peak	LF cf (Hz)	HF cf (Hz)
F2597A	31	M	58.8	1.45	6613	58.25	4739	41.74	11352.2	1.35	0.091	0.272
J2197A	31	F	68.5	1.9	7105	59.99	4737	40.00	11842	1.06	0.11	0.372
M0697A	33	M	67.8	1.45	6336	58.80	4439	41.19	10774.5	2.31	0.084	0.204
O2396A	35	F	72.6	1	5156	49.29	5303	50.70	10458.7	0.983	0.07	0.227
A0997A	35	F										
N1996A	37	F	59.6	0.718	4241	41.83	5896	58.16	10136.9	0.725	0.081	0.213
M0397A	37	F	63.7	0.729	4210	41.66	5895	58.33	10105.3	0.422	0.094	0.222
M2597A	37	F	73.9	1.22	5411	49.16	5596	50.84	11006.7	0.967	0.072	0.282
F1597B	40	F	68.8	1.27	6215	55.82	4917	44.16	11132.5	0.904	0.1	0.319
MA1397A	42	M	67.8	1.97	6022	63.14	3515	36.85	9537.43	2.16	0.099	0.27
MA0997A	42	F	61	0.977	5154	48.40	5503	51.68	10648.2	0.947	0.1	0.257
MA0797A	42	F	56.1	1.01	5432	48.60	5743	51.39	11174.8	0.895	0.089	0.301
F1597A	44	M	75.6	1.5	5662	58.24	4059	41.75	9720.36	2.49	0.087	0.156
F1797A	44	M	62	2.4	6049	69.94	2599	30.05	8647.97	4.29	0.093	0.15
D1996A	45	M	67.9	1.75	6988	62.36	4217	37.63	11205	2.23	0.097	0.265
A0297A	45	M	64	1.56	6248	60.90	4011	39.09	10259.3	2.13	0.078	0.204
A2297A	45	F	80.6	1.78	6345	63.35	3671	36.65	10015	3.64	0.085	0.195
A0897A	52	F	64.8	0.672	4200	39.67	6386	60.32	10586	0.324	0.097	0.302
N2896A	56	F	69.1	1	5782	49.83	5821	50.16	11602.6	0.972	0.077	0.251
A1597A	56	F	51.2	1.09	5391	51.58	5061	48.42	10451.4	1.2	0.08	0.263
A0397B	57	F	66.1	0.892	5493	45.11	6682	54.88	12175.1	1.06	0.081	0.286
A0397A	58	M	54.9	2.05	6278	67.13	3074	32.87	9351	2.92	0.077	0.231
A2997A	59	M	79.8	1.63	6374	59.72	4299	40.27	10672.9	3.21	0.067	0.206
A2897A	59	F	63.4	1.72	5691	61.47	3566	38.52	9256.88	2.29	0.08	0.24

Mean	66	1.37	5756	54.92	4770	45.03	10526	1.71	0.08	0.24
STD	7.46	0.47	805.8	8.51	1087	8.51	871.0	1.07	0.01	0.05
SEM	1.55	0.09	168.0	1.77	226.7	1.77	181.6	0.22	0.002	0.01

Subject Number	Age	Gender	Heart Rate (bts/min)	LF:HF Area	LF Area	LF Area (%)	HF Area	HF Area (%)	Total Area	LF:HF Peak	LF cf (Hz)	HF cf (Hz)
F2597A	31	F	98.6	3.36	7062	76.87	2124	23.12	9186.16	3.73	0.079	0.15
J2197A	31	F	77.2	1.57	5869	60.42	3843	39.56	9712.17	2.99	0.078	0.153
M0697A	33	M	86.5	2.57	7124	71.92	2781	28.07	9904.54	5.24	0.072	0.15
O2396A	35	F	77.6	1.36	6060	57.40	4496	42.58	10556.8	1.34	0.079	0.228
A0997A	35	F										
N1996A	37	F	75.6	1.07	5903	50.08	5884	49.92	11786.7	2.03	0.076	0.232
M0397A	37	F	79.7	0.897	5744	47.41	6369	52.57	12113.2	1.17	0.098	0.217
M2597A	37	F	76	1.76	6543	63.18	3812	36.81	10355	1.41	0.076	0.239
F1597B	40	F	83.4	2.04	7201	62.79	4266	37.20	11466.6	2.49	0.079	0.252
MA1397A	42	M	71.9	1.69	6288	62.61	3755	37.38	10042.8	2.72	0.07	0.209
MA0997A	42	F	75.6	2.87	7235	73.43	2617	26.56	9852.08	6.43	0.088	0.15
MA0797A	42	F	82	2.32	5785	69.89	2492	30.10	8276.95	4.42	0.083	0.303
F1597A	44	M	87.4	2.33	7633	69.26	3387	30.73	11020.4	3.53	0.086	0.238
D1996A	45	M	94.6	2.03	7064	66.65	3534	33.34	10597.8	3.68	0.068	0.177
F1797A	45	M	76	4.12	7618	80.03	1900	19.96	9517.97	3.95	0.071	0.15
A0297A	45	M	68.9	2.7	6186	71.85	2424	28.15	8609.53	5.42	0.086	0.15
A2297A	45	F	91.4	1.78	5872	64.39	3247	35.60	9118.91	2.43	0.09	0.168
A0897A	52	F	70.4	1.22	6223	53.41	5427	46.58	11649.7	1.46	0.085	0.216
N2896A	56	F	71.2	1.45	6943	57.66	5071	42.11	12041	3.26	0.077	0.17
A1597A	56	F	69.9	2.74	7587	72.06	2942	27.94	10528.1	3.04	0.079	0.27
A0397B	57	F	72	1.51	6903	57.10	5186	42.89	12088.8	2.4	0.083	0.232
A0397A	58	M	61.1	2.39	6791	70.27	2872	29.72	9663.49	4.52	0.071	0.189
A2997A	59	M	91.9	2.32	6068	68.80	2751	31.19	8819.57	5.61	0.058	0.189
A2897A	59	F	76.5	1.28	5681	56.19	4428	43.80	10109.3	2.48	0.067	0.15

Mean	78.93	2.05	6581	64.51	3722	35.47	10305	3.29	0.08	0.19
STD	9.28	0.78	659.3	8.57	1239	8.56	1152	1.46	0.009	0.04
SEM	1.93	0.16	137.4	1.78	258.4	1.78	240.3	0.30	0.002	0.009

v) Raw Spectral Data During 20 minutes of supine rest (top) and 10 minutes of quiet standing (bottom) in elderly (61+ years) subjects.

Subject Number	Age	Gender	Heart Rate (bts/min)	LF:HF Area	LF Area	LF Area (%)	HF Area	HF Area (%)	Total Arre	LF:HF Peak	LF cf (Hz)	HF cf (Hz)
D1696A	61	F	67.1	1.97	7780	65.51	4095	34.48	11875	3.59	0.072	0.188
A0297A	62	F	76	1.48	6631	59.29	4551	40.69	11182.2	1.99	0.088	0.161
N2296A	64	M	80.1	1.22	6121	55.03	5000	44.95	11121.1	1.33	0.069	0.15
MA0897A	64	F	61.5	0.954	5315	48.69	5600	51.30	10915.2	1.45	0.077	0.253
J2397A	64	F	57.5	1.5	6165	59.18	4252	40.81	10416.6	1.04	0.074	0.307
D0696A	65	M	70.9	1.41	5888	57.53	4346	42.46	10234.5	2.11	0.074	0.198
J3097A	65	F	65.9	1.21	5352	53.30	4688	46.69	10039.5	1.58	0.078	0.212
A2197A	65	F	71.8	1.65	6014	61.07	3833	38.92	9846.7	4	0.062	0.224
J1097A	66	F	65.3	0.987	5414	48.89	5659	51.10	11073.2	1.67	0.064	0.207
A1097A	66	F	69.5	0.894	4950	45.02	6044	54.97	10993.2	2.28	0.063	0.28
MA0697A	66	M										
F1897A	67	F	75.6	1.41	5916	58.36	4220	41.63	10136.5	3	0.075	0.15
A1497A	67	F	69.8	1.34	6343	56.65	4853	43.34	11195.8	2.63	0.072	0.218
A2197A	68	M	55.3	2.07	7381	66.18	3771	33.81	11151.7	2.81	0.089	0.215
MA0597B	68	F	60.6	0.795	6063	41.57	8519	58.42	14582	1.75	0.095	0.191
D1096B	68	F	60.1	0.932	4932	44.94	6042	55.05	10974.1	1.22	0.071	0.247
F1397A	68	F	68.3	1.09	5933	49.36	6085	50.63	12018.1	1.59	0.062	0.33
D1096A	69	M	57.7	2.16	6623	67.16	3238	32.83	9861.24	3.3	0.08	0.176
F1197A	69	M	79.2	1.82	7335	64.09	4109	35.90	11444.3	2.55	0.097	0.15
J2297A	69	F	72.1	1.63	6346	60.24	4188	39.75	10533.4	2.79	0.082	0.18
M1797A	70	F										
MA0597A	70	M	70	1.05	5967	51.01	5731	48.99	11697.7	1.29	0.075	0.233
D1296A	71	F										
F2597A	71	M	49.3	1.16	6534	53.02	5789	46.97	12323.5	1.72	0.07	0.214
F0497A	72	F	61.9	1.62	4712	61.55	2943	38.44	7654.73	1.98	0.063	0.233
D1896A	74	M	62.5	2.43	6630	68.90	2992	31.09	9622.11	2.57	0.07	0.304
D2096A	74	M	61.7	0.785	4616	43.28	6049	56.72	10664.3	0.727	0.089	0.197
F0797A	74	M	64.8	1.78	7506	60.96	4806	39.03	12311.8	4.04	0.06	0.163
J2097A	77	F	81.5	0.921	5256	47.48	5813	52.51	11068.7	1.36	0.063	0.235
J3197A	78	F	93.6	1.14	6670	51.50	6280	48.49	12950.7	1.99	0.064	0.335

Mean	67.76	1.38	6088	55.55	4944	44.44	11032	2.16	0.07	0.22
STD	9.39	0.44	849.0	7.76	1232	7.76	1260	0.88	0.01	0.05
SEM	1.80	0.08	163.4	1.49	237.1	1.49	242.6	0.17	0.002	0.01

Subject Number	Age	Gender	Heart Rate (bts/min)	LF:HF Area	LF Area	LF Area (%)	HF Area	HF Area (%)	Total Area	LF:HF Peak	LF cf (Hz)	HF cf (Hz)
D1696A	61	F	82	1.59	6323	61.37	3979	38.62	10302.1	1.98	0.087	0.162
A0297A	62	F	77.2	2.22	5750	68.91	2593	31.07	8343.11	1.87	0.1	0.15
N2296A	64	M	88.3	1.26	5508	56.08	4314	43.92	9821.39	1.27	0.072	0.167
MA0897A	64	F	74.8	1.53	5891	60.61	3827	39.38	9717.94	1.86	0.09	0.223
D0696A	65	M	73.5	1.71	7213	63.06	4225	36.93	11438.2	2.79	0.093	0.16
J2397A	65	F	68.9	1.27	5866	55.39	4723	44.60	10589.3	1.07	0.078	0.305
J3097A	65	F	68.7	1.75	5479	63.20	3190	36.79	8668.55	2.68	0.064	0.19
A2197A	65	F	83.7	1.49	5993	59.07	4151	40.91	10144.4	3.44	0.07	0.206
J1097A	66	F	72.9	1.88	6751	65.14	3611	34.84	10362.5	2.83	0.064	0.247
A1097A	66	F	73.9	1.23	6027	54.36	5059	45.63	11085.4	1.84	0.058	0.277
MA0697A	66	M										
F1897A	67	F	80.3	0.841	4429	45.59	5285	54.40	9714.63	0.874	0.072	0.201
A1497A	67	F	77.5	1.38	7394	57.84	5389	42.15	12782.7	1.44	0.047	0.237
A2197A	68	M	67.8	1.94	6094	65.54	3203	34.45	9297.05	4.1	0.077	0.15
MA0597B	68	F	63.8	1.13	7035	52.45	6375	47.53	13410.8	2.01	0.09	0.172
D1096B	68	F	69.1	1.23	5653	54.93	4638	45.06	10290.9	2.26	0.076	0.183
F1397A	68	F	67.5	0.882	4998	44.29	6285	55.70	11282.9	1.17	0.11	0.257
D1096A	69	M	63.6	2.13	6251	67.20	3050	32.79	9301.4	4.58	0.071	0.195
F1197A	69	M	97.8	2.5	7444	70.93	3050	29.06	10494	4.89	0.07	0.15
J2297A	69	F	74.8	2.39	7803	68.81	3537	31.19	11339.9	4.01	0.07	0.239
M1797A	70	F										
MA0597A	70	M	65.9	2.49	6653	71.01	2716	28.99	9368.67	3.86	0.098	0.15
D1296A	71	F										
F2597A	71	M	60.7	1.37	6649	56.36	5146	43.62	11795.6	3.38	0.065	0.175
F0497A	72	F	67.5	2.81	7071	72.23	2718	27.76	9788.85	5	0.07	0.15
D1896A	74	M	72.5	2.84	6694	71.53	2663	28.45	9357.28	4.79	0.072	0.282
D2096A	74	M	65.9	1.05	5005	48.56	5300	51.43	10305.1	1.41	0.069	0.259
F0797A	74	M	66.1	1.78	7445	62.66	4435	37.33	11880.4	2.5	0.083	0.274
J2097A	77	F	98.9	1.32	5957	56.73	4542	43.26	10499.3	2.47	0.054	0.24
J3197A	78	F	111	1.47	4787	54.32	4025	45.67	8812.11	2.45	0.061	0.444

Mean	75.35	1.68	6228	60.30	4149	39.69	10377	2.69	0.07	0.21
STD	11.96	0.56	885.3	7.87	1076	7.87	1212	1.25	0.01	0.06
SEM	2.30	0.10	170.3	1.51	207.0	1.51	233.2	0.24	0.002	0.01

vi) Time domain HRV parameters derived from 24-hour holter monitoring in pediatric (5-12 years) subjects.

Subject Number	Age	Gender	Heart Rate (bts/min)	N-N Interval (msec)	SDNN (msec)	SDNN Index (msec)	SDANN (msec)	R-MSSD	pNN50 (%)	Intervals Accepted (%)
X13061	5	F	92.065	651.71	154.8	80.5	136.22	83.84	34.4	86.4
D2196B	6	F	92.88	646.01	140.45	82.4	118.84	75.3	34.7	90.2
X10072	6	M								
X12061	6	F	96.549	621.45	132.99	64.62	119.32	49.1	21.4	87.6
X14121	7	F	92.115	651.36	112.81	76.36	83.31	61.99	33.7	94.7
X23082	7	M	87.26	687.6	121.03	52.45	110.65	32.129	8.91	92.5
X05072	7	F	84.55	709.67	165.25	98.15	140.87	88.3	38.2	89.8
X19061	8	F	93.879	639.12	95.05	48.37	80.99	30.06	7.74	97.8
D2296A	9	F	86.19	696.14	136.77	93.05	97.8	73.341	32.9	90.1
X10071	9	F	88.63	676.98	146.79	75.03	131.89	66.133	29.6	84.8
X05062	9	M	78.692	762.46	162.82	81.46	139.02	49.81	20	97.6
X13091	9	M	88.055	681.39	118.82	66.45	101.29	60.02	23.4	94
X14081	9	M	86.408	694.38	131.77	64.43	112.95	42.23	18.1	97.1
D2196A	10	F	86.29	695.34	140.86	111.2	95.25	94.3	39.2	81.4
X16082	10	M	77.879	770.42	170.96	92.3	140.92	65.33	32.1	93.2
X23081	10	M	86.804	691.21	134.89	64.95	120.28	46.29	17.5	94.1
X31052	10	M	78.69	762.46	162.82	81.46	139.02	49.81	20	97.6
X07062	11	M								
X16081	12	F	83.24	720.8	162.46	96.54	134.66	84.747	34.1	87.2
X23083	12	M	87.004	689.62	143.49	63.17	133.57	43.65	15.8	94.5
X27061	12	M	75.411	795.64	225.37	116.2	204.79	95.14	38.5	88.2
X09081	12	M	90.413	663.62	132.6	74.24	109.74	38.6	12.6	96.4

Mean	86.65	695.36	144.64	79.16	122.56	61.50	25.64	91.76
STD	5.69	47.07	27.19	18.07	27.16	20.59	10.23	4.74
SEM	1.27	10.52	6.08	4.04	6.07	4.60	2.28	1.06

vii) Time domain HRV parameters derived from 24-hour holter monitoring in adolescent (13-17 years) subjects.

Subject Number	Age	Gender	Heart Rate (bts/min)	N-N Interval (msec)	SDNN (msec)	SDNN index (msec)	SDANN (msec)	R-MSSD	pNN50 (%)	Accepted Intervals (%)
J1897A	13	F	80.731	743.21	117.3	61.9	97.363	38.107	14	99.1
D2296B	13	F	73.76	813.47	174.21	130.8	122.98	100.82	40.8	82.5
X31059	13	M	77.234	776.86	190.8	97.59	163.99	62.32	30	95.1
X28082	13	M								
X05071	13	F	73.657	814.59	204.78	109.6	172.77	66.81	31.4	94
X51072	14	F	74.098	809.74	177.11	76.49	151.75	47.61	21.8	98.1
X31071	14	F	75.856	790.97	232.92	102.7	213.73	78.04	35.5	94.9
X11111	15	M	77.212	777.08	164.29	93.03	133.48	55.429	27.1	98.4
J2597B	15	M	71.24	842.28	145.94	97.73	107.01	62.88	38.1	94.7
X28062	15	M	82.883	723.91	135.72	72.02	114.64	45.45	17.8	97.7
X28081	15	F	92.338	649.79	123.31	61.45	111.28	44.37	16.7	96.5
X28061	15	M	88.733	676.19	132.29	85.57	108.44	59.93	21.7	95
B24059	16	F	76.603	783.26	220.35	99.96	199.13	86.4	41.2	89.9
X24072	16	F	74.099	809.73	124.41	77.48	90.844	45.854	22.4	99.5
X02081	16	M	69.353	865.15	213.71	88.29	192.09	41.42	18.1	99.3
X24073	16	M	76.99	779.25	170.22	81.04	153.54	46.96	21.3	99.1
J2597A	17	F	63.73	941.44	218.28	97.17	193.79	81.29	41.3	92
X24059	17	F	88.106	681	142.39	72.05	124.72	49.99	20.1	94.1
X26071	17	F	76.309	786.27	182.26	67.84	166.57	35.91	13.1	98.9
X21081	17	F	72.59	826.55	191.24	81.87	172.43	45	20.7	99.4
X26072	17	F	93.564	641.4	117.04	46.72	108.81	56.63	14.2	81.2
Mean			77.95	776.60	168.92	85.06	144.96	57.56	25.36	94.97
STD			7.70	74.21	37.58	19.36	37.76	17.50	9.62	5.23
SEM			1.72	16.59	8.40	4.32	8.44	3.91	2.15	1.17

viii) Time domain HRV parameters derived from 24-hour holter monitoring in adult (18-30 years) subjects.

Subject Number	Age	Gender	Heart Rate (bts/min)	N-N Interval (msec)	SDNN (msec)	SDNN index (msec)	SDANN (msec)	R-MSSD	pNN50 (%)	Accepted Intervals (%)
F0397A	18	F	75.08	799.19	154.33	88.62	128.8	59.9	30	97.6
O2896A	18	F	67.08	894.47	172.39	80.83	151.5	51.83	29.6	99.1
N0296A	18	F	62.43	961.16	190.75	89.19	182.86	89.49	41.1	84.6
N0596A	18	M	69.72	860.59	192.82	89.82	177.66	59.36	31.6	92.7
N2796A	18	F	76.355	785.8	151.37	83.66	123.8	55.871	24.6	94
O1896A	19	F	65.89	910.6	193.15	107.7	148.88	74.55	39	96.7
N2696A	19	F	76.43	784.99	157.6	70.5	140.52	48.23	25	97.8
X17071	20	F	69.781	859.84	144.34	79.3	121.81	51.33	24.8	99.2
X12072	20	M								
X29051	21	F	88.98	674.31	118.56	58.5	102.23	36.89	7.68	97.5
X30051	21	F	85.333	703.13	129.75	64.5	117.14	53.462	11.9	89.4
S1196A	21	F								
N0796A	22	M	69.268	866.2	210.94	88.79	186.75	63.1	32.3	97.9
N1896A	22	F	75.619	793.46	176.37	88.75	144.95	60.81	31.6	97.9
N1196A	23	M	65.76	911.99	199.1	99.76	176.19	67.868	40.2	89.1
MA1597A	24	F	82.256	729.43	203.99	61.79	211.84	41.645	14.3	95.6
O1596A	24	M	70.09	855.96	230.03	85.18	214.11	36.3	13.3	96.4
MA1297A	24	M	64.606	928.71	164.93	95.73	122.86	56.928	32.2	98.8
N1296A	24	M	63.197	949.41	189.67	130.1	155.63	121.91	51.8	56.9
MA1797A	25	M	61.554	974.76	160.76	97.56	124.9	57.749	25.6	91.1
J1597A	25	M	64.488	930.41	203.88	113.7	168.58	79.154	37.1	92.6
D0396A	26	M	63.963	938.05	194.14	119.9	161.31	76.163	35.5	86.2
J2897A	26	F	71.25	842.1	187.45	76.83	168.93	51.718	27.5	97.8
N0996A	27	M	62.604	958.41	154.42	87.86	125.09	45.514	20.4	98.2
N1496A	27	M	79.607	753.71	116.57	53.98	101.23	28.952	8.12	99.7
D0296A	28	M								

Mean	70.92	855.07	173.79	87.50	150.32	59.50	27.61	93.33
STD	7.77	87.85	29.98	19.04	31.94	19.81	11.23	9.04
SEM	1.62	18.31	6.25	3.97	6.66	4.13	2.34	1.88

ix) Time domain HRV parameters derived from 24-hour holter monitoring in middle aged (31-60 years) subjects.

Subject Number	Age	Gender	Heart Rate (bts/min)	N-N Interval (msec)	SDNN (msec)	SDNN index (msec)	SDANN (msec)	R-MSSD	pNN50 (%)	Accepted Intervals (%)
J2197A	31	F	72.027	833.02	166.89	72.92	148.35	46.572	20.6	99.3
F2597A	31	M	65.539	915.49	191.63	87.94	169.91	37.358	13	94.9
M0697A	33	M	67.585	887.77	180.14	77.84	165.48	35.339	12	98.5
O2396A	35	F	80.365	746.59	112.77	38.94	108.34	20.495	2.42	99.8
A0997A	35	F	69.798	859.63	144.34	78.89	119.64	52.771	28.3	95.3
N1996A	37	F	73.63	814.88	194.08	82.38	180.68	47.117	19.1	99.2
M0397A	37	F	73.523	816.08	137.46	71.59	116.3	40.763	16.4	99.4
M2597A	37	F	73.138	820.37	128.84	47.93	117.13	24.818	4.85	99.8
F1597A	40	F	84.072	713.67	128.29	58.27	114.86	39.603	14.3	89.6
MA1397A	42	M	69.493	863.39	124.01	68.37	97.054	33.202	10.5	99.7
MA0997A	42	F	70.643	849.34	150.16	74.8	122.85	34.392	11.6	96.9
MA0797A	42	F	66.422	903.31	160.78	84.26	157.65	54.255	23.6	94.5
F1597B	44	M	80.005	749.95	116.59	52.46	103.71	30.967	8.68	97.7
F1797A	44	M	62.236	964.07	165.46	91.73	132.5	40.412	17.2	98.7
D1996A	45	M	81.476	736.41	120.44	68.48	97.006	31.827	8.81	98.5
A0297A	45	M	64.286	933.33	163.83	80.75	135.64	32.876	10.4	98.9
A2297A	45	F	84.412	710.8	122.84	48.66	112.1	21.315	2.56	99
A0897A	52	F	66.91	896.73	146.88	76.74	127.09	34.075	10	90.8
N2896A	56	F	74.416	806.28	183.24	55.21	174.72	34.538	10.9	96.6
A1597A	56	F	75.029	799.69	213.97	77.02	203.85	44.229	15.7	97.7
A0397B	57	F	67.107	894.09	155.02	56.06	140.59	36.27	12.1	98.8
A0397A	58	M	64.75	926.65	131.53	51.42	119.49	22.839	3.28	99.7
A2897A	59	F	71.663	837.25	161.75	53.18	153.56	23.216	3.25	99.3
A2997A	59	M	79.076	758.76	120.68	37.02	109.36	10.965	0.11	99.8

Mean	72.40	834.89	150.90	66.36	134.49	34.59	11.65	97.6
STD	6.43	72.64	27.87	15.74	28.84	10.51	7.05	2.77
SEM	1.31	14.82	5.68	3.21	5.88	2.14	1.44	0.56

x) Time domain HRV parameters derived from 24-hour holter monitoring in elderly (61+ years) subjects.

SubjectNumber	Age	Gender	Heart Rate (bts/min)	N-N Interval (msec)	SDNN (msec)	SDNN index (msec)	SDANN (msec)	R-MSSD	pNN50 (%)	Accepted Intervals (%)
D1696A	61	F	74.456	805.75	181.92	53.41	178.4	22.319	3.76	98.9
A0297B	62	F	78.378	765.52	114.26	39.32	109.32	15.759	0.729	98.2
N2296A	64	M	80.038	749.65	109.32	42.96	97.739	21.515	3.58	99.6
MA0897A	64	F	70.991	845.17	148.98	62.41	132.48	32.089	10.9	97.9
J2397A	64	F	67.436	889.73	131.95	58.98	111.33	34.991	9.75	98.3
D0696A	65	M	72.172	831.35	110.63	51.08	93.728	23.737	2.75	99.1
J3097A	65	F	68.414	877.02	119.25	44.5	110.86	32.259	9.08	94.4
A2197A	65	F	77.926	769.96	111.77	49.59	96.965	24.145	3.38	98.4
J1097A	66	F	72.786	824.34	110.72	46.82	97.265	19.663	1.68	95.6
A1097A	66	F	72.14	831.72	78.709	44.53	64.144	52.66	15.5	79.8
MA0697A	66	M								
F1897A	67	F	76.606	783.23	104.34	44.75	93.293	34.44	10.6	93.7
A1497A	67	F	69.512	863.16	163.24	50.89	147.35	32.243	8.31	95.8
D1096B	68	F	57.673	1040.3	151.62	62.12	142.48	64.534	17.6	81.6
F1397A	68	F	68.828	871.74	86.291	41.62	72.195	44.317	6.79	90.3
A2197A	68	M	60.474	992.16	181.26	67.14	168.08	31.225	9.54	93.4
MA0597B	68	F	72.494	827.65	130.31	55.88	115.49	31.36	5.62	98.6
D1096A	69	M	62.043	967.07	147.58	59.75	130.31	32	9.1	99.4
J2297A	69	F	82.532	726.99	109.4	26.32	102.15	13.041	0.481	99.5
F1197A	69	M	83.067	722.31	105.84	40.15	96.592	21.476	3.67	99.7
M1797A	70	F	69.045	869	117.64	38.24	113	22.669	2.43	96.3
MA0597A	70	M	64.677	927.68	103.88	40.32	91.145	20.106	1.89	99.7
D1296A	71	F								
F2597A	71	M	58.087	1032.9	142	50.84	130.62	29.352	7.63	97.4
F0497A	72	F	71.978	833.59	105.26	54.54	88.603	32.257	8.59	92.2
D1896A	74	M								
D2096A	74	M	61.24	979.75	154.37	52.69	135.91	38.677	8.79	92.2
F0797A	74	M	70.775	847.76	139.85	53.02	124.06	35.532	6.37	95.3
J2097A	77	F	75.598	793.67	141.48	44.59	131.81	26.48	6.37	99.2
J3197A	78	F	98.018	612.13	100.53	23.46	100.14	25.672	5.17	97.8

Mean	71.75	847.45	126.01	48.14	113.90	30.15	6.66	95.64
STD	8.66	98.63	26.67	10.22	26.69	10.99	4.24	5.06
SEM	1.66	18.98	5.13	1.96	5.13	2.11	0.81	0.97

xi) Frequency domain variables between 4-5 am (top) and 8-9 am (bottom) derived from 24 hour ambulatory ECG monitoring in pediatric subjects (5-12 years).

Subject Number	Age	Gender	Heart Rate (bts/min)	LF:HF Area	LF Area	LF Area (%)	HF Area	HF Area (%)	LF:HF Peak	LF cf (Hz)	HF cf (Hz)
X13061	5	F	109	1.61	6719	59.5	4688	40.5	2.58	0.074	0.245
D2196B	6	F	69.2	0.664	3964	35.2	7313	64.8	0.637	0.11	0.297
X12061	6	F	76.5	1.03	5422	47.4	5902	52.6	1.19	0.071	0.249
X10072	6	M									
X14121	7	F	80	0.688	4513	39	6769	61	0.762	0.086	0.251
X05072	7	F	62.2	0.611	4134	37	6925	63	0.522	0.094	0.304
X23082	7	M	75	1.84	6636	62	4136	38	2.77	0.059	0.273
X19061	8	F	80.3	1.28	6059	54.3	5103	45.7	1.29	0.091	0.298
D2296A	9	F	74.9	0.781	4570	42	6149	58	0.639	0.087	0.254
X10071	9	F	77.1	1.08	6240	49.8	6222	50.2	1.35	0.092	0.297
X05062	9	M									
X13091	9	M	75	0.811	5289	43.9	6699	56.1	0.995	0.093	0.261
X14081	9	M	73.6	0.939	4473	44.2	5768	55.8	1.45	0.088	0.211
D2196A	10	F	76.5	0.551	3148	31.7	6499	68.3	0.49	0.12	0.271
X16082	10	M	67.4	1.13	5448	50.8	5361	49.2	1.95	0.081	0.228
X23081	10	M	75.5	1.51	6388	58.6	4544	41.4	1.17	0.085	0.335
X31052	10	M	66.5	1.23	5993	52.5	5494	47.5	1.73	0.074	0.24
X07062	11	M									
X16081	12	F	69.3	0.96	5474	46.7	6167	53.3	0.995	0.1	0.263
X23083	12	M	70	1.11	5489	50.8	5309	49.2	1.29	0.08	0.256
X27061	12	M	55.8	0.924	5277	45.8	6142	54.2	0.94	0.092	0.291
X09081	12	M	79.3	1.91	7509	63.3	4326	36.7	2.64	0.082	0.272

Mean	74.37	1.09	5407	48.1	5764	51.87	1.33	0.09	0.27
STD	10.50	0.39	1087	9.00	915.8	9.00	0.71	0.01	0.03
SEM	2.41	0.09	249.5	2.07	210.1	2.06	0.16	0.003	0.007

Subject Number	Age	Gender	Heart Rate (bts/min)	LF:HF Area	LF Area	LF Area (%)	HF Area	HF Area (%)	LF:HF Peak	LF cf (Hz)	HF cf (Hz)
X13061	5	F	96	1.26	6031	52.8	5470	47.2	1.94	0.09	0.25
D2196B	6	F	81.5	0.816	5188	42.1	6982	57.9	0.99	0.11	0.323
X12061	6	F	107	1.42	6605	57.3	4949	42.7	2.34	0.073	0.252
X10072	6	M									
X14121	7	F	94.3	1.28	6707	54.8	5449	45.2	1.38	0.082	0.291
X05072	7	F	90.5	1.24	6230	53.3	5553	46.7	2.37	0.085	0.213
X23082	7	M	93.7	1.64	6866	61.1	4476	38.9	2.72	0.083	0.233
X19061	8	F	103	1.49	6588	58.4	4810	41.6	2.29	0.089	0.206
D2296A	9	F	79	0.766	4891	42.6	6603	57.4	0.755	0.1	0.26
X10071	9	F	113	1.36	5895	55.6	4793	44.4	2.37	0.079	0.242
X05062	9	M									
X13091	9	M	97.8	1.59	7283	59.9	4856	40.1	2.45	0.082	0.288
X14081	9	M	86.2	0.843	5070	43.3	6475	56.7	1.02	0.073	0.274
D2196A	10	F	78.7	0.806	5055	43.3	6632	56.7	0.708	0.12	0.28
X16082	10	M	72.9	1.34	6393	52.2	5782	47.8	2.16	0.072	0.232
X23081	10	M	107	2.06	6784	65.6	3566	34.4	3.13	0.079	0.228
X31052	10	M	97.7	1.66	6803	60.8	4360	39.2	2.32	0.089	0.192
X07062	11	M									
X16081	12	F	117	2.21	7121	67.7	3459	32.3	4.25	0.077	0.179
X23083	12	M	99.5	1.53	6397	59.6	4385	40.4	2.54	0.079	0.225
X27061	12	M	88.1	1.76	6626	61.1	4312	38.9	3.23	0.092	0.222
X09081	12	M	110	1.77	6773	62.8	4070	37.2	2.45	0.093	0.181

Mean	95.41	1.41	6279	55.5	5104	44.5	2.18	0.09	0.24
STD	12.38	0.41	732.4	7.85	1038	7.85	0.90	0.01	0.04
SEM	2.84	0.09	168.0	1.80	238.1	1.80	0.21	0.003	0.01

xii) Frequency domain variables between 12-1 pm (top) and 4-5 pm (bottom) derived from 24 hour ambulatory ECG monitoring in pediatric subjects (5-12 years).

Subject Number	Age	Gender	Heart Rate (bts/min)	LF:HF Area	LF Area	LF Area (%)	HF Area	HF Area (%)	LF:HF Peak	LF cf (Hz)	HF cf (Hz)
X13061	5	F	120	1.83	7116	62.8	4193	37.2	2.65	0.077	0.267
D2196B	6	F	88	1.52	6592	56.7	5067	43.3	1.26	0.1	0.353
X12061	6	F	120	1.77	6708	61	4329	39	2.55	0.069	0.221
X10072	6	M									
X14121	7	F	87.9	0.871	5448	44.4	6827	55.6	1.02	0.093	0.327
X05072	7	F	97.2	1.35	6486	55.5	5305	44.5	2.64	0.089	0.2
X23082	7	M	117	1.98	7039	64.9	3823	35.1	2.83	0.08	0.186
X19061	8	F	115	1.76	6597	62.7	4008	37.3	2.74	0.093	0.19
D2296A	9	F	102	1.77	7149	59.4	5052	40.6	2.09	0.078	0.262
X10071	9	F	109	1.42	6639	56.1	5348	43.9	2.48	0.079	0.251
X05062	9	M									
X13091	9	M	101	1.08	6183	50.6	6045	49.4	1.75	0.092	0.242
X14081	9	M	112	1.45	6846	58	5011	42	2.5	0.088	0.192
D2196A	10	F	88.9	0.966	5575	46.3	6511	53.7	1.44	0.11	0.22
X16082	10	M	93.3	1.26	6225	53.2	5525	46.8	2.03	0.089	0.205
X23081	10	M	108	2.27	6965	68.2	3321	31.8	3.89	0.08	0.19
X31052	10	M	109	1.5	6612	59	4632	41	2.26	0.082	0.198
X07062	11	M									
X16081	12	F	109	1.69	6547	60.3	4427	39.7	2.89	0.087	0.202
X23083	12	M	98.8	1.46	6549	57	4990	43	2.25	0.087	0.204
X27061	12	M	101	1.67	6287	60.6	4154	39.4	2.7	0.089	0.19
X09081	12	M	102	1.7	6429	61.4	4060	38.6	2.66	0.087	0.194
Mean			104.2	1.54	6525	57.8	4875	42.2	2.35	0.09	0.23
STD			10.3	0.35	453.4	5.98	920.9	5.98	0.66	0.01	0.05
SEM			2.36	0.08	104.0	1.37	211.2	1.37	0.15	0.002	0.01

Subject Number	Age	Gender	Heart Rate (bts/min)	LF:HF Area	LF Area	LF Area (%)	HF Area	HF Area (%)	LF:HF Peak	LF cf (Hz)	HF cf (Hz)
X13061	5	F	110	1.55	7514	58.8	5152	41.2	1.9	0.083	0.302
D2196B	6	F	109	1.24	6396	54.2	5337	45.8	2.06	0.087	0.196
X12061	6	F	113	1.66	7096	59.4	4817	40.6	2.16	0.085	0.224
X10072	6	M									
X14121	7	F	106	1.37	6802	55.8	5425	44.2	1.27	0.098	0.321
X05072	7	F	97.8	1.23	6425	53.7	5571	46.3	2.11	0.096	0.201
X23082	7	M	94	1.91	6823	62.8	4066	37.2	3.26	0.082	0.205
X19061	8	F	98.5	1.7	7001	60.2	4669	39.8	2.46	0.098	0.235
D2296A	9	F	107	1.79	7316	60.7	4818	39.3	1.94	0.087	0.283
X10071	9	F	98.4	1.45	6860	57.2	5170	42.8	2.33	0.086	0.225
X05062	9	M									
X13091	9	M	91.3	1.19	6568	52.6	5980	47.4	1.81	0.089	0.276
X14081	9	M	90.1	1.36	6515	56.1	5093	43.9	2.11	0.08	0.261
D2196A	10	F	92.9	1.08	5982	50	6071	50	1.8	0.1	0.214
X16082	10	M	86	0.882	5648	45.1	6830	54.9	1.06	0.11	0.258
X23081	10	M	97.3	1.7	6709	60.8	4400	39.2	2.84	0.082	0.196
X31052	10	M	89	1.47	6431	58.4	4631	41.6	1.94	0.095	0.192
X07062	11	M									
X16081	12	F	108	1.77	6598	62	4198	38	2.85	0.094	0.188
X23083	12	M	96.2	1.42	6628	57.8	4686	42.2	2.41	0.091	0.207
X27061	12	M	81.6	1.03	5920	48.1	6446	51.9	1.32	0.1	0.256
X09081	12	M	100	2.06	7297	66.4	3755	33.6	3.31	0.079	0.211
Mean			98.21	1.47	6659	56.85	5111	43.15	2.15	0.09	0.23
STD			8.77	0.32	483.3	5.31	810.6	5.30	0.61	0.008	0.04
SEM			2.01	0.07	110.9	1.22	186.0	1.22	0.14	0.002	0.009

xiii) Frequency domain variables between 8-9 pm (top) and 12-1 am (bottom) derived from 24 hour ambulatory ECG monitoring in pediatric subjects (5-12 years).

Subject Number	Age	Gender	Heart Rate (bts/min)	LF:HF Area	LF Area	LF Area (%)	HF Area	HF Area (%)	LF:HF Peak	LF cf (%)	HF cf (%)
X13061	5	F	78.2	0.793	4413	40.5	6274	59.5	0.546	0.096	0.332
D2196B	6	F	109	0.986	5537	49	5890	51	1.93	0.095	0.209
X12061	6	F	131	1.6	6594	60.7	4334	39.3	3	0.085	0.188
X10072	6	M									
X14121	7	F	107	1.48	6871	57.5	5086	42.5	1.13	0.095	0.32
X05072	7	F	104	1.33	6548	56	5138	44	2.44	0.089	0.183
X23082	7	M	85.1	1.71	7487	61	4895	39	2.12	0.09	0.276
X19061	8	F	101	1.98	7087	64.7	3892	35.3	2.38	0.089	0.328
D2296A	9	F	103	1.38	6408	57	4863	43	2.04	0.08	0.224
X10071	9	F	96.7	1.36	6747	56	5278	44	1.85	0.092	0.24
X05062	9	M									
X13091	9	M	94.4	1.23	6573	52.3	6116	47.7	1.39	0.1	0.293
X14081	9	M	83.1	1.07	6035	49.8	6068	50.2	1.36	0.083	0.263
D2196A	10	F	101	1.11	6376	51.2	6111	48.8	1.79	0.089	0.193
X16082	10	M	89.9	1.09	5914	51.1	5773	48.9	1.55	0.11	0.201
X23081	10	M	91.7	1.21	6446	52.7	5749	47.3	1.73	0.093	0.259
X31052	10	M	81.5	1.34	6767	56.1	5240	43.9	1.91	0.091	0.228
X07062	11	M									
X16081	12	F	98.1	1.49	6332	58	4681	42	2.8	0.094	0.189
X23083	12	M	95.5	1.39	6520	55.6	5209	44.4	1.44	0.08	0.281
X27061	12	M	89	1.44	6694	56	5411	44	1.98	0.097	0.236
X09081	12	M	94.6	1.74	6679	61.6	4266	38.4	2.4	0.084	0.215

Mean	96.52	1.35	6422	55.0	5277	44.90	1.88	0.09	0.25
STD	12.02	0.29	643.7	5.50	684.9	5.50	0.59	0.007	0.05
SEM	2.76	0.07	147.7	1.26	157.1	1.26	0.13	0.002	0.01

Subject Number	Age	Gender	Heart Rate (bts/min)	LF:HF Area	LF Area	LF Area (%)	HF Area	HF Area (%)	LF:HF Peak	LF cf (%)	HF cf (%)
X13061	5	F	75	0.867	5240	43.6	6666	56.4	0.947	0.095	0.307
D2196B	6	F	89.3	1.47	7459	57.3	5590	42.7	1.38	0.079	0.396
X12061	6	F	79.3	1.06	5320	48.7	5422	51.3	1.34	0.07	0.276
X10072	6	M									
X14121	7	F	82.8	0.573	4202	35	7769	65	0.642	0.11	0.292
X05072	7	F	75.8	1.36	6740	55	5412	45	1.05	0.092	0.354
X23082	7	M	73.7	1.11	5215	49	5326	51	1.12	0.062	0.305
X19061	8	F	80.6	1.11	5578	50.6	5371	49.4	0.969	0.081	0.294
D2296A	9	F	72.9	0.625	3825	37.2	6333	62.8	0.518	0.093	0.263
X10071	9	F	69.7	1.1	6119	49.6	6262	50.4	1.19	0.1	0.313
X05062	9	M									
X13091	9	M	78.9	0.748	4885	41.9	6703	58.1	0.752	0.091	0.269
X14081	9	M	75.3	0.827	4714	43.6	6069	56.5	1.03	0.073	0.231
D2196A	10	F	89.5	1.1	5742	47.8	6472	52.2	1.41	0.12	0.232
X16082	10	M	67.8	0.745	4751	41.4	6606	58.6	0.717	0.11	0.247
X23081	10	M	74.3	2.08	7194	65.4	3832	34.6	2.11	0.086	0.297
X31052	10	M	67.6	1.07	5302	48.8	5502	51.2	1.37	0.095	0.232
X07062	11	M									
X16081	12	F	67.8	0.765	5069	42.2	6936	57.8	1.16	0.11	0.25
X23083	12	M	70.3	0.757	4489	42.4	6049	57.6	0.517	0.09	0.267
X27061	12	M	55.7	0.86	5507	45.5	6590	54.5	0.891	0.11	0.276
X09081	12	M	79.6	1.92	6415	63.7	3673	36.3	2.14	0.08	0.277

Mean	75.05	1.06	5461	47.8	5925	52.2	1.12	0.09	0.28
STD	7.99	0.41	971.2	8.07	1001	8.07	0.45	0.02	0.04
SEM	1.83	0.09	222.8	1.85	229.6	1.85	0.10	0.004	0.01

xiv) Frequency domain variables between 4-5 am (top) and 8-9 am (bottom) derived from 24 hour ambulatory ECG monitoring in adolescent subjects (13-17 years).

Subject Number	Age	Gender	Heart Rate (bts/min)	LF:HF Area	LF Area	LF Area (%)	HF Area	HF Area (%)	LF:HF Peak	LF cf (%)	HF cf (%)
J1897A	13	F	66.8	1.31	6127	55.6	4941	44.4	1.62	0.08	0.281
D2296B	13	F	67	0.649	3955	39	6113	61	0.431	0.12	0.219
X05071	13	F	56	1.7	6885	61.4	4313	38.6	2.43	0.085	0.24
A310595	13	M	96	1.46	6298	56	4934	44	2.4	0.089	0.212
X28082	13	M									
X51072	14	F	66.9	1.01	5331	49.4	5485	50.6	1.35	0.079	0.237
X31071	14	F	59.5	1.13	5806	50.6	5552	49.4	1.56	0.085	0.255
X28081	15	F	74.8	0.661	4019	38.3	6339	61.7	0.594	0.11	0.219
X11111	15	M	68.3	1.34	5592	55.6	4411	44.4	1.42	0.11	0.181
J2597B	15	M	64.4	0.949	4897	46.3	5544	53.7	0.968	0.096	0.228
X28062	15	M	75.4	1.02	4832	46.7	5486	53.3	1.29	0.08	0.222
X28061	15	M	70.2	0.749	4374	41.3	6096	58.7	0.751	0.12	0.214
B240595	16	F	59.9	0.638	4097	37	6490	63	0.606	0.077	0.239
X24072	16	F	67.6	1.47	5822	56.6	4444	43.4	1.64	0.077	0.274
X02081	16	M	53.5	1.38	5472	55.5	4238	44.5	1.81	0.08	0.22
X24073	16	M	61.6	1.99	6904	64.7	3734	35.3	3.17	0.074	0.248
J2597A	17	F	49.4	0.69	4524	38.7	6889	61.3	0.823	0.11	0.242
A240595	17	F	68.6	1.33	6400	55.4	5139	44.6	1.58	0.08	0.293
X26071	17	F	56.9	1.16	5402	48.8	5373	51.2	1.7	0.064	0.24
X21081	17	F	59.4	1.66	7242	60.9	4654	39.1	2.61	0.073	0.259
X26072	17	F	79.2	0.823	4567	41.2	6662	58.8	0.931	0.13	0.342

Mean	66.07	1.16	5427	49.9	5341	50.1	1.48	0.09	0.24
STD	10.35	0.39	1009	8.59	891.9	8.59	0.74	0.02	0.03
SEM	2.31	0.09	225.7	1.92	199.4	1.92	0.16	0.004	0.008

Subject Number	Age	Gender	Heart Rate (bts/min)	LF:HF Area	LF Area	LF Area (%)	HF Area	HF Area (%)	LF:HF Peak	LF cf (%)	HF cf (%)
J1897A	13	F	77.2	1.67	6635	60.7	4326	39.3	2.31	0.087	0.265
D2296B	13	F	68.4	0.814	4632	43.9	5870	56.1	0.737	0.13	0.215
X05071	13	F	101	1.95	6990	64.7	3817	35.3	2.81	0.085	0.198
A310595	13	M	97.6	1.9	6981	63.9	3934	36.1	2.76	0.073	0.246
X28082	13	M									
X51072	14	F	106	2.44	6452	68.4	3071	31.6	5.33	0.073	0.196
X31071	14	F	96.6	2.01	6717	65.8	3522	34.2	4.1	0.074	0.186
X28081	15	F	76.6	0.639	4014	38.7	6339	61.3	0.508	0.099	0.224
X11111	15	M	101	2.48	6914	68.1	3372	31.9	3.73	0.079	0.184
J2597B	15	M	64.6	0.918	5324	47	5906	53	0.965	0.087	0.239
X28062	15	M	82.9	1.52	6149	57.2	4778	42.8	2.08	0.092	0.25
X28061	15	M	90.9	1.32	6211	55.7	4978	44.3	2.08	0.09	0.219
B240595	16	F	119	1.66	6465	61.1	4124	38.9	3.3	0.082	0.241
X24072	16	F	84.8	2.03	6751	64.9	3728	35.1	4.08	0.073	0.236
X02081	16	M	83.3	2.5	7183	70.4	3034	29.6	4.16	0.081	0.198
X24073	16	M	78.7	1.68	6687	60.9	4252	39.1	2.62	0.086	0.194
J2597A	17	F	52.7	0.803	5121	43.5	6746	56.5	1.11	0.084	0.233
A240595	17	F	106	1.94	6364	64.8	3481	35.2	3.1	0.091	0.204
X26071	17	F	91.4	1.64	6077	58.5	4394	41.5	2.8	0.086	0.23
X21081	17	F	87.5	2.21	7424	67	3746	33	3.42	0.074	0.197
X26072	17	F	121	1.56	5364	58.1	3959	41.9	2.82	0.075	0.368

Mean	89.36	1.68	6222	59.2	4368	40.8	2.74	0.09	0.23
STD	17.40	0.56	895.6	9.14	1080	9.14	1.26	0.01	0.04
SEM	3.89	0.13	200.3	2.04	241.6	2.04	0.28	0.003	0.009

xv) Frequency domain variables between 12-1 pm (top) and 4-5 pm (bottom) derived from 24 hour ambulatory ECG monitoring in adolescent subjects (13-17 years).

Subject Number	Age	Gender	Heart Rate (bts/min)	LF:HF Area	LF Area	LF Area (%)	HF Area	HF Area (%)	LF:HF Peak	LF cf (%)	HF cf (%)
J1897A	13	F	88.9	1.45	6527	57.6	4878	42.4	2.33	0.087	0.18
D2296B	13	F	84.3	1.22	6557	53.4	5758	46.6	1.6	0.1	0.224
X05071	13	F	95.3	1.61	6478	60.2	4358	39.8	2.8	0.085	0.185
A310595	13	M	97.9	1.66	6773	60.2	4512	39.8	2.48	0.088	0.232
X28082	13	M									
X51072	14	F	89.9	2.28	6722	67.8	3263	32.2	4.12	0.087	0.233
X31071	14	F	91.6	1.43	6627	56.8	5078	43.2	2.28	0.098	0.185
X28081	15	F	97.1	0.994	5728	48.9	5961	51.1	0.985	0.1	0.251
X11111	15	M	74.9	1.5	6748	58.4	4777	41.6	2.13	0.1	0.213
J2597B	15	M	82.5	1.52	6600	59.4	4501	40.6	2.42	0.1	0.18
X28062	15	M	92.8	1.49	6517	58.7	4624	41.3	2.67	0.088	0.18
X28061	15	M	94	1.6	6905	60.6	4469	39.4	2.39	0.098	0.19
B240595	16	F	117	1.81	6800	62.8	4124	37.2	3.98	0.081	0.198
X24072	16	F	79.6	2.25	7584	66.7	3832	33.3	2.39	0.08	0.312
X02081	16	M	94.8	2.73	7053	72.5	2669	27.5	4.15	0.085	0.19
X24073	16	M	93.9	2.24	6994	65.5	3749	34.5	3.26	0.084	0.209
J2597A	17	F	79.3	1.8	7379	63.2	4269	36.8	2.29	0.083	0.289
A240595	17	F	90.4	1.4	6176	56.5	4775	43.5	1.91	0.1	0.255
X26071	17	F	108	3.11	7150	75.1	2381	24.9	5.41	0.079	0.182
X21081	17	F	88.2	1.89	6761	64.3	3787	35.7	2.66	0.088	0.172
X26072	17	F	114	1.4	5756	55.7	4663	44.3	2.35	0.085	0.255
Mean			92.7	1.77	6690	61.2	4321	38.8	2.73	0.09	0.22
STD			10.8	0.52	457.5	6.27	884.3	6.27	1.01	0.008	0.04
SEM			2.42	0.12	102.3	1.40	197.7	1.40	0.23	0.002	0.009

Subject Number	Age	Gender	Heart Rate (bts/min)	LF:HF Area	LF Area	LF Area (%)	HF Area	HF Area (%)	LF:HF Peak	LF cf (%)	HF cf (%)
J1897A	13	F	90.8	1.54	6603	58.3	4798	41.7	2	0.089	0.235
D2296B	13	F	81.6	1.24	6499	53.4	5674	46.6	1.6	0.1	0.231
X05071	13	F	90.2	1.51	6593	57.4	4998	42.6	2.05	0.096	0.222
A310595	13	M	93.2	1.59	6632	60.4	4382	39.6	2.32	0.084	0.18
X28082	13	M									
X51072	14	F	79.4	2.04	6777	65.2	3711	34.8	4.24	0.086	0.248
X31071	14	F	73.3	1.17	6427	52.4	5861	47.6	1.6	0.12	0.212
X28081	15	F	108	1.41	6312	56.7	4881	43.3	2.35	0.1	0.213
X11111	15	M	87.8	1.84	6958	63.7	3970	36.3	2.69	0.097	0.197
J2597B	15	M	78.5	1.86	7261	64.2	4044	35.8	2.19	0.097	0.206
X28062	15	M	104	1.88	6374	63.5	3734	36.5	3.29	0.069	0.189
X28061	15	M	104	1.66	6823	61.6	4315	38.4	2.53	0.089	0.191
B240595	16	F	77.7	1.03	5800	48.4	6081	51.6	1.29	0.094	0.282
X24072	16	F	71.6	1.55	6236	59.5	4244	40.5	1.51	0.071	0.298
X02081	16	M	83.9	3.17	6817	74.9	2305	25.1	5.94	0.082	0.171
X24073	16	M	85.6	2.09	6898	64.4	3918	35.6	3	0.083	0.253
J2597A	17	F	58	0.936	5222	47.3	5790	52.7	0.976	0.1	0.282
A240595	17	F	112	2.46	6931	69.8	3017	30.2	3.84	0.084	0.181
X26071	17	F	94.2	2.28	6938	68.7	3190	31.3	3.56	0.086	0.18
X21081	17	F	89.5	2.06	6895	66.9	3420	33.1	3.19	0.09	0.172
X26072	17	F	114	1.52	5780	57.1	4318	42.9	2.09	0.086	0.312
Mean			88.8	1.74	6538	60.7	4332	39.3	2.61	0.09	0.22
STD			14.4	0.53	488.6	7.07	1010	7.07	1.17	0.01	0.04
SEM			3.22	0.12	109.2	1.58	225.9	1.58	0.26	0.003	0.01

xvi) Frequency domain variables between 8-9 pm (top) and 12-1 am (bottom) derived from 24 hour ambulatory ECG monitoring in adolescent subjects (13-17 years).

Subject Number	Age	Gender	Heart Rate (bts/min)	LF:HF Area	LF Area	LF Area (%)	HF Area	HF Area (%)	LF:HF Peak	LF cf (%)	HF cf (%)
J1897A	13	F	93.4	1.29	5990	54.5	5088	45.5	2.29	0.09	0.187
D2296B	13	F	82.9	1.11	6185	51.4	5874	48.6	1.45	0.12	0.199
X05071	13	F	90.8	1.37	6324	55.6	5113	44.4	1.98	0.087	0.19
A310595	13	M	79	1.57	6933	57.6	5111	42.4	1.99	0.076	0.235
X28082	13	M									
X51072	14	F	75.3	1.82	6816	62.3	4207	37.7	2.1	0.086	0.324
X31071	14	F	101	1.83	6641	63.1	3941	36.9	0.287	0.089	0.182
X28081	15	F	103	1.33	6428	55.5	5164	44.5	2.04	0.1	0.223
X11111	15	M	82.9	1.61	6504	59.1	4492	40.9	2.16	0.094	0.218
J2597B	15	M	78.2	1.65	6890	60.2	4580	39.8	1.81	0.1	0.243
X28062	15	M	88.6	1.53	6790	58.2	4947	41.8	2.59	0.086	0.244
X28061	15	M	98	1.31	6600	55.3	5370	44.7	1.82	0.097	0.201
B240595	16	F	99.4	1.54	6343	57.9	4625	42.1	2.47	0.089	0.19
X24072	16	F	79.3	2.4	7413	67.8	3587	32.2	3.24	0.083	0.276
X02081	16	M	76.5	2.58	7181	70.4	3010	29.6	4.55	0.082	0.211
X24073	16	M	100	2.04	6906	65.9	3588	34.1	3.1	0.078	0.176
J2597A	17	F	67.4	1.34	6556	55.8	5193	44.2	1.38	0.1	0.295
A240595	17	F	91.7	1.61	7028	60.2	4674	39.8	1.8	0.1	0.268
X26071	17	F	77.8	1.47	6620	55.6	5410	44.4	2.23	0.11	0.216
X21081	17	F	96.1	2.06	7246	66.4	3641	33.6	3.27	0.076	0.172
X26072	17	F	92.1	1.39	5863	53.1	5185	46.9	1.63	0.091	0.319

Mean	87.67	1.64	6662	59.2	4640	40.7	2.21	0.09	0.23
STD	10.32	0.38	408.1	5.20	755.3	5.20	0.88	0.01	0.05
SEM	2.31	0.09	91.2	1.16	168.9	1.16	0.20	0.003	0.01

Subject Number	Age	Gender	Heart Rate (bts/min)	LF:HF Area	LF Area	LF Area (%)	HF Area	HF Area (%)	LF:HF Peak	LF cf (Hz)	HF cf (Hz)
J1897A	13	F	78.8	1.11	5365	50.5	5367	49.5	1.23	0.092	0.279
D2296B	13	F	62.9	0.593	3407	35.7	5719	64.3	0.488	0.15	0.225
X05071	13	F	64.3	1.7	6425	61.1	4115	38.9	3.09	0.083	0.228
A310595	13	M	59.6	0.747	4677	41.5	6453	58.5	0.797	0.094	0.221
X28082	13	M									
X51072	14	F	59.8	1.31	6060	55.6	4869	44.4	1.91	0.079	0.236
X31071	14	F	53.4	0.806	4627	41	6313	59	0.69	0.083	0.272
X28081	15	F	89.3	1.05	5758	49.3	5714	50.7	1.16	0.097	0.221
X11111	15	M	72.4	1.04	4812	48.9	4901	51.1	1.03	0.095	0.193
J2597B	15	M	65.9	0.677	3750	39.6	5669	60.4	0.371	0.095	0.232
X28062	15	M	68.7	0.717	4502	39.2	6799	60.8	0.729	0.085	0.249
X28061	15	M	78.9	1.69	6713	61.1	4212	38.9	2.62	0.079	0.226
B240595	16	F	64.3	0.833	4342	42.2	5657	57.8	1.12	0.082	0.267
X24072	16	F	70.1	2.02	7134	64.6	3876	35.4	2.37	0.075	0.296
X02081	16	M	58.1	2.34	7085	68.8	3216	31.2	2.85	0.076	0.241
X24073	16	M	63.7	1.52	6893	58.7	4775	41.3	1.69	0.082	0.278
J2597A	17	F	52.9	0.732	4350	38.5	6610	61.5	0.683	0.085	0.273
A240595	17	F	81.4	1.3	5766	54.8	4746	45.2	1.06	0.095	0.302
X26071	17	F	67.7	1.19	5771	49.6	5884	50.4	1.42	0.099	0.262
X21081	17	F	57	2.29	7136	67.8	3374	32.2	3.08	0.07	0.285
X26072	17	F	73.8	0.745	5107	41.7	7108	58.3	1.03	0.12	0.29

Mean	67.15	1.22	5484	50.5	5268	49.5	1.47	0.09	0.25
STD	9.70	0.54	1166	10.5	1126	10.5	0.88	0.02	0.03
SEM	2.17	0.12	260.7	2.35	251.9	2.35	0.20	0.004	0.007

xvii) Frequency domain variables between 4-5 am (top) and 8-9 am (bottom) derived from 24 hour ambulatory ECG monitoring in adult subjects (18-30 years).

Subject Number	Age	Gender	Heart Rate (bts/min)	LF:HF Area	LF Area	LF Area (%)	HF Area	HF Area (%)	LF:HF Peak	LF cf (Hz)	HF cf (Hz)
F0397A	18	F	58.6	0.862	4449	43.6	5527	56.4	0.922	0.081	0.243
O2896A	18	F	52.4	0.966	5072	47.9	5498	52.1	1.13	0.069	0.23
N0296A	18	F	44.7	0.77	4431	41	6162	59	0.857	0.097	0.224
N2796A	18	F	61.7	0.778	4481	42.5	5845	57.5	0.686	0.092	0.21
N0596A	18	M	56.2	1.47	6108	55.5	4856	44.5	2.05	0.082	0.251
O1896A	19	F	60.1	1.55	6614	59	4610	41	2.12	0.094	0.23
N2696A	19	F	63.4	1.16	5952	52.2	5385	47.8	1.27	0.083	0.247
X17071	20	F	67.9	1.52	5561	59.1	3864	40.9	1.73	0.07	0.289
X12072	20	M									
X29051	21	F	74	1.18	5491	52.9	4725	47.1	1	0.087	0.254
X30051	21	F	68.3	0.655	3323	34	6588	66	0.952	0.07	0.301
S1196A	21	F	39.9	1.37	6361	55.9	5057	44.1	2.07	0.059	0.251
N1896A	22	F	63.4	1.24	5496	52	4765	48	1.1	0.088	0.261
N0796A	22	M	54.2	0.503	3274	32.7	6693	67.3	0.331	0.069	0.232
N1196A	23	M	51.1	1.08	5557	49.5	5528	50.5	1.38	0.076	0.209
MA1597A	24	F	55.6	1.01	5427	47.9	5801	52.1	1.69	0.061	0.255
O1596A	24	M	49.4	2.06	7390	65.3	3821	34.7	2.47	0.075	0.243
MA1297A	24	M	56.9	0.737	4000	41.2	5586	58.8	0.427	0.073	0.245
N1296A	24	M	50.2	1.01	6073	49.6	6134	50.4	1.5	0.08	0.203
MA17967A	25	M	51.8	1.68	6300	61.5	4013	38.5	2.56	0.093	0.162
J1597A	25	M	56.3	1.22	6323	52.8	5726	47.2	1.69	0.084	0.225
J2897A	26	F	54.7	0.934	4961	45.7	5692	54.3	1.3	0.075	0.234
D0396A	26	M	44.4	1.28	5848	52.9	5043	47.1	1.68	0.076	0.229
N0996A	27	M	54	1.63	6113	61.2	3897	38.8	2.3	0.08	0.207
N1496A	27	M	72.9	1.81	6219	62.1	3735	37.9	2.22	0.067	0.299
D0296A	28	M	40.9	0.991	5439	48.2	5847	51.8	1.12	0.086	0.236
Mean			56.12	1.18	5450	50.6	5215	49.4	1.46	0.08	0.24
STD			9.03	0.39	1009	8.50	866.4	8.49	0.63	0.01	0.03
SEM			1.81	0.08	201.9	1.70	173.3	1.70	0.13	0.002	0.006

Subject Number	Age	Gender	Heart Rate (bts/min)	LF:HF Area	LF Area	LF Area (%)	HF Area	HF Area (%)	LF:HF Peak	LF cf (Hz)	HF cf (Hz)
F0397A	18	F	72	1.25	5329	51.7	5053	48.3	2.2	0.074	0.224
O2896A	18	F	78.2	1.61	6639	59.2	4555	40.8	2.55	0.075	0.258
N0296A	18	F	68.4	1.75	6039	58.5	4462	41.5	2.87	0.073	0.232
N2796A	18	F	80.6	1.35	5031	50.5	5148	49.5	2.09	0.079	0.204
N0596A	18	M	78	1.75	6912	62.4	4207	37.6	3.22	0.086	0.193
O1896A	19	F	62	1.48	6630	58	4819	42	1.57	0.089	0.27
N2696A	19	F	103	1.83	6657	61.6	4253	38.4	3.16	0.084	0.229
X17071	20	F	75.1	2.17	6999	67.7	3337	32.3	3.96	0.079	0.225
X12072	20	M									
X29051	21	F	105	2.44	6874	69.9	2954	30.1	4.82	0.08	0.195
X30051	21	F	115	2.82	6855	72.4	2664	27.6	4.22	0.083	0.235
S1196A	21	F	77.8	1.93	7006	63.6	4037	36.4	3.02	0.091	0.242
N1896A	22	F	73.7	1.95	6583	63.4	3862	36.6	3.68	0.073	0.227
N0796A	22	M	59.1	0.843	4457	42.8	5793	57.2	0.819	0.066	0.219
N1196A	23	M	104	1.79	6336	62.2	3833	37.8	3.5	0.073	0.209
MA1597A	24	F	121	1.88	6077	60.3	3995	39.7	3.4	0.063	0.286
O1596A	24	M	106	2.27	6296	67.7	2960	32.3	4.06	0.087	0.182
MA1297A	24	M	71.9	1.51	6339	58.1	4641	41.9	2.27	0.076	0.201
N1296A	24	M	67.1	1.05	4631	51.2	4411	48.8	1.37	0.09	0.237
MA17967A	25	M	51.9	1.6	6411	59.9	4295	40.1	2.33	0.081	0.195
J1597A	25	M	56.8	1.5	6666	58.5	4710	41.5	1.85	0.096	0.206
J2897A	26	F	96.8	1.29	5896	53.9	4885	46.1	2.01	0.079	0.232
D0396A	26	M	76.3	2.04	7237	65.3	3901	34.7	3.68	0.085	0.212
N0996A	27	M	60.5	1.89	6315	63.7	3628	36.3	3.61	0.073	0.17
N1496A	27	M	66.8	1.82	6248	61.2	4045	38.8	2.73	0.07	0.276
D0296A	28	M	81.7	1.6	6422	59.2	4466	40.8	2.48	0.076	0.181
Mean			80.34	1.74	6275	60.1	4196	39.9	2.86	0.08	0.22
STD			19.10	0.43	721.0	6.56	728.7	6.56	0.98	0.008	0.03
SEM			3.82	0.09	144.2	1.31	145.7	1.31	0.20	0.002	0.006

xviii) Frequency domain variables between 12-1 pm (top) and 4-5 pm (bottom) derived from 24 hour ambulatory ECG monitoring in adult subjects (18-30 years).

Subject Number	Age	Gender	Heart Rate (bts/min)	LF:HF Area	LF Area	LF Area (%)	HF Area	HF Area (%)	LF:HF Peak	LF cf (Hz)	HF cf (Hz)
F0397A	18	F	83.6	1.4	6156	56.1	4866	43.9	2.19	0.092	0.209
O2896A	18	F	84.5	1.65	6820	60.5	4494	39.5	2.56	0.083	0.267
N0296A	18	F	72.9	2.9	7288	72.9	2694	27.1	5.39	0.077	0.324
N2796A	18	F	81.6	1.63	6578	61.2	4215	38.8	2.64	0.087	0.161
N0596A	18	M	70.3	1.83	6923	63.7	3959	36.3	2.7	0.1	0.199
O1896A	19	F	55.8	1.19	5807	53	5124	47	1.47	0.083	0.251
N2696A	19	F	80.9	1.35	6296	55.1	5146	44.9	2	0.082	0.278
X17071	20	F	83.1	2.31	6983	68.2	3295	31.8	4.33	0.088	0.196
X12072	20	M									
X29051	21	F	118	1.91	7169	65.1	3859	34.9	3.44	0.094	0.228
X30051	21	F	90	2.07	6785	65.9	3553	34.1	3.31	0.087	0.208
S1196A	21	F	72.7	2.16	7187	65.6	3837	34.4	2.51	0.089	0.282
N1896A	22	F	76.6	1.95	7225	64.8	3957	35.2	2.47	0.094	0.267
N0796A	22	M	90.8	1.73	6787	62.8	4030	37.2	2.99	0.089	0.236
N1196A	23	M	71.5	1.7	6517	60.4	4338	39.6	2.73	0.09	0.194
MA1597A	24	F	101	1.57	6531	59.1	4545	40.9	2.89	0.085	0.219
O1596A	24	M	76	2.71	7187	72.6	2704	27.4	4.28	0.084	0.17
MA1297A	24	M	65.3	1.12	5699	51.9	5316	48.1	1.91	0.1	0.163
N1296A	24	M	65.6	1.07	5668	50.9	5490	49.1	1.86	0.1	0.16
MA17967A	25	M	62.2	1.25	5984	54	5178	46	2.18	0.082	0.181
J1597A	25	M	70.4	1.69	6939	61.5	4371	38.5	2.22	0.087	0.18
J2897A	26	F	86.1	1.34	6193	54.6	5073	45.4	1.84	0.088	0.22
D0396A	26	M	64.9	1.73	6876	61.5	4325	38.5	2.74	0.09	0.208
N0996A	27	M	65.1	1.99	6642	65.7	3471	34.3	3.02	0.091	0.157
N1496A	27	M	95.3	2.41	6959	69	3087	31	4.68	0.078	0.276
D0296A	28	M	61	1.15	6033	51.4	5797	48.6	1.43	0.094	0.206

Mean	77.81	1.75	6609	61.1	4268	38.9	2.79	0.09	0.22
STD	14.18	0.49	501.7	6.44	852.2	6.44	0.99	0.006	0.05
SEM	2.84	0.10	100.4	1.29	170.4	1.29	0.20	0.001	0.01

Subject Number	Age	Gender	Heart Rate (bts/min)	LF:HF Area	LF Area	LF Area (%)	HF Area	HF Area (%)	LF:HF Peak	LF cf (Hz)	HF cf (Hz)
F0397A	18	F	73.4	1.18	6179	52.2	5665	47.8	1.52	0.11	0.218
O2896A	18	F	74.5	1.32	6655	55.6	5294	44.4	2.14	0.092	0.236
N0296A	18	F	73.2	2.1	6813	63.2	4109	36.8	3.54	0.078	0.31
N2796A	18	F	84.2	1.66	6706	61.1	4339	38.9	2.61	0.09	0.186
N0596A	18	M	80.1	1.65	6764	60.6	4403	39.4	2.43	0.097	0.185
O1896A	19	F	80.1	1.57	6699	58.9	4702	41.1	2.25	0.1	0.223
N2696A	19	F	89.9	1.76	6716	61.3	4342	38.7	2.97	0.095	0.234
X17071	20	F	62.4	1.25	6122	52.7	5574	47.3	1.68	0.076	0.28
X12072	20	M									
X29051	21	F	86.9	1.67	6602	61.7	4116	38.3	3.23	0.099	0.19
X30051	21	F	83.1	2.03	7196	68.1	3656	31.9	3.91	0.079	0.208
S1196A	21	F	54.3	1.18	5977	52.5	5445	47.5	1.66	0.096	0.223
N1896A	22	F	81.3	1.9	6931	62.7	4106	37.3	1.46	0.086	0.305
N0796A	22	M	91.6	1.84	6442	63	3878	37	3.98	0.088	0.182
N1196A	23	M	107	1.3	5660	55.4	4671	44.6	2.13	0.079	0.181
MA1597A	24	F	78.3	1.22	6089	53	5373	47	1.97	0.08	0.234
O1596A	24	M	72.9	2.63	7525	71.5	2979	28.5	3.93	0.084	0.177
MA1297A	24	M	64.2	1.32	6317	55.6	5109	44.4	1.87	0.085	0.18
N1296A	24	M	66.1	0.91	5412	47.1	6089	52.9	1.67	0.1	0.173
MA17967A	25	M	70.2	1.31	6031	54.4	5304	45.6	2.6	0.08	0.175
J1597A	25	M	70.9	1.58	6660	60.1	4439	39.9	2.12	0.09	0.185
J2897A	26	F	84.3	1.57	6581	59.8	4444	40.2	2.71	0.087	0.205
D0396A	26	M	67.4	1.97	6736	63.9	3955	36.1	2.98	0.099	0.218
N0996A	27	M	76.4	2.32	6303	66.8	3174	33.2	3.05	0.083	0.19
N1496A	27	M	86.6	2.26	7254	67.7	3460	32.3	3.86	0.093	0.222
D0296A	28	M	58.3	1.13	5840	50.8	5734	49.2	1.56	0.1	0.194

Mean	76.70	1.63	6488	59.2	4574	40.8	2.55	0.09	0.21
STD	11.61	0.43	500.6	6.12	838.3	6.12	0.83	0.009	0.04
SEM	2.32	0.09	100.1	1.22	167.7	1.22	0.17	0.002	0.008

xix) Frequency domain variables between 8-9 pm (top) and 12-1 am (bottom) derived from 24 hour ambulatory ECG monitoring in adult subjects (18-30 years).

Subject Number	Age	Gender	Heart Rate (bts/min)	LF:HF Area	LF Area	LF Area (%)	HF Area	HF Area (%)	LF:HF Peak	LF cf (Hz)	HF cf (Hz)
F0397A	18	F	83.5	1.32	6133	55.3	4990	44.7	2.01	0.11	0.188
O2896A	18	F	79.5	1.56	6938	59.7	4650	40.3	2.31	0.084	0.285
N0296A	18	F	60.4	1.59	7078	59.9	4766	40.1	1.83	0.082	0.325
N2796A	18	F	83.1	1.63	6549	60.6	4314	39.4	2.37	0.091	0.232
N0596A	18	M	74.9	1.55	6530	59.5	4459	40.5	2.21	0.1	0.193
O1896A	19	F	77.4	1.51	6661	58.7	4757	41.3	2.15	0.096	0.206
N2696A	19	F	75.6	1.08	5551	49.3	5739	50.7	1.7	0.078	0.257
X17071	20	F	79.7	2.46	6942	70.5	2922	29.5	4.33	0.076	0.2
X12072	20	M									
X29051	21	F	91.3	2.21	6734	67.8	3208	32.2	3.71	0.089	0.203
X30051	21	F	88.3	2.63	7175	71.6	2878	28.4	3.81	0.095	0.199
S1196A	21	F	71.5	1.52	6218	57.8	4666	42.2	2.45	0.094	0.196
N1896A	22	F	83.1	2.02	7336	65.6	3834	34.4	2.16	0.096	0.29
N0796A	22	M	87.1	1.77	6605	62.4	4035	37.6	3.27	0.093	0.203
N1196A	23	M	64.3	1.47	6462	57.8	4641	42.2	1.66	0.084	0.243
MA1597A	24	F	101	2.01	6933	64.4	3861	35.6	3.46	0.072	0.27
O1596A	24	M	74	2.7	6991	71.2	2902	28.8	3.8	0.085	0.207
MA1297A	24	M	63.9	1.59	6250	59.6	4262	40.4	2.01	0.087	0.17
N1296A	24	M	60.1	0.814	5011	44.1	6445	55.9	1.24	0.1	1.66
MA17967A	25	M	67.8	1.34	6353	55.8	5155	44.2	2.43	0.085	0.172
J1597A	25	M	70.2	1.48	6040	57.7	4474	42.3	2.01	0.085	0.196
J2897A	26	F	73.9	1.4	6418	55.8	5085	44.2	1.67	0.086	0.321
D0396A	26	M	62.2	2	6842	65.1	3629	34.9	2.19	0.096	0.284
N0996A	27	M	60.9	1.96	6463	64.8	3506	35.2	2.82	0.09	0.17
N1496A	27	M	75.1	2.44	7397	69.4	3243	30.6	3.68	0.086	0.252
D0296A	28	M	55.5	1.08	5728	48.7	6042	51.3	1.15	0.093	0.249

Mean	74.57	1.73	6533	60.5	4338	39.5	2.50	0.09	0.29
STD	11.21	0.49	559.9	7.04	959.5	7.04	0.87	0.008	0.29
SEM	2.24	0.10	112.0	1.41	191.9	1.41	0.17	0.002	0.06

Subject Number	Age	Gender	Heart Rate (bts/min)	LF:HF Area	LF Area	LF Area (%)	HF Area	HF Are(%)	LF:HF Peak	LF cf (Hz)	HF cf (Hz)
F0397A	18	F	79.8	1.58	6829	58.7	4865	41.3	1.91	0.1	0.228
O2896A	18	F	59.2	1.05	5175	50.1	5146	49.9	1.21	0.067	0.222
N0296A	18	F	47.6	0.779	4461	38.8	7066	61.2	1.23	0.082	0.253
N2796A	18	F	71.5	1.3	6315	55.3	5105	44.7	1.3	0.1	0.272
N0596A	18	M	68.9	1.55	6471	59.4	4515	40.6	2.27	0.1	0.236
O1896A	19	F	59.8	1.34	6636	55.6	5364	44.4	1.64	0.1	0.22
N2696A	19	F	64.4	0.972	5603	48.3	5902	51.7	1.08	0.07	0.258
X17071	20	F	64.4	1.59	6537	58.7	4407	41.3	1.54	0.069	0.283
X12072	20	M									
X29051	21	F	89.5	3.59	7326	67.7	3594	32.3	3.59	0.084	0.254
X30051	21	F	74.4	0.896	4296	42.6	5443	57.4	1.35	0.065	0.313
S1196A	21	F	41.3	1.52	6374	58.8	4457	41.2	2.5	0.06	0.244
N1896A	22	F	62.8	0.951	5029	46.6	5719	53.4	0.833	0.091	0.265
N0796A	22	M	60.1	1.1	5669	49.5	5695	50.5	1.72	0.071	0.247
N1196A	23	M	75.1	1.77	6711	62.6	4041	37.4	2.58	0.086	0.229
MA1597A	24	F	62.3	0.982	5048	46.8	5639	53.2	1.25	0.07	0.276
O1596A	24	M	53.9	1.86	6790	63.2	4096	36.8	2.15	0.086	0.242
MA1297A	24	M	59.9	1.22	6006	53.7	5129	46.3	2.05	0.078	0.184
N1296A	24	M	47.6	0.904	5620	47	6311	53	1.04	0.098	0.228
MA17967A	25	M	57.4	1.93	6604	64.8	3659	35.2	2.84	0.083	0.229
J1597A	25	M	59.1	1.36	6144	55.2	5002	44.8	1.79	0.082	0.21
J2897A	26	F	57	0.897	4716	44.7	5717	55.3	1.01	0.079	0.264
D0396A	26	M	51	1.58	6586	60.1	4331	39.9	1.71	0.092	0.267
N0996A	27	M	69.9	1.88	6438	63.2	3823	36.8	2.99	0.087	0.208
N1496A	27	M	85.5	2.42	7039	69.5	3101	30.5	4.59	0.087	0.213
D0296A	28	M	49.3	1.31	5967	54	4878	46	1.85	0.08	0.227

Mean	62.87	1.45	6015	54.1	4920	45.0	1.92	0.08	0.24
STD	11.92	0.60	831.3	8.10	931.1	8.10	0.89	0.01	0.03
SEM	2.38	0.12	166.3	1.62	186.2	1.62	0.18	0.002	0.006

xx) Frequency domain variables between 4-5 am (top) and 8-9 am (bottom) derived from 24 hour ambulatory ECG monitoring in middle aged subjects (31-60 years).

Subject Number	Age	Gender	Heart Rate (bts/min)	LF:HF Area	LF Area	LF Area (%)	HF Area	HF Area (%)	LF:HF Peak	LF cf (Hz)	HF cf (Hz)
J2197A	31	F	58.5	1.38	6484	57	4901	43	1.42	0.068	0.299
F2597A	31	M	55.4	1.64	5966	60.4	3830	39.6	2.4	0.072	0.193
M0697A	33	M	56.8	1.9	6474	63.8	3680	36.2	3.02	0.069	0.193
O2396A	35	F	62.8	1.06	5243	50.3	5113	49.7	1.27	0.063	0.213
A0997A	35	F	59.7	1.22	5908	53.8	5055	46.2	1.32	0.074	0.278
N1996A	37	F	55	1.96	6647	64.9	3665	35.1	3.08	0.074	0.21
M0397A	37	F	60.7	0.815	4382	43.9	5508	56.1	0.712	0.083	0.187
M2597A	37	F	60	1.07	5383	49.9	5388	51.1	1.08	0.066	0.258
F1597A	40	F	69.1	0.935	5228	46.5	6030	53.5	1.13	0.073	0.254
MA0997A	42	F	63.3	2.21	6212	67.5	2989	32.5	3.37	0.079	0.252
MA0797A	42	F	50.9	0.902	5194	46.5	5926	53.5	0.829	0.072	0.261
MA1397A	42	M	63.3	1.68	6001	61.2	3774	38.8	1.85	0.068	0.257
F1597B	44	M	69	1.82	6466	61.7	4040	38.3	2.52	0.074	0.267
F1797A	44	M	59.4	2.5	6829	69.3	2941	30.7	4.12	0.076	0.199
A2297A	45	F	70	1.56	6226	59.8	4148	40.2	2.22	0.07	0.235
D1996A	45	M	72	1.11	5235	50.9	5087	49.1	1.23	0.087	0.231
A0297A	45	M	54.3	1.87	6218	62.8	3658	37.2	2.84	0.064	0.243
A0897A	52	F	55.6	1.3	5041	53.7	4499	46.3	2.22	0.058	0.245
N2896A	56	F	58.3	2.4	6911	67.9	3290	32.1	3.07	0.064	0.327
A1597A	56	F	57	2.03	7280	65.7	3845	34.3	3.66	0.072	0.225
A0397B	57	F	55.7	1.1	4947	47.8	5607	52.2	1.97	0.067	0.226
A0397A	58	M	55.2	1.88	6339	63.2	3821	36.8	2.94	0.064	0.243
A2897A	59	F	57.6	1.91	6410	63.7	3676	36.3	2.89	0.075	0.214
A2997A	59	M	66.8	1.53	5702	58.7	4036	41.3	3.81	0.052	0.221

Mean	60.3	1.57	5946	57.9	4354	42.1	2.29	0.07	0.24
STD	5.65	0.49	726.5	7.67	919.3	7.72	1.00	0.008	0.03
SEM	1.15	0.10	148.3	1.57	187.7	1.57	0.21	0.002	0.007

Subject Number	Age	Gender	Heart Rate (bts/min)	LF:HF Area	LF Area	LF Area (%)	HF Area	HF Area (%)	LF:HF Peak	LF cf (Hz)	HF cf (Hz)
J2197A	31	F	90.1	1.75	6861	62	4188	38	3.39	0.081	0.198
F2597A	31	M	70.7	2.29	6575	68.1	3096	31.9	3.75	0.063	0.197
M0697A	33	M	54.2	1.66	6278	60.8	4048	39.2	2.9	0.068	0.216
O2396A	35	F	90.4	1.46	6490	58	4725	42	2.7	0.081	0.23
A0997A	35	F	64.7	1.3	6075	54.6	5098	45.4	1.92	0.073	0.24
N1996A	37	F	102	1.66	6063	60.5	3992	39.5	3.03	0.078	0.198
M0397A	37	F	86.6	1.49	5850	58.6	4234	41.4	2.72	0.087	0.168
M2597A	37	F	91.4	1.61	6459	60	4261	40	3.51	0.071	0.241
F1597A	40	F	69.5	1.1	5342	50.9	5067	49.1	1.2	0.077	0.249
MA0997A	42	F	68.4	2.6	7575	71.5	3008	28.5	3.62	0.074	0.22
MA0797A	42	F	68.5	2.08	6826	65.2	3752	34.8	3.85	0.073	0.265
MA1397A	42	M	66	1.5	6497	59.3	4484	40.7	2.58	0.088	0.205
F1597B	44	M	67.7	1.01	4848	46.7	5355	53.3	1.09	0.071	0.259
F1797A	44	M	64.3	2.71	6895	71.9	2661	28.1	4.51	0.072	0.221
A2297A	45	F	100	2	6466	65.2	3459	34.8	3.66	0.074	0.171
D1996A	45	M	83.1	1.76	6609	61	4262	39	2.63	0.083	0.179
A0297A	45	M	78.9	1.81	6757	63.4	3921	36.6	3.55	0.083	0.22
A0897A	52	F	75.1	1.63	6583	60.9	4223	39.1	2.78	0.063	0.24
N2896A	56	F	70.1	2.05	7700	66.3	3922	33.7	2.45	0.078	0.305
A1597A	56	F	57.7	1.26	5486	53.3	4629	46.7	1.62	0.071	0.247
A0397B	57	F	67.5	1.32	6625	54.9	5426	45.1	2.23	0.07	0.246
A0397A	58	M	78	2.08	6289	66.9	3117	33.1	4.24	0.073	0.167
A2897A	59	F	82.7	1.48	5423	57.3	4259	42.7	3.17	0.084	0.177
A2997A	59	M	76.8	2.3	6062	67.4	2941	32.6	6.16	0.054	0.21

Mean	76.0	1.75	6359	61.0	4088	39.0	3.05	0.07	0.22
STD	12.6	0.44	658.2	6.26	762.9	6.26	1.10	0.008	0.03
SEM	2.57	0.09	134.3	1.28	155.7	1.28	0.23	0.002	0.007

xxi) Frequency domain variables between 12-1 pm (top) and 4-5 pm (bottom) derived from 24 hour ambulatory ECG monitoring in middle aged subjects (31-60 years).

Subject Number	Age	Gender	Heart Rate (bts/min)	LF:HF Area	LF Area	LF Area (%)	HF Area	HF Area (%)	LF:HF Peak	LF cf (Hz)	HF cf (Hz)
J2197A	31	F	74.3	1.46	6345	57.3	4767	42.7	2.42	0.084	0.192
F2597A	31	M	70.9	2.47	6557	70.1	2807	29.9	4.44	0.08	0.178
M0697A	33	M	85.4	1.59	6468	60.5	4232	39.5	3.3	0.09	0.17
O2396A	35	F	92.1	1.33	6483	56	5127	44	2.56	0.085	0.201
A0997A	35	F	70.4	1.21	6216	53.4	5407	46.6	2.06	0.089	0.215
N1996A	37	F	77.5	1.83	7021	62.6	4213	37.4	2.65	0.078	0.223
M0397A	37	F	86.9	1.3	6005	55.3	4873	44.7	1.85	0.096	0.201
M2597A	37	F	75.5	1.63	6313	60.5	4207	39.5	1.96	0.085	0.225
F1597A	40	F	90.3	0.925	5264	45.9	6265	54.1	1.63	0.11	0.211
MA0997A	42	F	65.4	2.33	6612	68	3093	32	3.92	0.068	0.289
MA0797A	42	F	76.3	2.51	7293	70.3	3118	29.7	4.87	0.074	0.195
MA1397A	42	M	75.4	1.75	6028	62.3	3660	37.7	3.16	0.086	0.186
F1597B	44	M	85.7	1.42	5983	57.3	4597	42.7	3.21	0.084	0.202
F1797A	44	M	69.9	2.3	6623	68.3	3140	31.7	3.94	0.079	0.198
A2297A	45	F	102	1.61	6125	60.1	4069	39.9	2.59	0.085	0.153
D1996A	45	M	93.3	1.35	6170	56.2	4909	43.8	2.19	0.088	0.169
A0297A	45	M	64.3	2.12	6863	66.2	3561	33.8	3.04	0.075	0.216
A0897A	52	F	78	1.63	5871	58.3	4644	41.7	2.81	0.085	0.206
N2896A	56	F	111	0.985	4747	46.5	5250	53.5	2.03	0.1	0.257
A1597A	56	F	82	1.65	6073	60.4	4007	39.6	2.83	0.078	0.199
A0397B	57	F	78.1	1.36	6304	55.3	5074	44.7	2.38	0.074	0.231
A0397A	58	M	67.7	1.76	6196	62.3	3893	37.7	3.4	0.08	0.176
A2897A	59	F	84	1.68	6518	61	4314	39	2.8	0.083	0.17
A2997A	59	M	94.1	2.35	6286	68.6	2910	31.4	5.32	0.069	0.162

Mean	81.3	1.69	6265	60.1	4255	39.9	2.97	0.08	0.2
STD	11.7	0.45	520.1	6.56	884.4	6.56	0.96	0.009	0.03
SEM	2.39	0.09	106.2	1.34	180.5	1.34	0.20	0.002	0.006

Subject Number	Age	Gender	Heart Rate (bts/min)	LF:HF Area	LF Area	LF Area (%)	HF Area	HF Area (%)	LF:HF Peak	LF cf (Hz)	HF cf (Hz)
J2197A	31	F	75.2	1.94	6924	64	3917	36	2.67	0.079	0.285
F2597A	31	M	72.3	1.95	6778	65.6	3553	34.4	3.34	0.077	0.159
M0697A	33	M	64.6	1.54	6207	58.7	4448	41.3	2.16	0.082	0.234
O2396A	35	F	87.8	1.37	6226	56.1	4898	43.9	1.91	0.096	0.192
A0997A	35	F	78.7	1.4	5945	54.5	5070	45.5	1.98	0.075	0.238
N1996A	37	F	90.6	2.05	6424	66.3	3286	33.7	3.87	0.077	0.173
M0397A	37	F	86.4	1.18	5859	53.2	5180	46.8	1.92	0.099	0.171
M2597A	37	F	83.7	1.63	6785	60.6	4411	39.4	2.43	0.079	0.267
F1597A	40	F	91.1	1.13	5660	50.8	5440	49.2	1.78	0.086	0.251
MA0997A	42	F	86	1.8	6479	63	3851	37	4.31	0.081	0.248
MA0797A	42	F	73.7	2.38	6910	68	3353	32	4.15	0.079	0.24
MA1397A	42	M	77.4	1.77	6676	62.5	3992	37.5	3.38	0.087	0.159
F1597B	44	M	80	1.62	6965	60.4	4642	39.6	3.02	0.082	0.171
F1797A	44	M	68.7	2.29	6948	68.4	3255	31.6	4.72	0.076	0.192
A2297A	45	F	90	1.59	6504	60.6	4200	39.4	2.87	0.088	0.164
D1996A	45	M	98	1.62	5845	58.8	4189	41.2	2.95	0.085	0.208
A0297A	45	M	84.3	2.41	6725	69.7	2887	30.3	4.67	0.08	0.257
A0897A	52	F	76.4	1.31	5318	54.2	4722	45.8	2.07	0.083	0.216
N2896A	56	F	93.1	1.85	6838	61.4	4279	38.6	1.89	0.079	0.311
A1597A	56	F	96.3	1.27	5399	54.4	4501	45.6	2.43	0.075	0.214
A0397B	57	F	77.5	1.49	6450	58.7	4495	41.3	2.99	0.076	0.167
A0397A	58	M	69	1.88	6455	63.8	3718	36.2	3.6	0.076	0.195
A2897A	59	F	84.6	1.96	6509	64.5	3584	35.5	3.03	0.081	0.174
A2997A	59	M	74.9	1.72	5887	61.3	3626	38.7	4.12	0.059	0.236

Mean	81.7	1.71	6363	60.8	4145	39.2	3.01	0.08	0.21
STD	9.05	0.36	496.9	5.11	666.1	5.11	0.93	0.008	0.04
SEM	1.85	0.07	101.4	1.04	136.0	1.04	0.19	0.002	0.009

xxii) Frequency domain variables between 8-9 pm (top) and 12-1 am (bottom) derived from 24 hour ambulatory ECG monitoring in middle aged subjects (31-60 years).

Subject Number	Age	Gender	Heart Rate (bts/min)	LF:HF Area	LF Area	LF Area (%)	HF Area	HF Area (%)	LF:HF Peak	LF cf (%)	HF cf (%)
J2197A	31	F	82.5	1.72	6776	61.8	4152	38.2	3.09	0.079	0.183
F2597A	31	M	65.4	1.7	6703	61.7	4153	38.3	2.46	0.093	0.173
M0697A	33	M	69.7	1.93	6710	64.7	3747	35.3	2.95	0.094	0.242
O2396A	35	F	84.8	1.36	6526	56.3	5124	43.7	1.94	0.072	0.236
A0997A	35	F	74.4	1.08	5839	50.4	5825	49.6	1.61	0.088	0.23
N1996A	37	F	90.1	1.5	6001	59	4143	41	2.55	0.092	0.153
M0397A	37	F	72.1	1.09	5938	51.1	5651	48.9	1.4	0.089	0.209
M2597A	37	F	69.3	1.34	5751	54	4832	46	1.66	0.082	0.252
F1597A	40	F	88.9	1.29	5446	52.1	5195	47.9	1.49	0.092	0.295
MA0997A	42	F	73.1	2.67	7124	70.9	2912	29.1	5.16	0.062	0.308
MA0797A	42	F	72.3	1.96	7027	64.4	3977	35.6	3.11	0.076	0.296
MA1397A	42	M	78.7	1.87	6264	64.3	3486	35.7	2.97	0.089	0.162
F1597B	44	M	92.2	1.65	6290	59.6	4336	40.4	2.7	0.084	0.199
F1797A	44	M	64.1	2.31	6706	68.3	3120	31.7	3.69	0.073	0.219
A2297A	45	F	86.6	1.42	5991	57.8	4322	42.2	2.22	0.088	0.171
D1996A	45	M	89.5	1.81	6183	63	3684	37	3.02	0.086	0.173
A0297A	45	M	65.4	2.47	6858	69	3090	31	3.79	0.079	0.219
A0897A	52	F	67.3	1.58	6398	59.8	4328	40.2	3	0.066	0.195
N2896A	56	F	72.7	1.86	6672	62.2	3969	37.8	2.7	0.07	0.277
A1597A	56	F	88.1	1.5	5895	57.4	4429	42.6	2.43	0.077	0.214
A0397B	57	F	67.3	1.01	5644	47.9	6157	52.1	2	0.096	0.192
A0397A	58	M	70.1	1.91	6730	64.1	3865	35.9	2.27	0.086	0.246
A2897A	59	F	68.7	2.56	6864	70.8	2839	29.2	3.88	0.076	0.169
A2997A	59	M	79.6	2.22	6412	66.2	3413	33.8	6.19	0.06	0.252
Mean			76.4	1.74	6364	60.7	4197	39.3	2.85	0.08	0.22
STD			9.20	0.46	467.4	6.43	900.6	6.43	1.12	0.01	0.05
SEM			1.88	0.09	95.40	1.31	183.8	1.31	0.23	0.002	0.009

Subject Number	Age	Gender	Heart Rate (bts/min)	LF:HF Area	LF Area	LF Area (%)	HF Area	HF Area (%)	LF:HF Peak	LF cf (Hz)	HF cf (Hz)
J2197A	31	F	61.6	1.75	7062	62.5	4179	37.5	1.73	0.071	0.265
F2597A	31	M	52.2	1.42	5605	56.3	4272	43.7	1.96	0.071	0.209
M0697A	33	M	62.3	1.54	6368	58.9	4536	41.1	2.42	0.077	0.239
O2396A	35	F	76.8	1.4	5075	55	4154	45	2.07	0.069	0.219
A0997A	35	F	78.8	1.11	5860	50.8	5765	49.2	1.73	0.097	0.192
N1996A	37	F	58.1	1.82	6642	62.6	4048	37.4	2.8	0.087	0.224
M0397A	37	F	64.5	0.746	4116	41.5	5789	58.5	0.633	0.076	0.195
M2597A	37	F	66.1	1.41	5847	56.2	4594	43.8	2.02	0.061	0.256
F1597A	40	F	91.6	1.79	5131	46.9	5947	53.1	1.79	0.087	0.298
MA0997A	42	F	65.6	2.37	6859	68.5	3200	31.5	3.41	0.08	0.272
MA0797A	42	F	71.7	1.16	4978	48.5	5358	51.5	1.55	0.064	0.296
MA1397A	42	M	65.2	2.02	6152	63.3	3608	36.7	2.34	0.072	0.252
F1597B	44	M	81.8	1.84	6926	63.5	4033	36.5	1.94	0.076	0.328
F1797A	44	M	59	1.65	6274	59.6	4141	40.4	1.96	0.078	0.243
A2297A	45	F	79.1	1.8	6543	62.2	3959	37.8	2.76	0.076	0.239
D1996A	45	M	70.2	1.69	6240	58.8	4400	41.2	2.72	0.08	0.24
A0297A	45	M	53.5	1.65	6098	59.1	4289	40.9	2.41	0.068	0.261
A0897A	52	F	56.7	0.954	4705	46.7	5096	53.3	1.01	0.064	0.234
N2896A	56	F	62.3	2.69	7153	70.8	2944	29.2	3.62	0.067	0.33
A1597A	56	F	62.1	1.45	6605	57.8	4773	42.2	2.16	0.09	0.217
A0397B	57	F	56.9	0.987	5043	47.3	5635	52.7	1.42	0.069	0.242
A0397A	58	M	56.8	1.81	6522	62.8	4008	37.2	2.38	0.075	0.238
A2897A	59	F	74.2	1.52	6323	58.1	4745	41.9	2.35	0.079	0.244
A2997A	59	M	77	1.37	5592	55.7	4449	44.3	3.03	0.057	0.243
Mean			66.8	1.58	5988	57.2	4496	42.8	2.18	0.07	0.25
STD			10.1	0.44	806.6	7.24	785.9	7.24	0.69	0.009	0.04
SEM			2.07	0.09	164.6	1.48	160.4	1.48	0.14	0.002	0.007

xxiii) Frequency domain variables between 4-5 am (top) and 8-9 am (bottom) derived from 24 hour ambulatory ECG monitoring in elderly subjects (61+ years).

Subject Number	Age	Gender	Heart Rate (bts/min)	LF:HF Area	LF Area	LF Area (%)	HF Area	HF Area (%)	LF:HF Peak	LF cf (Hz)	HF cf (Hz)
D1696A	61	F	55.4	2.3	5988	64.8	3366	35.2	4.83	0.061	0.2
A0297B	62	F	62	1.97	6204	64.5	3368	35.5	1.84	0.098	0.152
MA0897A	64	F	57.7	1.3	5146	53.9	4404	46.1	2.08	0.065	0.226
J2397A	64	F	60.3	1.94	6507	64.7	3530	35.3	2.69	0.069	0.265
N2296A	64	M	68.2	1.41	5358	54.1	4552	45.9	2.36	0.059	0.214
J3097A	65	F	58.2	1.17	5933	52.7	5369	47.3	2.2	0.069	0.27
A2197A	65	F	70.5	1.43	5650	56.5	4542	43.5	2.46	0.064	0.222
D0696A	65	M	66.7	1.61	5837	59.9	3893	40.1	3.21	0.07	0.212
J1097A	66	F	62.2	1.05	4711	46.6	5512	53.4	1.79	0.059	0.188
A1097A	66	F	67.6	0.932	5495	46.6	6623	53.4	1.84	0.083	0.22
MA0697A	66	M									
F1897A	67	F	67.7	0.991	4504	44.4	5934	55.6	1.87	0.068	0.23
A1497A	67	F	60.3	1.09	5061	50.2	5132	49.8	2.16	0.062	0.197
D1096B	68	F	51.1	1.13	5635	45.9	6575	54.1	1.65	0.095	0.286
F1397A	68	F	65.6	0.722	4042	40.2	6424	59.8	0.9	0.081	0.288
MA0597B	68	F	65.7	1.92	6010	59	4570	41	4.01	0.07	0.256
A2197A	68	M	46.8	2.48	6631	69.3	3011	30.7	4.73	0.064	0.19
J2297A	69	F	71.1	1.83	6032	63.2	3549	36.8	3.97	0.056	0.319
D1096A	69	M	52.3	2.64	6852	71.4	2835	28.6	4.17	0.06	0.285
F1197A	69	M	76.9	1.93	6309	65	3411	35	5.14	0.068	0.166
M1797A	70	F	61.7	1.68	6206	61.7	3841	38.3	3.23	0.069	0.179
MA0597A	70	M	65.6	1.26	6224	54.8	5184	45.2	2.91	0.07	0.232
D1296A	71	F	60.6	0.535	4572	34.7	8563	65.3	0.766	0.1	0.255
F2597A	71	M	52.3	1.28	5704	53.9	4975	46.1	2.4	0.064	0.214
F0497A	72	F	73.4	1.69	5801	60.1	3916	39.9	3.05	0.07	0.208
D1896A	74	M									
D2096A	74	M	54.8	0.976	5237	47.3	5736	52.7	1.49	0.071	0.231
F0797A	74	M	62.9	2.35	5663	65.8	3101	34.2	7.07	0.054	0.213
J2097A	77	F	66.8	0.612	3933	36.2	6899	63.8	0.774	0.064	0.234
J3197A	78	F	81.2	0.961	5645	46.9	6469	53.1	1.73	0.062	0.308

Mean	63.1	1.47	5603	54.8	4831	45.2	2.76	0.07	0.23
STD	7.95	0.57	739	9.86	1436	9.86	1.47	0.01	0.04
SEM	1.50	0.11	139	1.86	271	1.86	0.28	0.002	0.008

Subject Number	Age	Gender	Heart Rate (bts/min)	LF:HF Area	LF Area	LF Area (%)	HF Area	HF Area (%)	LF:HF Peak	LF cf (Hz)	HF cf (Hz)
D1696A	61	F	85.7	1.52	6739	59.8	4488	40.2	3.19	0.074	0.215
A0297B	62	F	72.4	1.7	6171	60.9	4047	39.1	3.71	0.068	0.198
MA0897A	64	F	73.1	1.75	6281	62.3	3862	37.7	3.88	0.076	0.189
J2397A	64	F	82	1.42	5646	55.5	4638	44.5	2.49	0.082	0.258
N2296A	64	M	83.2	2.25	6521	66.2	3267	33.8	4.94	0.062	0.225
J3097A	65	F	71.8	1.8	7275	62.7	4325	37.3	3.63	0.067	0.288
A2197A	65	F	76.4	1.86	6314	63.4	3724	36.6	3.41	0.07	0.18
D0696A	65	M	73.1	1.93	6346	64.4	3530	35.6	3.54	0.069	0.199
J1097A	66	F	69.8	1.35	5395	53.5	5186	46.5	2.85	0.052	0.25
A1097A	66	F	65.4	0.995	5962	49.2	6144	50.8	1.93	0.082	0.209
MA0697A	66	M									
F1897A	67	F	89.9	1.16	5406	50.2	5297	49.8	2.45	0.073	0.198
A1497A	67	F	88.4	1.02	5158	48.9	5315	51.1	2.29	0.082	0.235
D1096B	68	F	68.6	1.38	6240	55.1	5252	44.9	2.71	0.071	0.233
F1397A	68	F	71.6	0.901	5282	42.8	7218	57.2	1.1	0.1	0.296
MA0597B	68	F	75.7	1.35	6492	55.4	5519	44.6	2.79	0.072	0.253
A2197A	68	M	60.6	1.92	6187	63.9	3688	36.1	4.75	0.071	0.168
J2297A	69	F	95	1.54	5746	56.1	4676	43.9	2.65	0.059	0.36
D1096A	69	M	62.4	2.13	6700	65.6	3743	34.4	5.53	0.073	0.228
F1197A	69	M	94.7	1.75	6018	60.9	3865	39.1	3.64	0.07	0.224
M1797A	70	F	72.6	1.56	6415	59.4	4476	40.6	3.34	0.069	0.188
MA0597A	70	M	61.7	1.75	6357	62.8	3843	37.2	4.22	0.068	0.183
D1296A	71	F	64.8	0.767	4992	41.5	7012	58.5	1.14	0.081	0.257
F2597A	71	M	63.3	1.5	6120	58.9	4396	41.1	3.5	0.065	0.202
F0497A	72	F	84.6	1.35	5868	55.4	4787	44.6	2.49	0.072	0.242
D1896A	74	M									
D2096A	74	M	65.5	1.37	6655	56.3	5174	43.7	2.54	0.066	0.262
F0797A	74	M	72	1.63	6778	59.4	4730	40.6	3.02	0.064	0.239
J2097A	77	F	78.5	1.89	6403	61.4	4073	38.6	4.02	0.06	0.24
J3197A	78	F	95.4	0.962	4391	44.1	5506	55.9	1.71	0.068	0.386

Mean	75.7	1.51	6066	57	4706	43	3.12	0.07	0.24
STD	10.57	0.38	630.7	6.87	985	6.3	1.05	0.009	0.05
SEM	1.99	0.07	119.1	1.29	186	1.30	0.20	0.002	0.01

xxiv) Frequency domain variables between 12-1 pm (top) and 4-5 pm (bottom) derived from 24 hour ambulatory ECG monitoring in elderly subjects (61+ years).

Subject Number	Age	Gender	Heart Rate (bts/min)	LF:HF Area	LF Area	LF Area (%)	HF Area	HF Area (%)	LF:HF Peak	LF cf (Hz)	HF cf (Hz)
D1696A	61	F	95.8	2.24	6976	67.4	3446	32.6	5.04	0.069	0.21
A0297B	62	F	86.2	2.02	6470	65.6	3402	34.4	4.67	0.073	0.18
MA0897A	64	F	75.4	1.34	6171	56.1	4856	43.9	2.21	0.085	0.202
J2397A	64	F	66.2	1.48	6145	58.5	4446	41.5	2.47	0.076	0.217
N2296A	64	M	82.3	1.21	5789	53.7	4901	46.3	2.2	0.074	0.222
J3097A	65	F	85.9	0.937	4978	47.2	5690	52.8	1.58	0.084	0.296
A2197A	65	F	76.3	1.66	6224	58.9	4497	41.1	2.9	0.077	0.264
D0696A	65	M	78.2	1.51	5902	58.5	4210	41.5	2.64	0.087	0.24
J1097A	66	F	82.6	1.34	5733	54.3	5167	45.7	2.88	0.06	0.206
A1097A	66	F	77.1	0.828	5057	41.4	7065	58.6	1.31	0.052	0.343
MA0697A	66	M									
F1897A	67	F	83.8	1.18	5806	52.1	5334	47.9	2.12	0.078	0.229
A1497A	67	F	92	0.84	5197	44.7	6379	55.3	1.68	0.089	0.205
D1096B	68	F	63.7	1.23	6461	52.7	6120	47.3	2.44	0.084	0.246
F1397A	68	F	71	0.681	4270	38.3	6865	61.7	0.996	0.094	0.3
MA0597B	68	F	80	1.78	6699	58.5	4956	41.5	2.72	0.072	0.324
A2197A	68	M	69.6	1.99	6801	65.8	3503	34.2	4.82	0.073	0.171
J2297A	69	F	90.8	1.28	5430	52.2	5150	47.8	2.42	0.085	0.329
D1096A	69	M	68.6	1.42	6337	57.1	4871	42.9	3.03	0.08	0.197
F1197A	69	M	90.8	1.91	6086	63.2	3515	36.8	4.69	0.077	0.222
M1797A	70	F	75	1.42	6020	56.4	4919	43.6	3.31	0.072	0.245
MA0597A	70	M	68.5	1.92	6210	64.6	3460	35.4	7	0.069	0.171
D1296A	71	F	72.7	0.62	4619	37.3	7588	62.7	1.13	0.075	0.279
F2597A	71	M	62.5	1.15	5466	49.9	5566	50.1	2.36	0.078	0.202
F0497A	72	F	78.6	1.78	5217	59.7	3632	40.3	3.34	0.071	0.222
D1896A	74	M									
D2096A	74	M	66	1.23	6270	53.2	5444	46.8	1.91	0.077	0.251
F0797A	74	M	80.7	1.82	6977	62.3	4234	37.7	3.33	0.072	0.301
J2097A	77	F	98.4	1.47	6078	57.2	4664	42.8	3	0.077	0.234
J3197A	78	F	117	0.746	3094	35.6	5228	64.4	0.844	0.096	0.389

Mean	79.8	1.39	5802	54.4	4968	45.6	2.82	0.08	0.25
STD	12.1	0.44	861	8.70	1128	8.70	1.39	0.01	0.06
SEM	2.29	0.08	162	1.64	213	1.64	0.26	0.002	0.01

Subject Number	Age	Gender	Heart Rate (bts/min)	LF:HF Area	LF Area	LF Area (%)	HF Area	HF Area (%)	LF:HF Peak	LF cf (Hz)	HF cf (Hz)
D1696A	61	F	76.7	1.77	6537	60.9	4305	39.1	3.54	0.071	0.225
A0297B	62	F	84.6	1.79	6267	62.5	3773	37.5	3.86	0.073	0.188
MA0897A	64	F	83.4	2.15	6795	66.2	3606	33.8	3.95	0.063	0.192
J2397A	64	F	84.9	1.12	5569	50	5494	50	1.69	0.079	0.286
N2296A	64	M	73.3	1.3	5898	55.3	4705	44.7	1.95	0.08	0.2
J3097A	65	F	64.3	1.27	6137	53.9	5261	46.1	2.36	0.065	0.258
A2197A	65	F	80	1.28	6082	53.6	5565	46.4	2.3	0.087	0.204
D0696A	65	M	71.3	1.94	6558	63.2	3916	36.8	3.26	0.077	0.218
J1097A	66	F	82.1	1.12	5756	50.3	5852	49.7	2.21	0.078	0.226
A1097A	66	F	78.1	0.663	4072	37.4	7092	62.6	0.935	0.1	0.34
MA0697A	66	M									
F1897A	67	F	84.4	1.05	5087	45.2	6357	54.8	1.52	0.092	0.33
A1497A	67	F	65	0.991	5726	48.5	6050	51.5	1.45	0.072	0.256
D1096B	68	F	60.4	0.636	4459	36.3	7736	63.7	0.831	0.11	0.275
F1397A	68	F	72	0.749	4138	37.5	7386	62.5	1.04	0.082	0.334
MA0597B	68	F	76.2	1.4	6231	55.1	5188	44.9	2.7	0.072	0.292
A2197A	68	M	75.5	1.61	6255	57.9	4539	42.1	3.61	0.078	0.193
J2297A	69	F	87.6	1.81	6604	61	4289	39	2.9	0.077	0.316
D1096A	69	M	67.2	1.64	6419	60.5	4239	39.5	3.68	0.075	0.173
F1197A	69	M	85.8	1.5	6085	58.3	4375	41.7	3.19	0.073	0.223
M1797A	70	F	74.1	1.19	5673	51.5	5398	48.5	2.31	0.073	0.254
MA0597A	70	M	73	1.93	6397	64.4	3611	35.6	5.07	0.07	0.18
D1296A	71	F	72	0.706	4705	39.4	7374	60.6	0.989	0.1	0.313
F2597A	71	M	58.7	1.13	6070	51.5	5838	48.5	2.48	0.067	0.236
F0497A	72	F	75.6	1.33	5221	53.4	4677	46.6	2.86	0.068	0.211
D1896A	74	M									
D2096A	74	M	64	1.17	6021	51.4	5614	48.6	1.48	0.091	0.285
F0797A	74	M	85.3	1.86	7201	62.7	4222	37.3	2.93	0.075	0.292
J2097A	77	F	90	1.74	6655	61.8	4128	38.2	3.91	0.068	0.261
J3197A	78	F	112	0.851	3156	39.3	4989	60.7	0.906	0.092	0.396

Mean	77.1	1.35	5777	53.2	5199	46.8	2.50	0.08	0.26
STD	10.9	0.43	939	8.93	1185	8.93	1.13	0.01	0.06
SEM	2.06	0.08	177	1.68	224	1.68	0.21	0.002	0.01

xxv) Frequency domain variables between 8-9 pm (top) and 12-1 am (bottom) derived from 24 hour ambulatory ECG monitoring in elderly subjects (61+ years).

Subject Number	Age	Gender	Heart Rate (bts/min)	LF:HF Area	LF Area	LF Area (%)	HF Area	HF Area (%)	LF:HF Peak	LF cf (Hz)	HF cf (Hz)
D1696A	61	F	77.5	2.38	6697	68.6	3112	31.4	4.61	0.074	0.216
A0297B	62	F	88.7	1.35	6130	56	4759	44	2.5	0.072	0.228
MA0897A	64	F	75.7	1.83	6525	62.9	3918	37.1	3.3	0.072	0.196
J2397A	64	F	62.2	1.54	6239	59.2	4429	40.8	2.29	0.084	0.218
N2296A	64	M	92.7	1.53	6459	57.8	4673	42.2	2.9	0.068	0.228
J3097A	65	F	72.1	1.46	6093	54.7	5089	45.3	2.6	0.074	0.287
A2197A	65	F	81.1	1.48	6269	58.2	4574	41.8	2.94	0.079	0.227
D0696A	65	M	80	1.88	7009	62.1	4517	37.9	2.78	0.083	0.253
J1097A	66	F	84.8	1.38	5456	54.7	4710	45.3	2.47	0.069	0.238
A1097A	66	F	74.1	0.716	4915	40.7	7286	59.3	1.41	0.095	0.283
MA0697A	66	M									
F1897A	67	F	81.6	0.908	5084	45.7	5982	54.3	1.59	0.088	0.254
A1497A	67	F	59.2	0.939	5536	47.4	6005	52.6	1.13	0.068	0.252
D1096B	68	F	75.6	1.34	5705	53.1	5142	46.9	2.39	0.077	0.254
F1397A	68	F	66.1	0.993	4341	44.8	5349	55.2	2.02	0.083	0.286
MA0597B	68	F	75.9	1.52	6489	57.5	4934	42.5	2.88	0.078	0.281
A2197A	68	M	59.7	1.9	6509	64.4	3573	35.6	3.85	0.077	0.159
J2297A	69	F	78.7	1.87	7057	63.3	4087	36.7	3.45	0.07	0.292
D1096A	69	M	61.3	2.31	6996	64.1	4156	35.9	3.7	0.072	0.262
F1197A	69	M	80.3	1.86	6513	64.2	3642	35.8	3.83	0.069	0.174
M1797A	70	F	79.6	1.32	5958	55.4	4795	44.6	2.42	0.077	0.278
MA0597A	70	M	63.9	1.89	6361	64.2	3545	35.8	5.36	0.069	0.185
D1296A	71	F	68.6	0.53	3986	33.9	8309	66.1	1.16	0.12	0.242
F2597A	71	M	61.5	1.4	6381	56.8	4846	43.2	3.09	0.071	0.206
F0497A	72	F	62.1	2.06	6343	65.6	3345	34.4	3.51	0.068	0.202
D1896A	74	M									
D2096A	74	M	61.5	0.905	5585	45.3	6624	54.7	1.61	0.078	0.176
F0797A	74	M	78.8	1.86	6513	62.4	4073	37.6	3.55	0.072	0.257
J2097A	77	F	70.6	1.03	5661	49	5945	51	1.75	0.079	0.215
J3197A	78	F	103	0.752	3914	38.4	5943	61.6	1.08	0.05	0.381

Mean	74.2	1.46	5954	55.4	4905	44.6	2.72	0.08	0.24
STD	10.3	0.43	853	9.08	1208	9.08	1.05	0.01	0.05
SEM	2.05	0.09	161	1.72	228	1.72	0.20	0.002	0.007

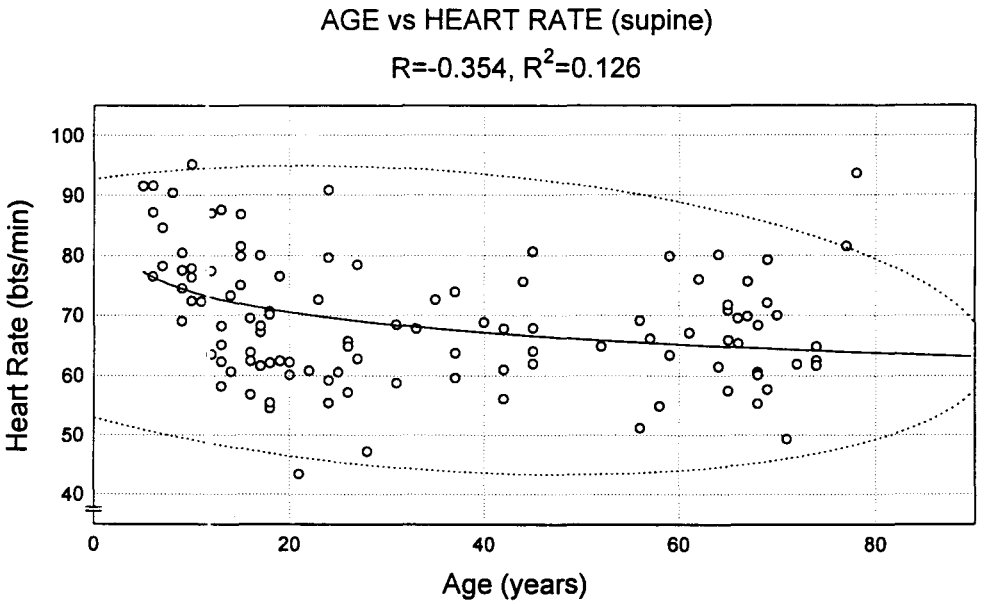
Subject Number	Age	Gender	Heart Rate (bts/min)	LF:HF Area	LF Area	LF Area (%)	HF Area	HF Area (%)	LF:HF Peak	LF cf (Hz)	HF cf (Hz)
D1696A	61	F	58.7	1.48	5456	54.3	4581	45.7	2.68	0.06	0.217
A0297B	62	F	71.6	1.02	5464	49.5	5582	50.5	1.29	0.086	0.177
MA0897A	64	F	65.4	1.13	5129	50.4	5196	49.6	1.56	0.069	0.235
J2397A	64	F	62.9	1.8	6225	62.5	3823	37.5	2.5	0.07	0.27
N2296A	64	M	75.1	1.21	5456	53.6	4705	46.4	1.66	0.07	0.209
J3097A	65	F	67.6	1.56	6416	58	4602	42	2.98	0.071	0.272
A2197A	65	F	80.5	2.92	6425	71.9	2571	28.1	6.71	0.069	0.207
D0696A	65	M	72.3	1.9	6841	62	4447	38	2.83	0.088	0.26
J1097A	66	F	71.6	0.999	4944	47.2	5672	52.8	1.98	0.043	0.205
A1097A	66	F	78.1	0.53	3370	31.3	7237	68.7	0.777	0.092	0.315
MA0697A	66	M									
F1897A	67	F	67.7	2.48	6822	68.6	3270	31.4	4.44	0.067	0.241
A1497A	67	F	57.7	1.09	5482	50.1	5551	49.9	1.98	0.067	0.207
D1096B	68	F	51.8	0.803	4443	40.7	6811	59.3	0.435	0.15	0.325
F1397A	68	F	65.7	1.43	5481	54	4793	46	2.43	0.061	0.256
MA0597B	68	F	59	2.05	6752	64.4	4027	35.6	3.93	0.071	0.224
A2197A	68	M	52.7	2.05	6534	65.6	3471	34.4	4	0.074	0.173
J2297A	69	F	70.9	2.51	6481	69.6	2954	30.4	6.08	0.061	0.273
D1096A	69	M	56.5	1.71	6622	61.1	4448	38.9	2.3	0.075	0.264
F1197A	69	M	75.3	0.996	5020	48.2	5726	51.8	2.02	0.092	0.186
M1797A	70	F	60.9	1.1	5311	50.7	5387	49.3	1.91	0.077	0.207
MA0597A	70	M	54.2	1.36	5892	56	4640	44	2.52	0.074	0.235
D1296A	71	F	56.6	0.765	4494	42.4	6430	57.6	0.993	0.067	0.242
F2597A	71	M	51.4	1.44	6234	57.3	4737	42.7	3.05	0.067	0.213
F0497A	72	F	75.1	1.35	6034	53.7	5411	46.3	3.27	0.073	0.236
D1896A	74	M									
D2096A	74	M	53	0.98	5484	48.8	5703	51.2	1.65	0.084	0.216
F0797A	74	M	60.4	1.81	6272	62.6	3699	37.4	4.57	0.062	0.206
J2097A	77	F	59.9	0.952	4900	46.8	5627	53.2	1.55	0.064	0.211
J3197A	78	F	81.7	0.904	4998	44.5	6111	55.5	1.7	0.051	0.288

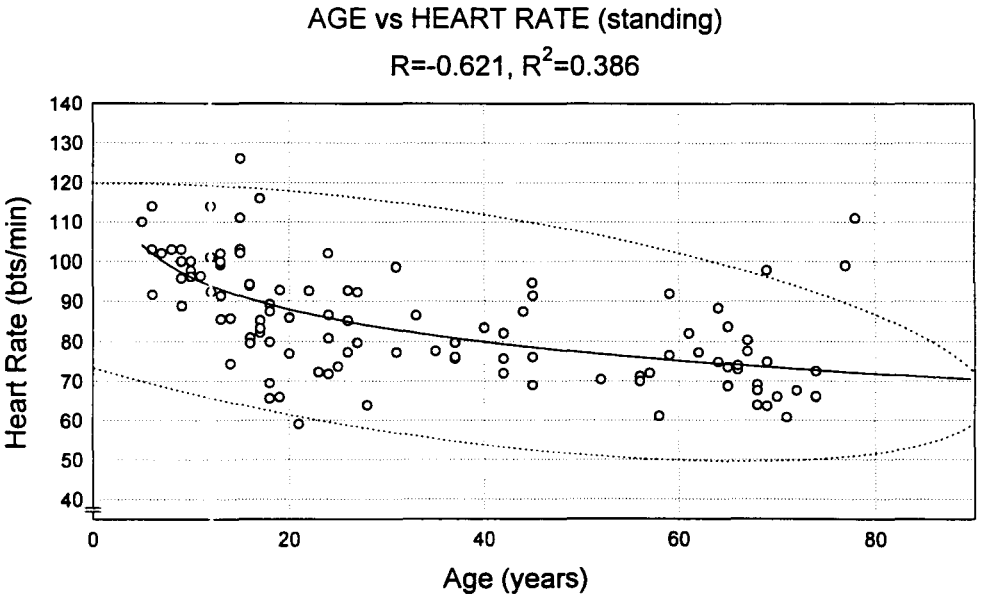
Mean	64.8	1.44	5677	54.5	4900	45.5	2.64	0.07	0.23
STD	9.23	0.58	845	9.51	1136	9.51	1.43	0.02	0.04
SEM	1.74	0.11	159	1.80	214	1.80	0.28	0.004	0.007

APPENDIX C:

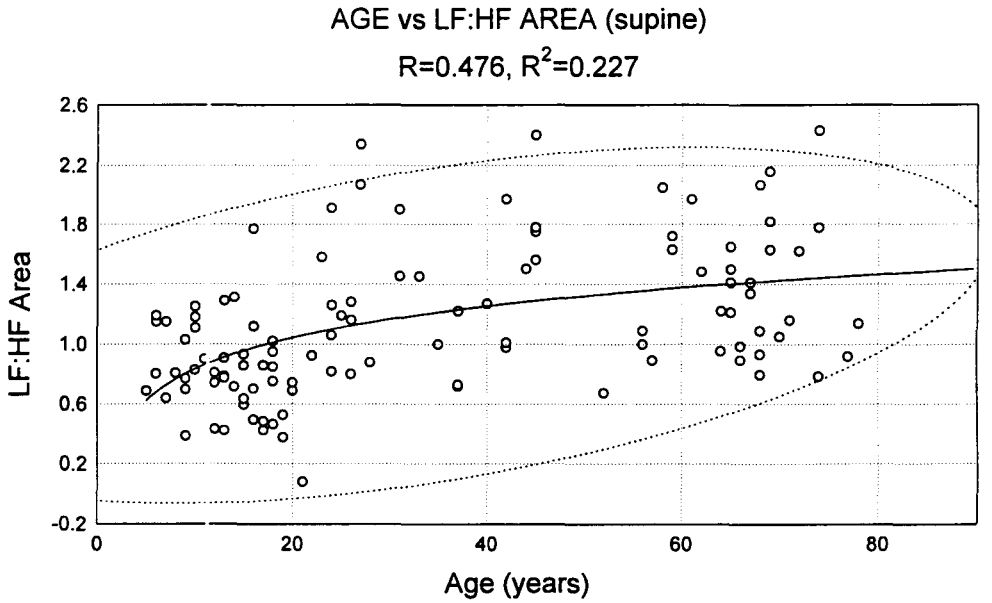
SCATTERPLOTS

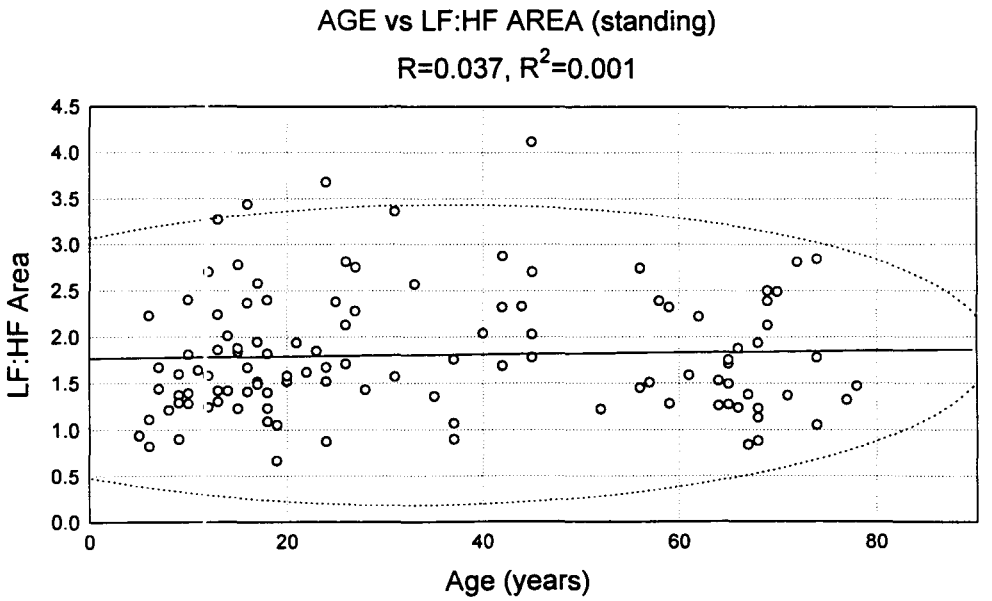
1) Scatterplots of Age vs HR



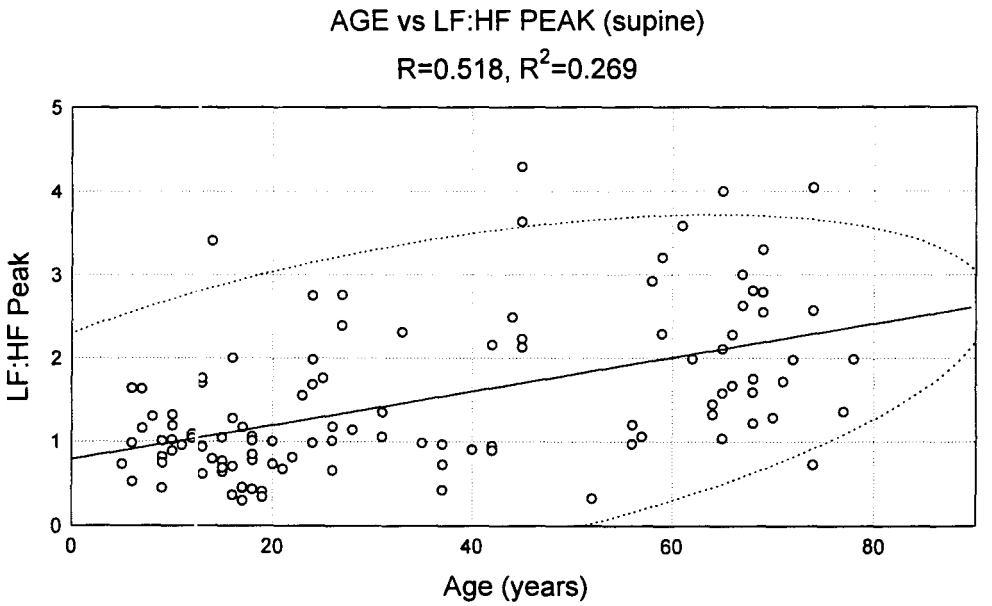


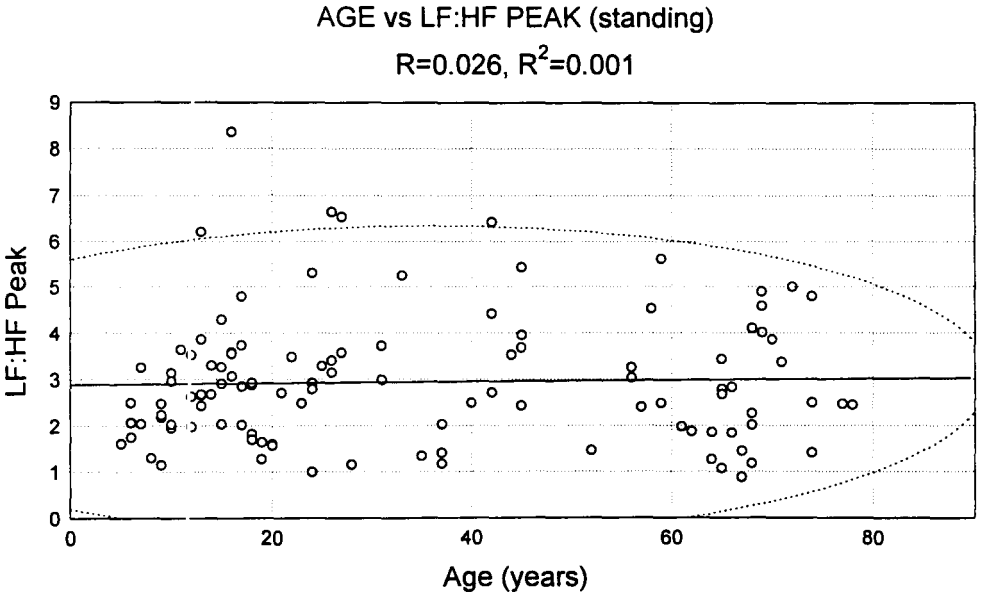
2) Scatterplots of Age vs LF:HF Area



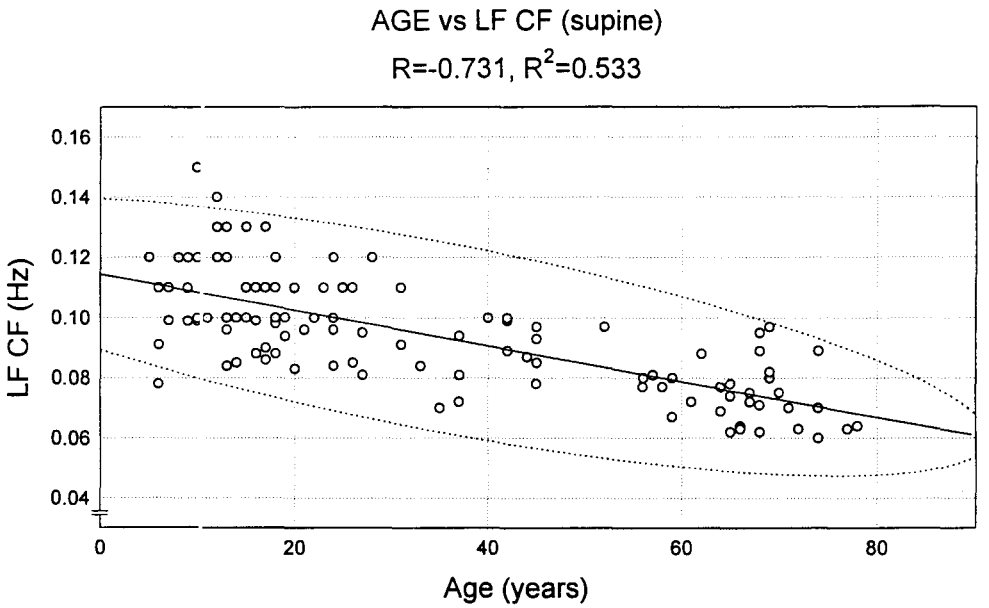


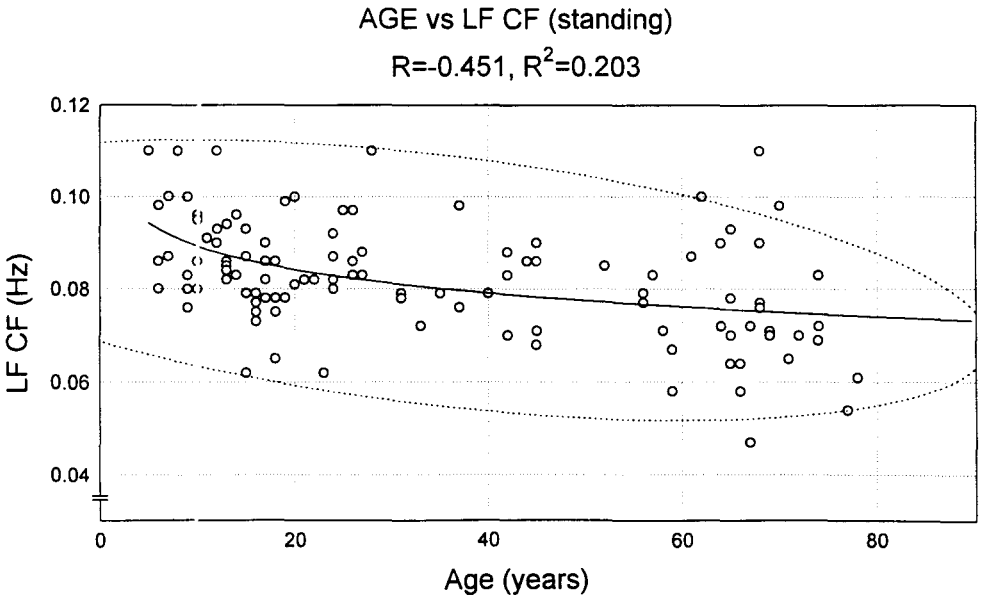
3) Scatterplots of Age vs LF:HF Peak



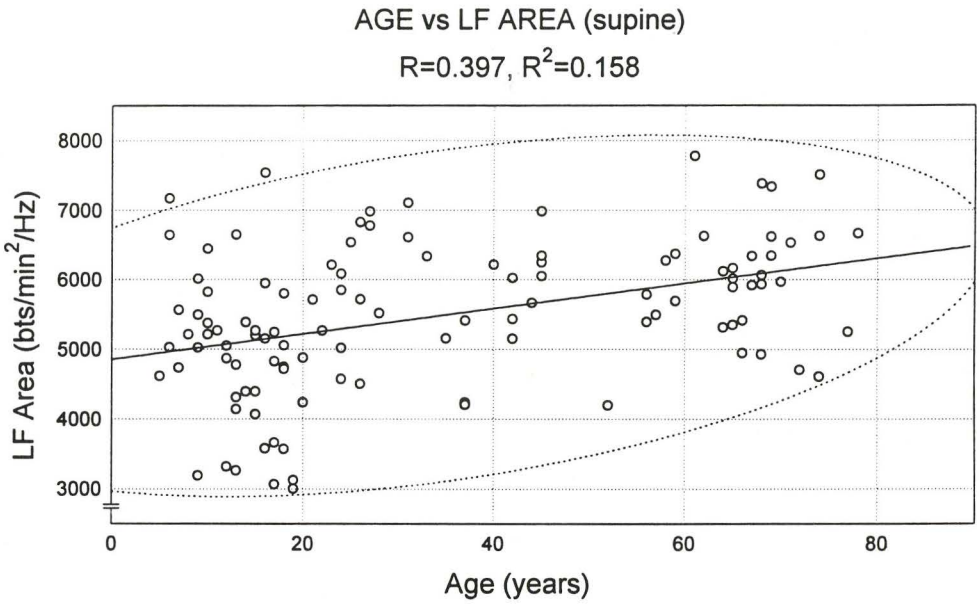


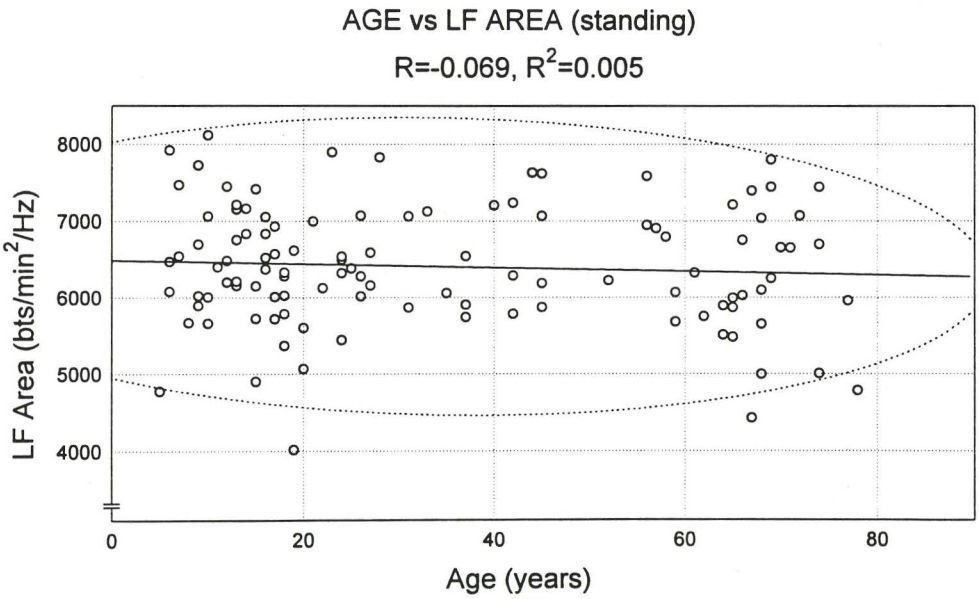
4) Scatterplots of Age vs LF CF



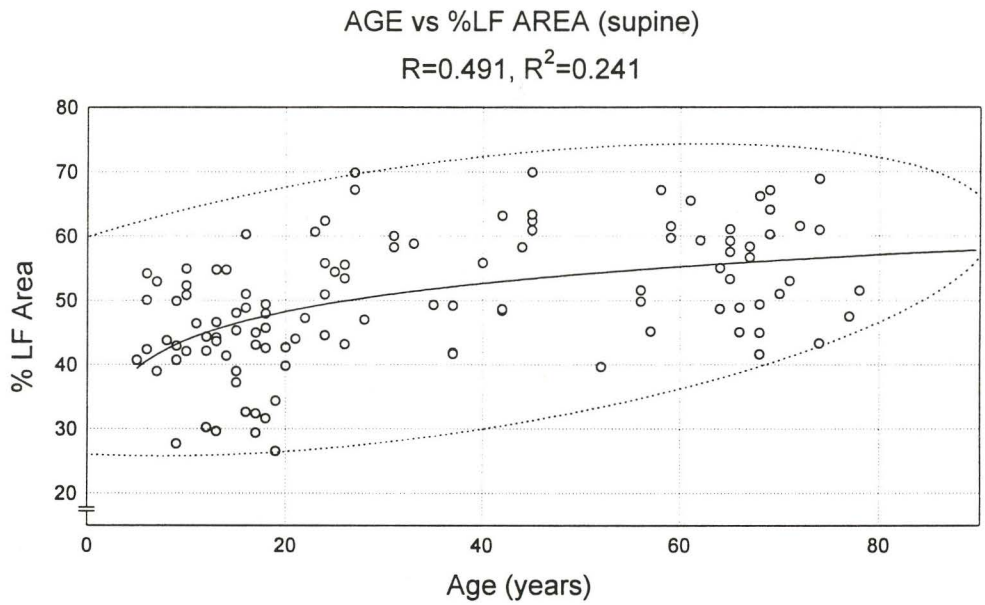


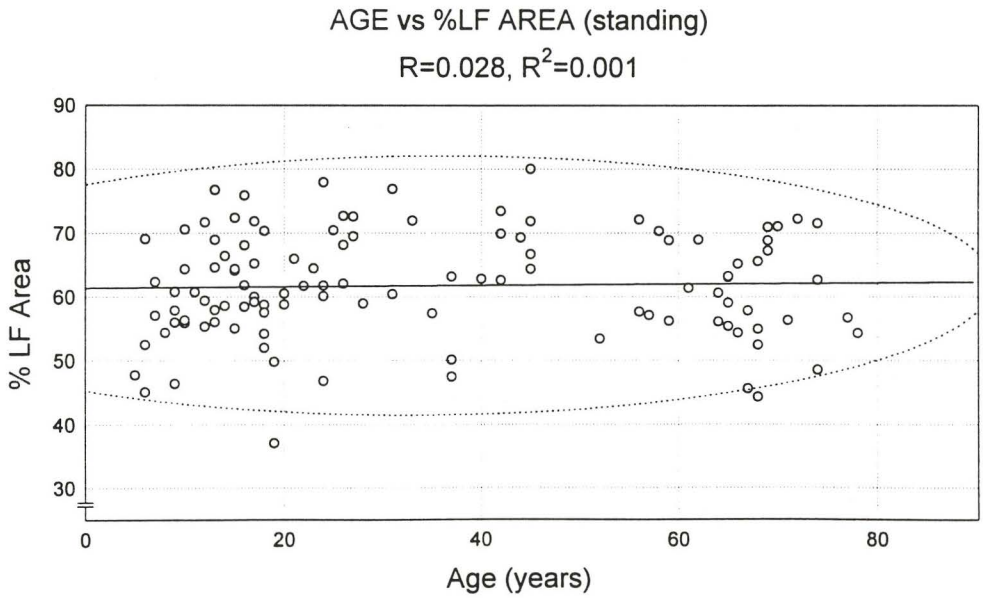
5) Scatterplots of Age vs LF Area



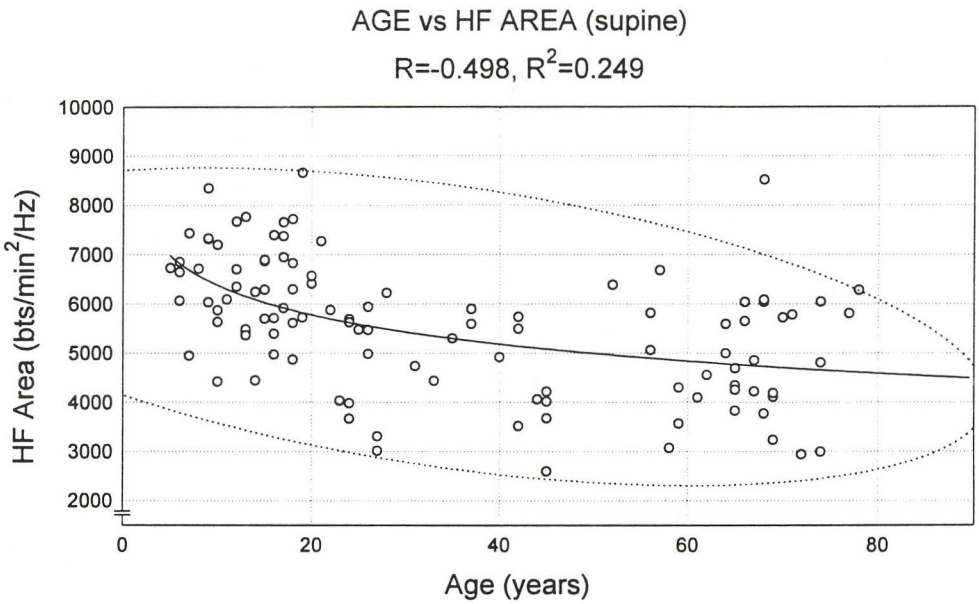


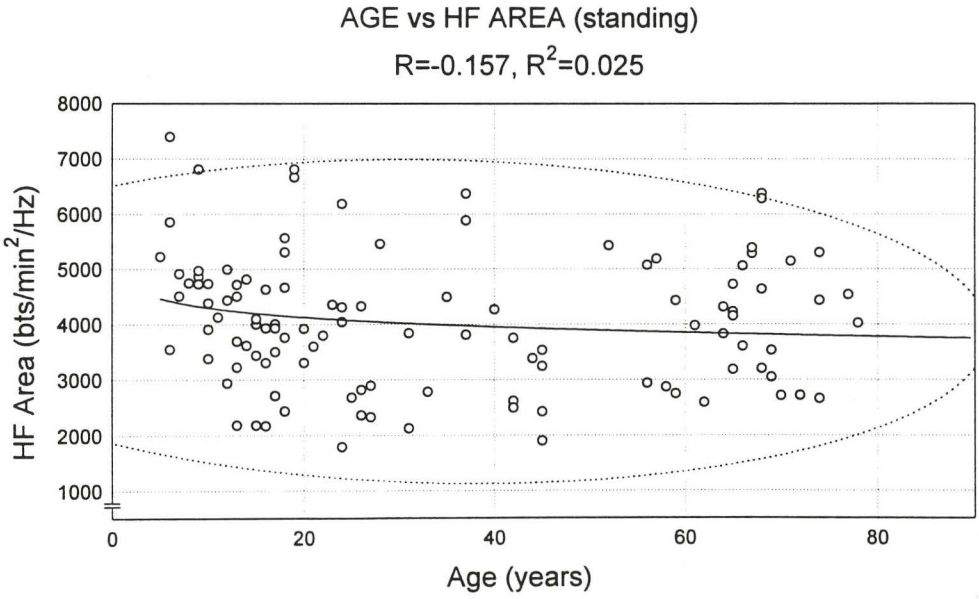
6) Scatterplots of Age vs % LF Area



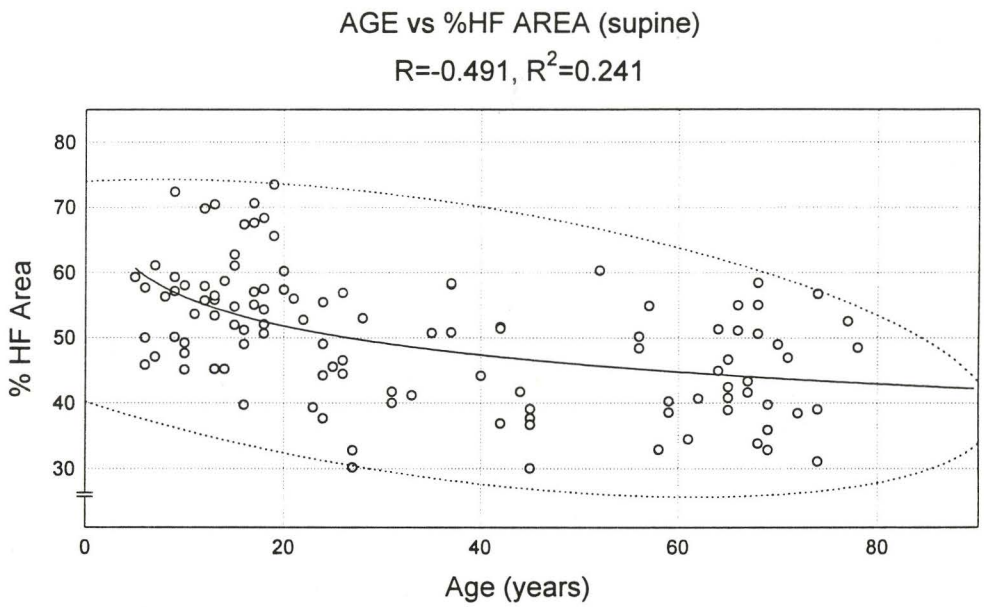


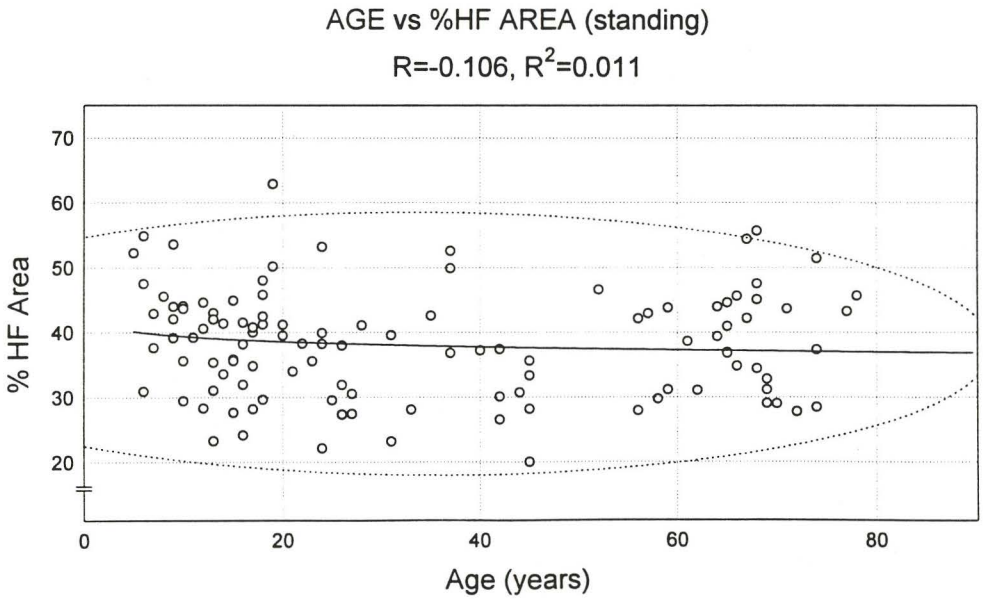
7) Scatterplots of Age vs HF Area



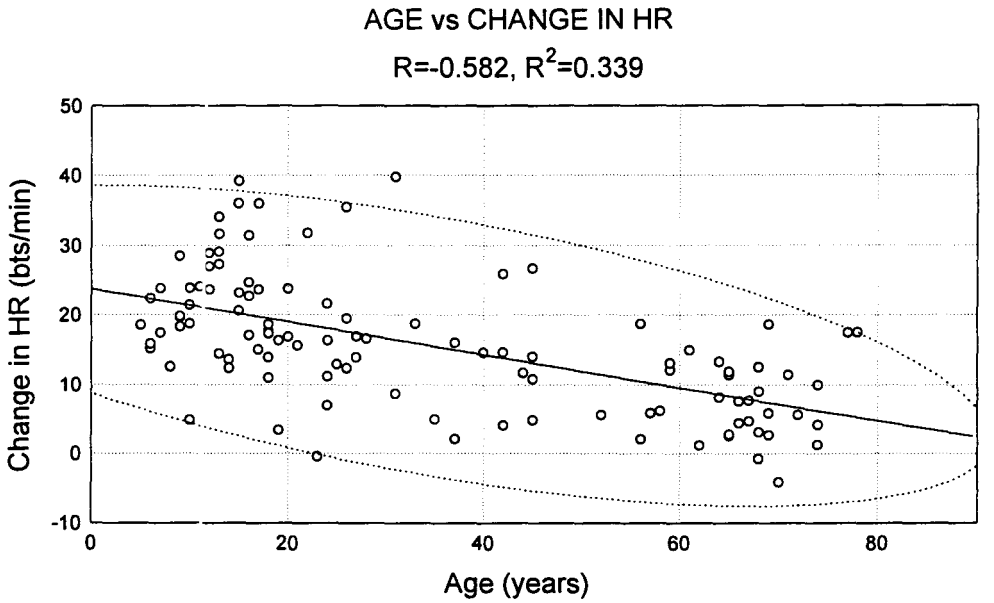


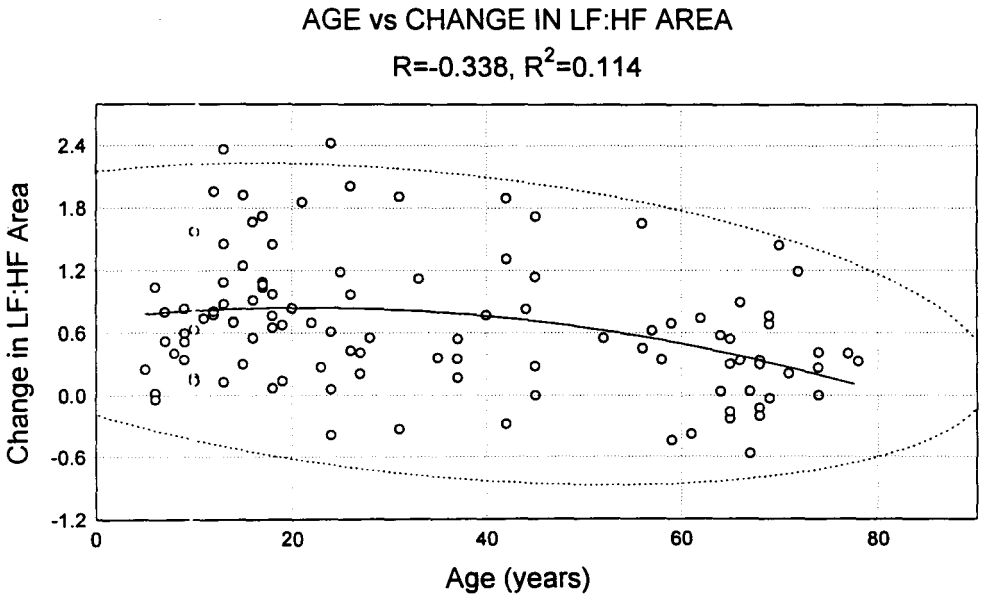
8) Scatterplots of Age vs % HF Area



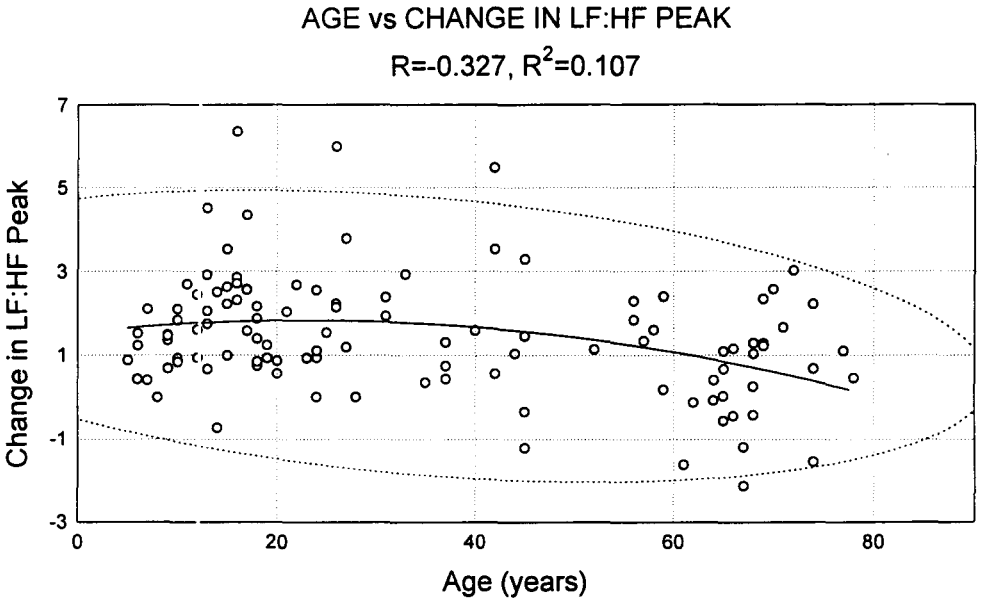


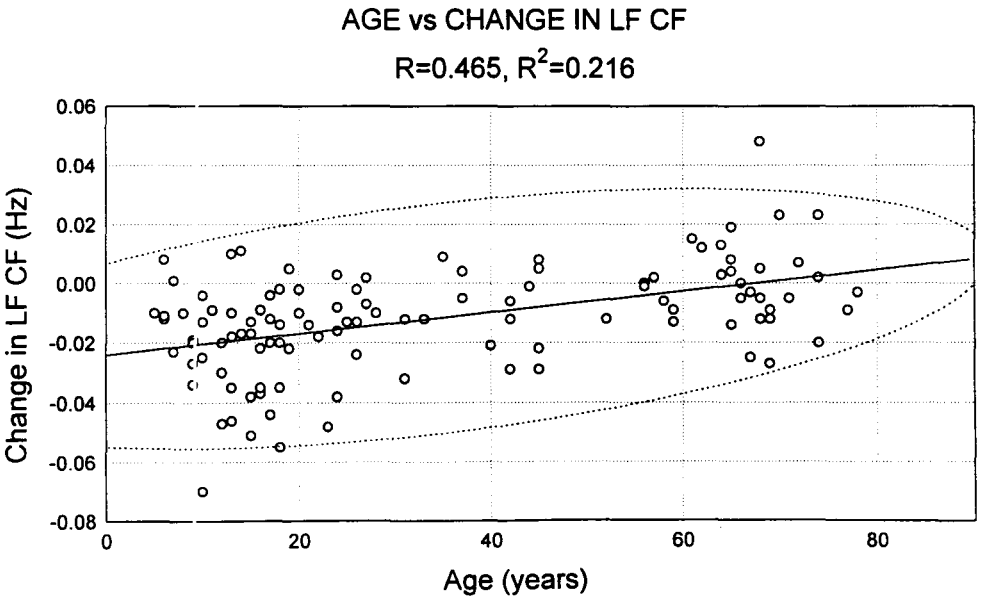
9) Scatterplots of Age vs HR & LF:HF Area Response to Orthostasis



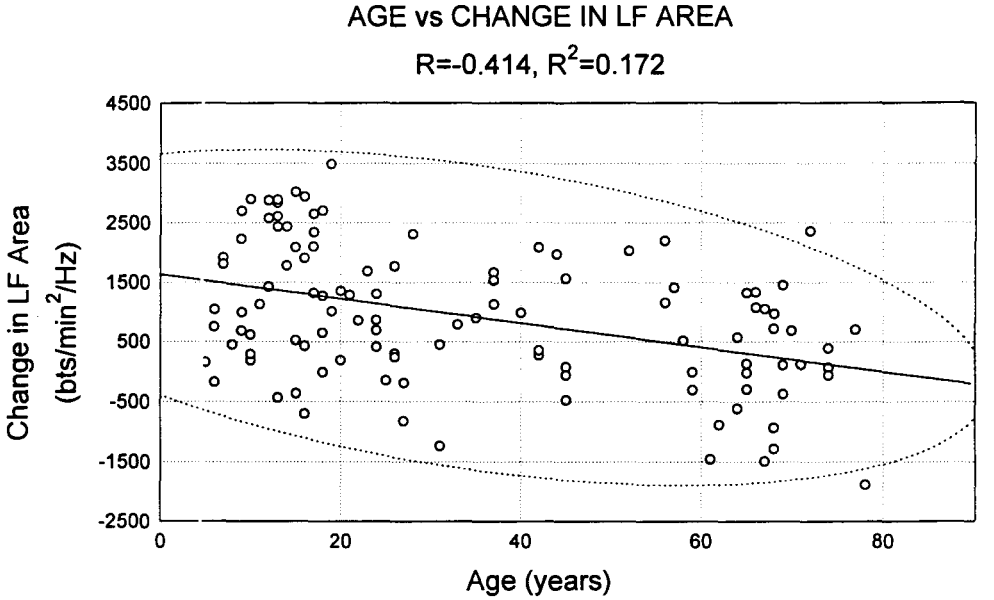


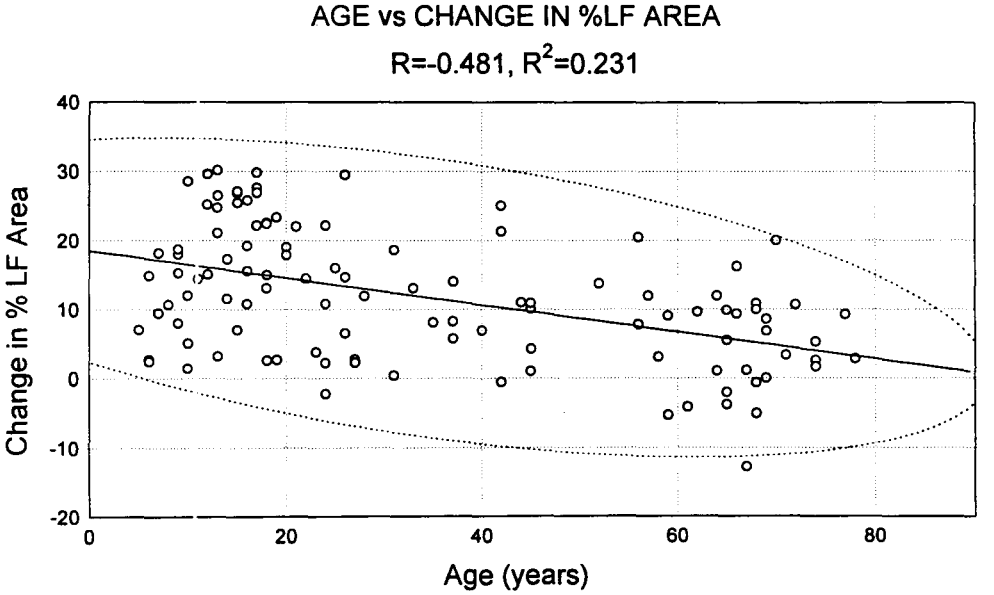
10) Scatterplots of Age vs LF:HF Peak & LF CF Response to Orthostasis



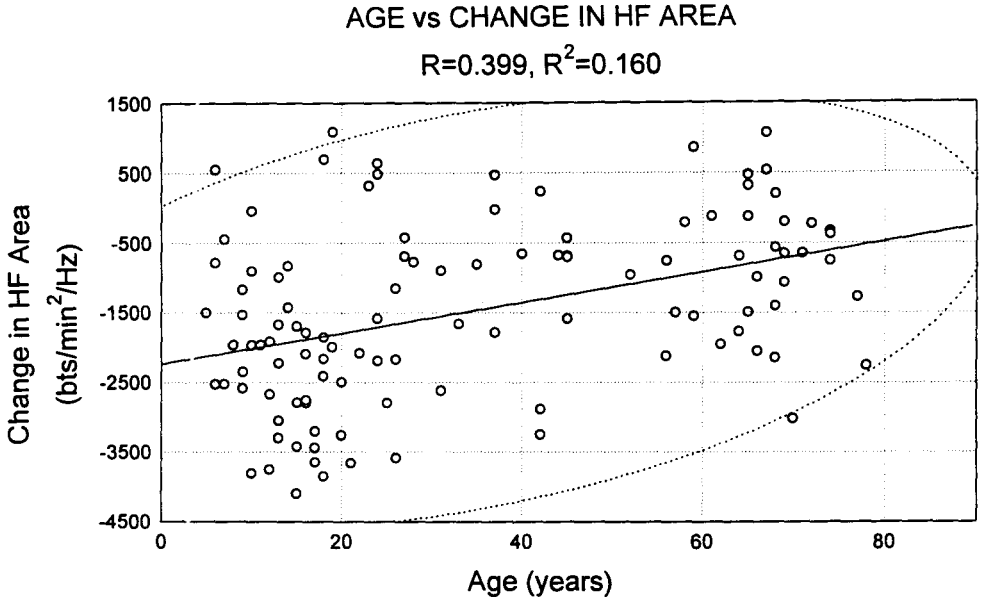


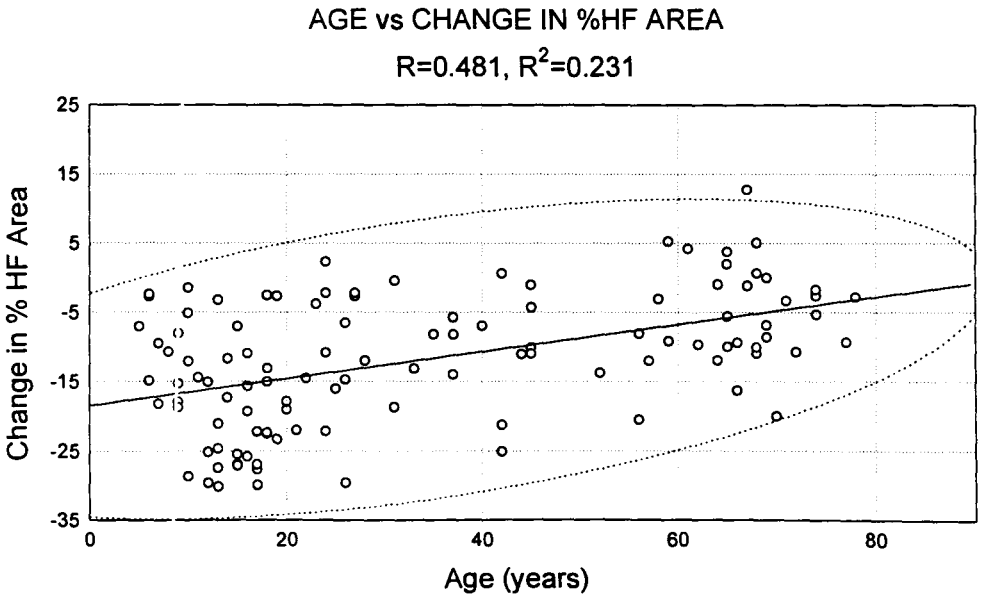
11) Scatterplots of Age vs LF Area & %LF Area Response to Orthostasis



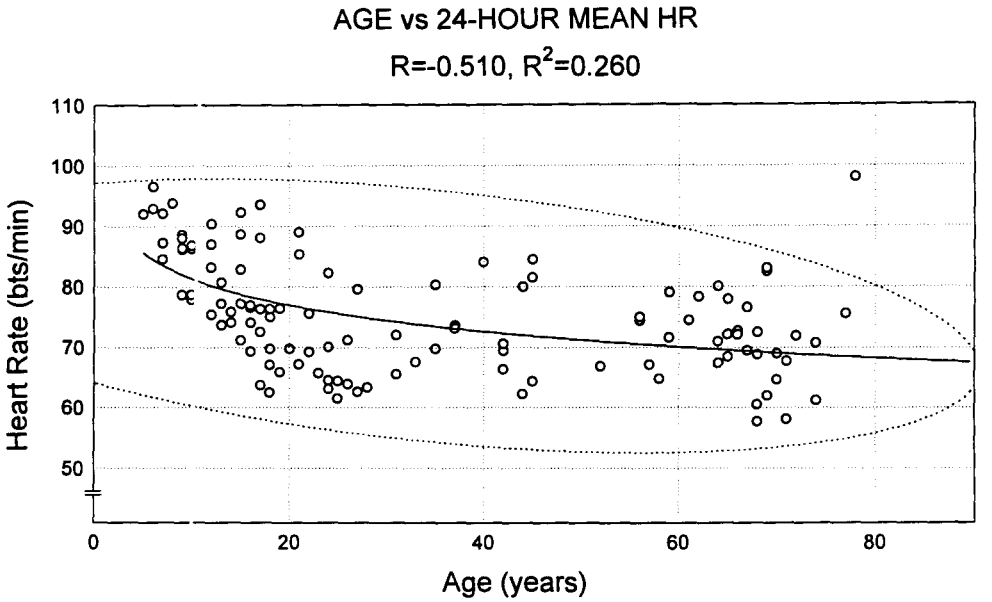


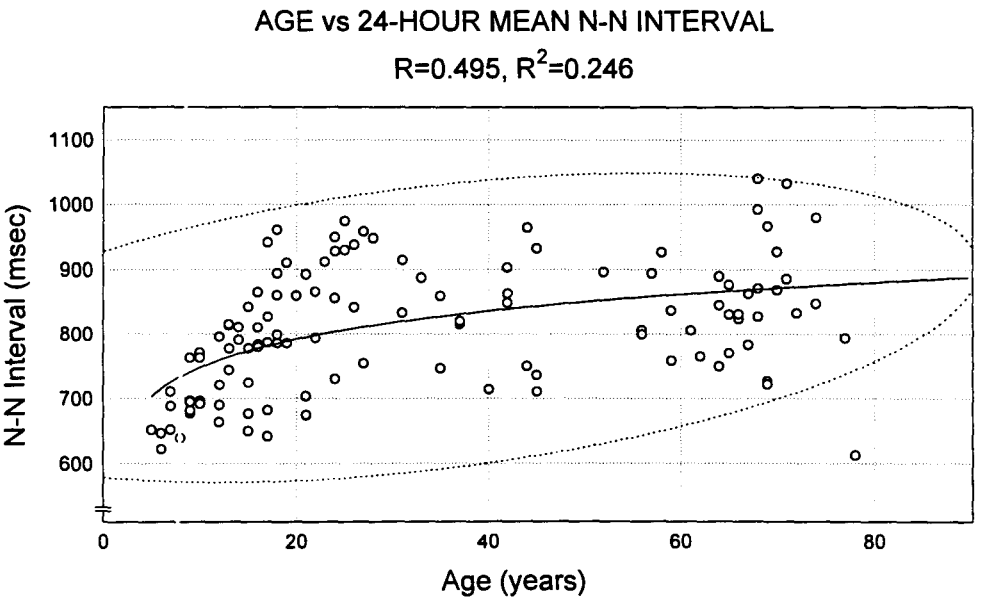
12) Scatterplots of Age vs HF Area & %HF Area Response to Orthostasis



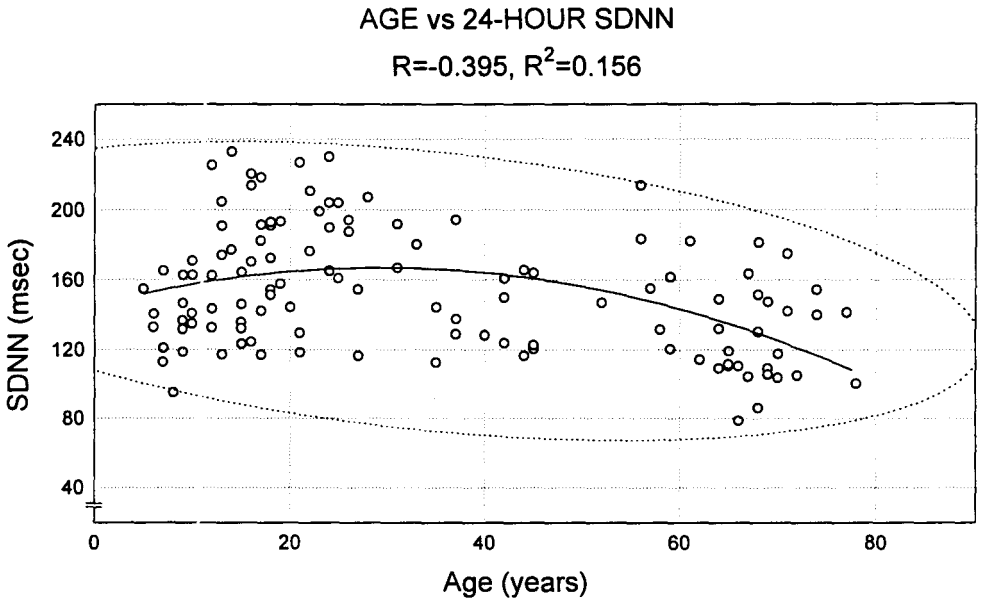


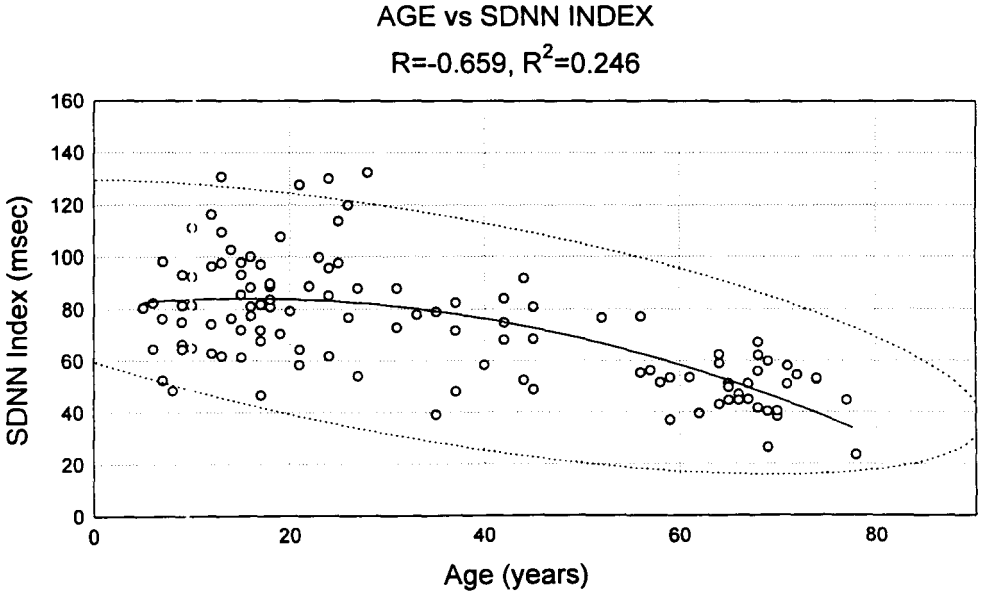
13) Scatterplots of Age vs 24-Hour Mean HR & N-N Interval



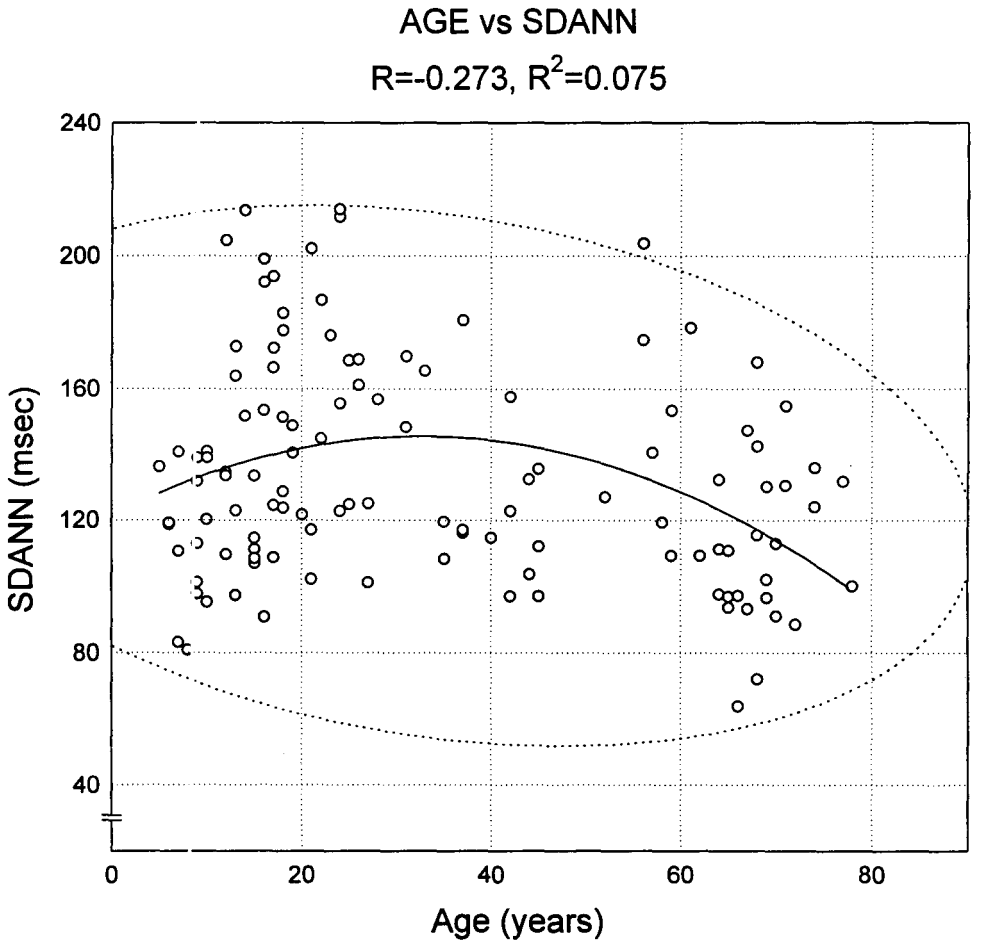


14) Scatterplots of Age vs 24-Hour SDNN & SDNN Index

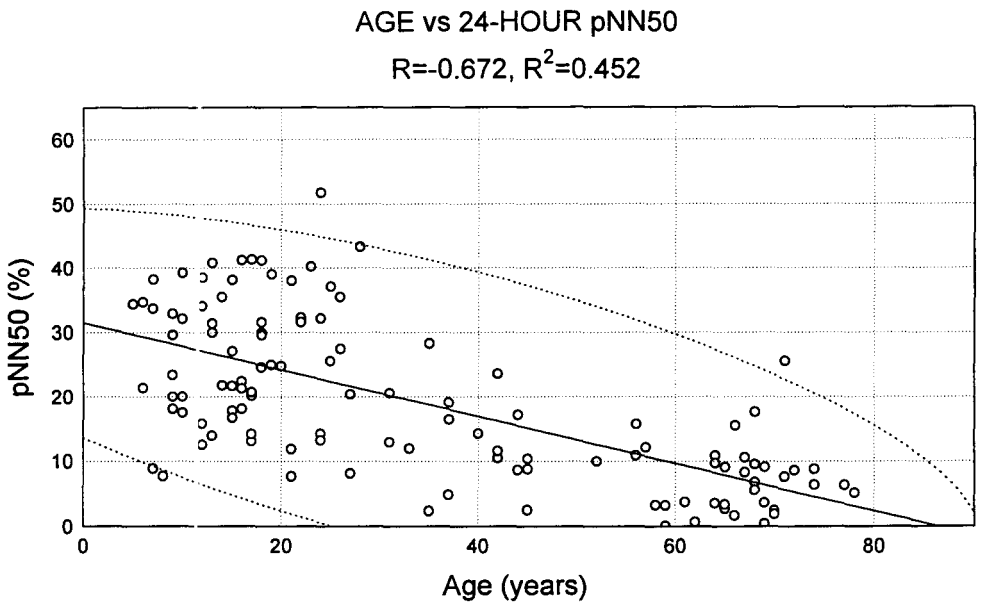


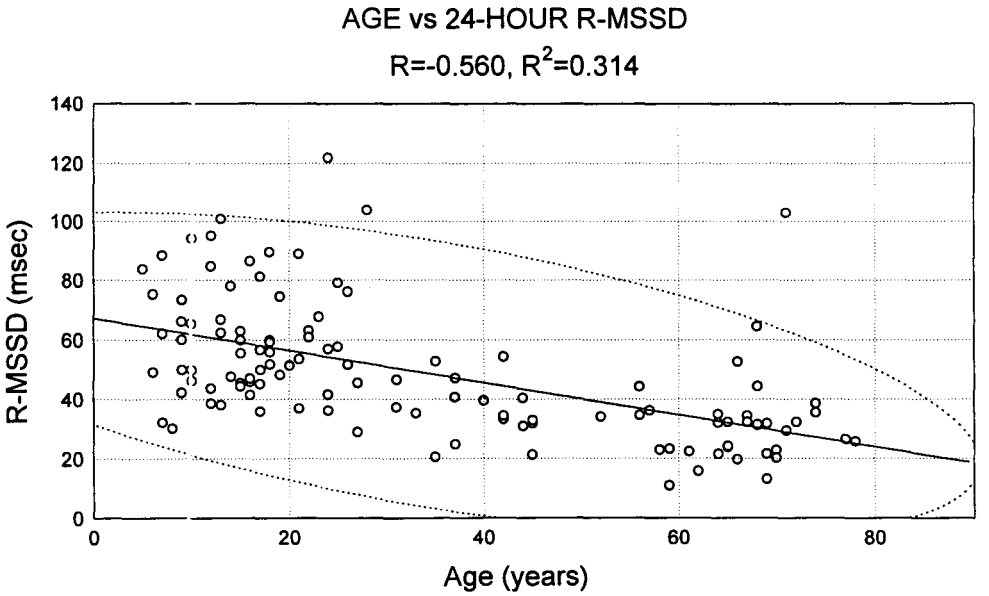


15) Scatterplot of Age vs 24-Hour SDANN



16) Scatterplots of Age vs 24-Hour pNN50 & R-MSSD





APPENDIX D:

Statistics - Multivariate Analysis of Variance and Chi-Square Summary Tables

MANOVA Summary Tables

1. Comparison of Power Spectral Measures in the Supine and Standing Postures (Mean Heart Rate (HR), Low Frequency Power (LF area), Fractional Low Frequency Power (%LF area), High Frequency Power (HF area), Fractional High Frequency Power (%HF area), LF:HF Area Ratio, LF:HF Peak Ratio and Low Frequency Central Frequency (LF CF)).

Effect	Wilks' Lambda	Rao's R	df 1	df 2	p-level
Age (A)	**0.178781**	**5.03819**	**40**	**350**	**0.000000**
Gender (G)	**0.694212**	**4.05244**	**10**	**92**	**0.000126**
Posture (P)	**0.112154**	**72.82990**	**10**	**92**	**0.000000**
A × G	0.705582	0.84459	40	350	0.737468
A × P	**0.265469**	**3.67131**	**40**	**350**	**0.000000**
G × P	**0.768030**	**2.77870**	**10**	**92**	**0.004800**
A × G × P	0.748100	0.69740	40	350	0.917761

2. Comparison of the Magnitude of Change in Power Spectral Parameters (Mean Heart Rate (HR), Low Frequency Power (LF area), Fractional Low Frequency Power (%LF area), High Frequency Power (HF area), Fractional High Frequency Power (%HF area), LF:HF Area Ratio, LF:HF Peak Ratio and Low Frequency Central Frequency (LF CF)) in Response to Orthostasis.

Effect	Wilks' Lambda	Rao's R	df 1	df 2	p-level
Age (A)	**0.109005**	**3.071152**	**80**	**325**	**0.000000**
Gender (G)	**0.572423**	**3.062539**	**20**	**82**	**0.000192**
A × G	0.399505	1.066760	80	325	0.343261

3. Comparison of 24-Hour Mean Heart Rate and N-N Interval, SDNN, SDANN, SDNN Index, pNN50 and R-MSSD Time Domain Measures Derived From Holter Monitor Recordings.

Effect	Wilks' Lambda	Rao's R	df 1	df 2	p-level
Age (A)	**0.113522**	**10.81618**	**28**	**365**	**0.000000**
Gender (G)	**0.728961**	**5.36476**	**7**	**101**	**0.000300**
A × G	0.681814	1.46327	28	365	0.063564

4. Comparison of Diurnal Variation in Power Spectral Indices (Mean Heart Rate (HR), Low Frequency Power (LF area), Fractional Low Frequency Power (%LF area) and LF:HF Area Ratio).

Effect	Wilks' Lambda	Rao's R	df 1	df 2	p-level
Age (A)	**0.245848**	**11.48691**	**16**	**315**	**0.000000**
Gender (G)	**0.750918**	**8.54134**	**4**	**103**	**0.000005**
Time (T)	**0.081718**	**48.88214**	**20**	**87**	**0.000000**
A × G	0.867805	0.93617	16	315	0.527875
A × T	**0.268663**	**1.70816**	**80**	**345**	**0.000580**
G × T	0.780324	1.22461	20	87	0.254674
A × G × T	0.372828	1.22767	80	345	0.109668

5. Comparison of Diurnal Variation in Power Spectral Indices (High Frequency Power (HF area), Fractional High Frequency Power (%HF area), LF:HF Peak Ratio and Low Frequency Central Frequency (LF CF)).

Effect	Wilks' Lambda	Rao's R	df 1	df 2	p-level
Age (A)	**0.263872**	**8.385559**	**20**	**339**	**0.000000**
Gender (G)	**0.854048**	**3.486251**	**5**	**102**	**0.005976**
Time (T)	**0.274520**	**8.668126**	**25**	**82**	**0.000000**
A × G	0.883135	0.647653	20	339	0.875386
A × T	**0.199771**	**1.642007**	**100**	**327**	**0.000639**
G × T	0.706358	1.363538	25	82	0.149751
A × G × T	0.276628	1.254327	100	327	0.072637

CHI-SQUARE ANALYSIS Summary Tables

1. Assessment of the prevalence of paradoxical heart rate responses to orthostasis.

	AGE		
	<30	**>55**	**TOTAL**
TYPICAL	60	40	100
ATYPICAL	1	2	3
TOTAL	61	42	103

χ^2=0.838, df=1; N.S.

2. Assessment of the prevalence of paradoxical LF:HF area responses to orthostatic stress.

	AGE		
	<30	**>55**	**TOTAL**
TYPICAL	59	34	93
ATYPICAL	2	8	10
TOTAL	61	42	103

χ^2=7.06, df=1; p<0.01

3. Assessment of the prevalence of paradoxical LF area responses to orthostatic stress.

	AGE		
	<30	**>55**	**TOTAL**
TYPICAL	53	29	82
ATYPICAL	8	13	21
TOTAL	61	42	103

χ^2=4.88, df=1; p<0.05

4. Assessment of the prevalence of paradoxical HF area responses to orthostatic stress.

	AGE		
	<30	**>55**	**TOTAL**
TYPICAL	55	36	91
ATYPICAL	6	6	12
TOTAL	61	42	103

χ^2=0.478, df=1; N.S.

5. Assessment of the prevalence of paradoxical LF:HF peak responses to orthostasis.

	AGE		
	<30	**>55**	**TOTAL**
TYPICAL	60	33	93
ATYPICAL	1	9	10
TOTAL	61	42	103

χ^2=11.11, df=1; p<0.001

6. Assessment of the prevalence of paradoxical LF CF responses to orthostasis.

	AGE		
	<30	**>55**	**TOTAL**
TYPICAL	54	26	80
ATYPICAL	7	16	23
TOTAL	61	42	103

χ^2=10.16, df=1; p<0.01