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THE RELATIONSHIP BETWEEN MODERATE ALCOHOL USE AND HEART RATE VARIABILITY IN OLDER WOMEN

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of the Ohio State University

By

Joan Ann Masters, MS, RN

****

The Ohio State University

1998

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ABSTRACT

Epidemiological studies suggest that moderate alcohol use, compared to both abstinence and higher levels of consumption, is associated with lower cardiovascular mortality. The lower incidence of cardiovascular mortality among lighter drinkers has been attributed to decreases in coronary artery disease (CAD). Given the high prevalence rates of CAD among the elderly, any suggestion of an alcohol-related cardioprotective effect raises questions regarding the consumption threshold for alcohol-related cardiovascular health/harm for this population. However, little knowledge exists concerning the effects of moderate level alcohol consumption on most cardiovascular phenomena. Specification of the effect of moderate alcohol consumption on numerous cardiovascular phenomena is essential for developing rational alcohol consumption recommendations for the middle and later adult years. This is especially true for older adults and women. These groups have a documented heightened vulnerability to alcohol-related harm but have been under-represented in alcohol studies.

Aging, alcohol abuse/dependence, and cardiac rhythm disturbances are three major public health challenges associated with excess morbidity and mortality. Perturbed autonomic tone is a factor that increases cardiac electrical instability, the risk of arrhythmogenesis, and the possibility of sudden cardiac death (SCD). Autonomic tone can be operationalized as measures of heart rate variability (HRV). Indirect relationships between HRV and both heavy alcohol use and aging are well documented in the
literature. However, there is a paucity of information about the effects of chronic moderate alcohol consumption on HRV (i.e. autonomic tone). Even less is known about the effect of chronic moderate alcohol consumption on the circadian variation in HRV.

Arrhythmogenesis, stroke, SCD, and angina exhibit a circadian variation similar to the variation in HRV. This finding suggests that altered HRV may be a trigger for these profound cardiovascular events. Research examining the circadian pattern of HRV in multiple disease states suggests that circadian analysis may reveal sentinel changes in autonomic tone that have prognostic significance. Specification of the nature of the relationship between chronic moderate alcohol consumption and HRV is essential for determining whether any amount of alcohol is physiologically neutral during the later adult years.

The purpose of this project was to describe the relationship between chronic moderate alcohol consumption and HRV in a group of 52 healthy community dwelling women over the age of 60 who self-reported varying amounts of chronic alcohol use or abstinence. This sample was recruited from social groups in a midsized city in the midwest and screened by a gerontological nurse practitioner and a cardiologist for clinical heart disease. This sample is a subset of subjects enrolled in an interdisciplinary study of older women who met the selection criteria established for this project. The larger parent project was funded by the NIAAA/NIH and the Andrus Foundation.
The concurrent validity of self-reported alcohol consumption was established by scores on a standardized assessment tool (the T-ACE) and γ-glutamyl transpepsidase (MCV) and mean corpuscular volume (MCV), hematological indices known to be affected by alcohol use. Measures of HRV were calculated from 24 hour Holter electrocardiogram (ECG) recordings scanned in semiautomatic mode and corrected for abnormal beats. Bivariate correlations and multiple regression were used to determine the direction and strength of the relationship between alcohol consumption rate and HRV for the sample and the unique contribution of alcohol consumption to HRV in the sample. Repeated Measures analysis of variance was used to identify circadian differences between the subjects with moderate alcohol consumption rates (> 15 standard drinks per month) and alcohol abstaining subjects (< 1 drink per month).

Moderate levels of alcohol consumption were associated with a dose dependent reduction in estimates of HRV. This reduction affected all estimates of HRV except HR. Alcohol consumption in this sample independently accounted for about one-third of the sample variance in global measures of HRV when the effects of the other independent variables were held constant. For moderate drinkers, lowered absolute values of [log] HF power and [log] LF power combined with a phase delay in circadian events to buffer the abrupt relative rise in sympathetic tone (i.e., LF/HF power) that began about 6:00 a.m. among abstainers. The LF/HF ratio among drinkers had a tendency toward higher values during the nighttime hours and lower values during the daytime hours compared to abstainers. The LF/HF ratio remained lower and more regular during the day in women who consumed alcohol than those who abstained from alcohol. Thus, the
quantitative effects of moderate alcohol consumption level on the absolute measures and rates of change in [log] HF and [log] LF power produced qualitative changes in autonomic function.

Overall, the findings of this study strongly suggest that moderate alcohol consumption, rather than unilateralley modifying vagal tone or sympathetic activity, is associated with a resetting of autonomic balance and a decrease in the flexibility of the autonomic nervous system. The balance between vagal tone and sympathetic input is a critical factor in determining the response of the cardiovascular system to stressors. Knowledge about the flexibility of autonomic function provides more comprehensive and useful information than can be derived from discriptions of isolated changes in sympathetic tone or decreases in vagal tone.

This project contributes to the precise definition of the threshold level of alcohol related health/harm during later adult years by initiating specification of the relationship among aging, moderate alcohol use, and perturbed autonomic tone and contributing to clarification of the mechanism underlying alcohol-related arrhythmogenesis and SCD.
Dedication

This project is dedicated to my husband, James M. Masters, who has spent countless hours with me over the past four years discussing the philosophy of science, research design and, statistical methods - and only asked for dinner occasionally.
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Special thanks and recognition are due to Professor Joanne S. Stevenson, my dissertation chairman and adviser, who liberally gave her time and energy throughout the course of this research as well as through the course of my doctoral studies. Dr. Stevenson's special knowledge and practical experience helped insure that the research was solidly grounded in a meaningful, nursing practice problem. Special thanks and recognition are also due to Dr. Robert L. Hamlin and Dr. Stephen Schaal. These two individuals have shown me remarkable patience and generosity over the past four years. Dr. Hamlin introduced me to the physiology of the cardiovascular system, allowed me into his laboratory, and was indispensable resource in formulating the methodology used in this study. Over the last four years my needs have made considerable demands on Dr. Hamlin's time and energies. He has always reacted to my intrusions with unbounded enthusiasm and unfailingly given the impression that it was a pleasure to hear from me one more time. He has my eternal admiration. Dr. Schaal's electrophysiological studies describing the effects of alcohol on arrhythmic thresholds provided the inspiration for this project. He has magnanimously given me access to the Holter ECG recordings used in this study and, equally as meaningfully, provided unwavering optimism during the entire research project. I am also extremely grateful to Dr. Debra K. Moser. Her recognized clinical expertise and familiarity with the complexities of heart rate variability research in human populations were indispensable to the formulation and execution of this project.
Her criticisms, insights, and encouragement have been invaluable. I owe these individuals a debt which cannot be repaid. Through their influence the conduct of this research has been a genuinely enjoyable experience.

My gratitude and appreciation are also due to my friends and colleagues in the doctoral program at the Ohio State University who have spent many hours of their time helping me to clarify my ideas and sharpen the focus of this dissertation. Special thanks in this regard are owed to Mary Lou Campbell and Mary Stange. Their contributions to my education and to this work have been substantial.

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The profound consequences of high levels of both acute and chronic alcohol consumption are well documented. High levels of alcohol consumption are associated with every known cause of cardiovascular and non-cardiovascular mortality (Zahari, 1991). The physical chemistry of ethyl alcohol explains the ability of this simple two carbon molecule to affect every organ system of the body, but organ systems have differential vulnerabilities to alcohol-related damage. Separate mechanisms, within one organ system, may exhibit differential dose and temporal exposure thresholds for alcohol-related effects. For example, in the cardiovascular system, the blood ethanol concentrations (BEC) level associated with arterial vasospasm (Altura, Altura, & Carella, 1983) are far below and more transitory than the BEC levels associated with cardiac muscle disease (Preedy & Richardson, 1994). The differential effects of alcohol on cardiovascular mechanisms can produce seemingly paradoxical outcomes. One such outcome is the unexpected beneficial effect of moderate level alcohol consumption on cardiovascular morbidity and mortality.
Epidemiological studies suggest that moderate alcohol use, compared to both abstinence and higher levels of consumption, is associated with lower cardiovascular mortality (Anderson, Cremona, Paxton, Turner, & Wallace, 1993; Klatsky, Armstrong, & Friedman, 1990; Klatsky, Armstrong, & Freidman, 1992; Stampfer, Graham, Willett, Speizer, Hennekens, 1988; Thun et al., 1997). Lower cardiovascular mortality rates among lighter drinkers when compared to abstainers and heavy drinkers may be partially attributable to an alcohol-related increase in high density lipoproteins (HDLs) and a subsequent reduced incidence of coronary artery disease (CAD). Other probable contributors to the cardioprotective effect of moderate alcohol consumption are: 1) alterations in cellular signaling in the vascular endothelium that inhibit the inflammatory response associated with fatty streaks in blood vessel and consequently prevent atherogenesis, 2) reductions in platelet function that interfere with thrombus formation and, 3) increased fibrinolytic activity (Klatsky, Armstrong, & Friedman, 1992; Zakhari, 1997).

CAD is the most common cause of death in the United States. Approximately eighty percent of the 500,000 annual CAD deaths occur in individuals aged 65 and older (McIntosh, 1994) and CAD has a significant impact on functional status among the elderly. The majority of older adults affected by CAD are women (Vaccarino, de Leon, & Berkman, 1997). Given the high prevalence rates of CAD among the elderly, and especially women, any suggestion of an alcohol-related reduction in CAD
raises important questions regarding the consumption threshold for alcohol-related cardiovascular health/harm for older adults.

The legitimacy of statements imputing a cardioprotective effect to moderate alcohol consumption has been questioned on the basis that early studies included "sick quitters" and other individuals who avoided alcohol because of illness in the abstainer (control) groups. This error artificially raised all-cause mortality rates among non-drinkers, masked alcohol-related mortality, and increased the apparent cardioprotective effect of moderate alcohol consumption. Other studies have failed to control for the effects of smoking, diabetes, obesity, and hypertension (Arria & Van Theil, 1992). Because past research has mostly employed samples of middle-aged men, the generalizability of most findings to older adults and women is limited by sample composition. These design flaws have greatly undermined the credibility of any claim of a cardioprotective effect for alcohol among older adults and women.

A recent well designed prospective study of alcohol consumption and mortality in middle-aged and older adults raises new questions about the cardioprotective effects of alcohol consumption among older women. This study described a "slight" reduction in cardiovascular and all-cause mortality among the 490,000 study subjects (Thun et al., 1997). Unexpectedly, the greatest benefit was pinpointed among older adults with preexisting cardiovascular disease. An alcohol related cardiovascular benefit was identified for all subject subgroups delineated by the study, except healthy women over the age of 60 who were free of cardiovascular disease at the inception of the study. The lack of significant findings in this subset of 143,000 subjects is a
strong argument that the threshold for alcohol-related cardiac benefit, if there is
indeed a cardiovascular benefit, is meaningfully higher in healthy older women than
in other populations. Other researchers have noted that middle-aged and older women
who drank more than three drinks per day incurred an increased risk of CAD
morbidity and mortality compared to women drinking at lower levels (relative risk =
2.6, CI 1.2-5.5) (Rehm, Bondy, Sembros, & Vuong, 1997). Thus, the threshold for
alcohol’s imputed cardioprotective effect seems to be higher for females compared to
males while the threshold for increased risk of CAD may be lower. The fact that
women incur organ level alcohol-related cardiovascular harm after briefer and lower
dose exposure to alcohol than males is well known.

Since little knowledge exists concerning the effects of moderate alcohol
consumption on many cardiovascular phenomena, it is possible that the therapeutic
index for alcohol consumption among women may be much narrower than is currently
appreciated or even that healthy women may experience alcohol-related harm at
consumption levels far below those associated with any possible cardioprotective
effect. These possibilities and the fact that little knowledge exists concerning the
cardiovascular effects of long-term moderate levels of alcohol consumption in older
adults and women, despite the documented exceptional vulnerability of these groups
for alcohol-related harm (Pfefferbaum, Lim, Zipursky, Mathalon, Rosenbloom, Lane,
Ha, & Sullivan, 1992; U.S. Department of Health and Human Services, 1993),
mandates a rigorous examination of alcohol-related cardiovascular risk among older
healthy older women. Specification of these effects is essential for developing rational alcohol consumption recommendations for the middle and later adult years.

Cardiac arrhythmias are an important source of excess morbidity and mortality among older adults. Supraventricular arrhythmias carry a risk of hemodynamic alteration, embolization, and cerebral vascular accident. Ventricular arrhythmias carry similar risks, are the proximate cause of sudden cardiac death (SCD), and account for half of the cardiovascular related deaths occurring in the United States each year. The prevalence of cardiac arrhythmias increases during aging. Age-related changes in cardiac electrophysiology result in conduction delays that promote re-entrant arrhythmias. One older adult out of every one hundred will develop atrial fibrillation. The increased incidence of cardiac, pulmonary, and other chronic diseases during senescence augments arrhythmic morbidity and mortality in older adults. As the relative and absolute numbers of older adults swell during the next quarter century, arrhythmic morbidity and mortality among older adults will become an increasingly critical clinical and public health challenge.

Alcohol abuse/dependence is a second significant clinical and public health challenge that will assume increased importance among older adults during the next quarter century. Currently the majority of older adults consume alcohol. Between 10% and 20% of older adults are daily drinkers, and the prevalence of alcohol abuse/dependence among older adults is estimated to be between 4% and 10% (Beresford, 1995). The proportion of older moderate drinkers, as well as the number of older adults experiencing alcohol abuse/dependence, is predicted to increase as the
current middle-aged cohort ("baby boomers") ages (Beresford, 1995; Glynn, Bouchard, LoCastro, & Laird, 1985).

Although the arrhythmogenic effects of alcohol have been well documented by experimental and clinical studies, the mechanism of alcohol-related arrhythmic risk is undefined for older adults as well as for other populations. Perturbed autonomic tone, which can be quantified by measures of heart rate variability (HRV), is a significant risk factor for arhythmogenesis. Parasympathetic (vagal) and sympathetic input to the sinoatrial node (SAN) is decreased after chronic high level alcohol consumption. Parasympathetic function seems to be more sensitive to alcohol's effects. The net result of these reductions is a shift toward sympathetic predominance. Alcohol-related perturbations in autonomic input are qualitatively similar to normal age-related changes in autonomic function and are generally considered to be proarrhythmogenic. During the next quarter century, alcohol related arrhythmogenesis will make an increasingly significant contribution to excess morbidity and mortality among the elderly.

Specification of the relationship between aging, moderate alcohol consumption, and autonomic tone (HRV) will help explain the mechanism of alcohol-related arrhythmogenesis as well as help delineate the threshold for alcohol related health/harm during the middle years and beyond.
Purpose

The purpose of this dissertation project was to describe the relationship between chronic moderate alcohol consumption and various estimates of autonomic tone (HRV) in healthy community-dwelling women over the age of 60.

Overview of the Study

This descriptive cross-sectional study examined the relationship between chronic alcohol consumption rate and HRV in a sample of healthy community-dwelling older women. The sample was composed of all qualified women enrolled in a more comprehensive interdisciplinary study of 135 older women. This study extended the objectives of the parent study by introducing HRV as a dependent variable. Twenty-four hour Holter ECG recordings from every qualifying subject were re-analyzed and standard geometric, time-domain, and frequency-domain measures of HRV were calculated for this subset of subjects. Bivariate correlations, standard multiple regression analysis, and repeated measures ANOVA were calculated to determine the relationship between long-term moderate alcohol consumption and HRV in the sample and the association between moderate alcohol consumption and the circadian pattern of HRV estimated by frequency-domain measures.

Research Questions

This project answered the following questions:

1) What is the direction and strength of the relationship between moderate alcohol consumption rate and heart rate variability in older women?
2) What is the unique contribution of moderate alcohol consumption to heart rate variability in older women?

3) What is the relationship between moderate alcohol consumption rate and the circadian rhythm of heart rate variability in older women?

Contributions of the Research

Previous research describing the relationship between alcohol and HRV has focused on middle-aged males, and mostly used controlled laboratory conditions. The few females included in these studies were mainly young or middle-aged women who consumed either very low quantities of alcohol (e.g., mean of 1.25 standard drinks/week) or very high quantities (i.e., alcohol dependent). This is the first study to describe the relationship between moderate alcohol consumption and heart rate variability in a healthy community-based sample of women over the age of 60 and the first to examine the effect of moderate alcohol consumption on the circadian pattern of heart rate variability in any population.

The use of twenty-four hour Holter ECG recordings collected during routine daily activities has not been reported in the alcohol/aging literature. Twenty-four analysis is critical because profound cardiac events occur with a circadian rhythm that parallels the circadian rhythm of HRV. This finding has led to speculation that perturbed HRV may be a trigger for these incidents. Also, the circadian rhythm of HRV is attenuated in many pathophysiological states associated with SCD and this attenuation has prognostic significance.
Circadian analysis of HRV is more sensitive to sentinel perturbations in autonomic tone than traditional tests for autonomic neuropathy and can reveal perturbations of automatic tone that are concealed by the averaging effects of global measures of HRV. Thus, this project provides clinically feasible estimates of the relationship between long-term moderate alcohol consumption and HRV in a previously unexamined population under everyday living conditions. This study identified an inverse relationship between moderate alcohol consumption and estimates of autonomic balance and detected a previously unsuspected indication of abnormal sympathovagal balance in subjects who self-reported moderate alcohol consumption.

The long-term goals of this line of research are:

1. to determine optimal levels of alcohol intake for the middle and later adult years.

2. to clarify the roles of aging and chronic moderate alcohol consumption in arrhythmogenesis and sudden cardiac death.

Definitions

Independent Variables/Theoretical Definitions

Chronic alcohol consumption consists of repeated acute episodes of alcohol consumption.

Acute alcohol consumption consists of one episode of alcohol use, during which the blood alcohol curve begins at baseline, reaches a peak, and returns to baseline. Alcohol consumption was reported by subjects as quantity (drinks per occasion) and frequency (number of drinking days per month). The number of
standard drinks per month was then calculated for each subject. The following equivalencies were used to convert this metric into grams: A standard drink = 12 grams of ethanol. Forty-five milliliters of 80 proof distilled spirits, 5 ounces of wine, or 12 ounces of beer = 12 grams of ethanol).

Moderate alcohol consumption is defined as "drinking that does not generally cause problems either for the drinker or society" (National Institute of Alcoholism and Alcohol Abuse, 1992) and adheres to the US Department of Agriculture/US Department of Health & Human Services (1990) guideline for women of 1 standard drink or less per day. The range of alcohol consumption of interest in this study was determined to be a minimum average of 1 drinks per month and a maximum average of 3 drinks per day. The optimal balance between alcohol-related cardiovascular benefit and risk lies somewhere in this consumption range.

Abstinence is defined as fewer than one drinking occasion per month and no more than one drink per occasion.

Independent Variable/Operational Definition

Alcohol Consumption Rate is reported in the pharmacological gold standard of grams of ethanol per square centimeter of body surface per day (g/cm²/day). Body surface in cm² = 71.8 x weight⁰.⁴²⁵ x height⁰.⁷²⁵ (Dubois & Dubois, 1916).

Dependent Variable/Theoretical Definition

Heart Rate Variability is an index of autonomic nervous system tone and reflects the variation in inter-beat intervals that results from the dynamic interaction between parasympathetic (vagal) and sympathetic input to the sinoatrial node (SAN).
Parasympathetic stimulation generally protects against arrhythmogenesis. Sympathetic stimulation generally facilitates arrhythmia development. HRV is an output signal produced by the integration of intrinsic cardiac factors, medullary cardiovascular reflexes, and supramedullary factors on heart rate (McDonald, 1980) and reflects the integrity of both the central and peripheral inputs to the sinoatrial node (SAN) as well as the health of the SAN. HRV can be expressed by geometric, time-domain, and frequency domain measures. These stochastic measures represent HRV by summary statistics such as means, standard deviations, and spectral power (Cowan, 1995). Although measures of heart rate variability are correlated, they are not surrogates, and many have specific applications (e.g. SDANN is the best gauge of circadian variation) (Cowan, 1995).

**Geometric measures** estimate HRV from geometrical forms usually based on the sample density histogram derived from RR or NN intervals. Global estimates based on these estimates are robust to imperfections in the ECG recording and felt to be more suitable for clinical data.

**Time-domain measures** of HRV are calculated from a time series of normal R-R intervals that reflect the variability in sino-atrial node firing. These measures can be categorized as those derived from beat-to-beat intervals and those measures based on the differences between successive cycle lengths.

**Frequency-domain measures** are derived from an interval function that has an amplitude and frequency that varies according to the values that comprise the time series used in time-domain analysis. Frequency-domain analysis assumes that this
interval function is the sum of several sinusoidal component signals of various amplitudes and frequencies. Fourier transformation converts an interval plot from the time-domain to the frequency-domain, produces a graphic depiction of the component signals of the interval function, and parses out the influence of the parasympathetic nervous system.

**Dependent Variable/Operational Definitions**

The operational definitions for both time and frequency domain measures of HRV adhere to the recommendations of the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (Task Force, 1993).

**HRV Triangular Index (HRVI)** is the integral (area) of the density distribution divided by the maximum (modal value) of the density distribution.

**Triangular Interpolation of the NN (TINN)** is the length of the base of a triangle derived from a minimum squared difference interpolation. The base of the triangle is equal to the horizontal axis of the density histogram of a triangle and a height equal to the peak of the maximum point (highest peak) of the sample density histogram.

**SDNN** is calculated as the standard deviation of all the inter-beat intervals between normal beats (NN intervals) over a 24 hour period. The SDNN includes all frequency components in a 24 period.
SDANN5 is calculated as the standard deviation of the mean of the NN intervals contained in a 5 minute epochs of NN intervals in a 24 hour period and assesses the HRV of cycles greater than 5 minutes.

ASDNN5 is calculated as the mean of the standard deviations of all 5 minute epochs of NN intervals in a 24 hour period and assesses the HRV of cycles of less than 5 minutes.

RMSSD is calculated as the square root of the mean of the squared differences between successive NN intervals and is an estimate of high frequency oscillations in HRV.

High Frequency (HF) equals the variance (power) in all NN intervals in the frequency range 0.15-0.4 Hz and reported as AUC in ms².

Low Frequency (LF) equals the variance (power) in all NN intervals in the frequency range between 0.04 - 0.15 HZ reported as AUC in ms².

Very Low Frequency (VLF) equals the variance (power) in all NN intervals in the frequency range 0.003-0.04 Hz and reported as AUC in ms².

Summary

The harmful effects of high level alcohol consumption are well known. Recent epidemiological studies suggest that moderate alcohol consumption may have a cardioprotective influence. This suggestion raises questions about the level of alcohol consumption that maximizes cardiac health while minimizing alcohol-related cardiac harm. Definition of the threshold for alcohol-related harm is critical for older adults. This burgeoning population has a documented heightened vulnerability for both
arrhythmogenesis and alcohol related harm. The vulnerability for alcohol-related harm is enhanced among women.

The threshold for alcohol-related cardiac harm for older adults may be partially revealed by the association among aging, autonomic tone, and alcohol consumption. HRV measures facilitate examination of this association. Specification of this relationship will support rational recommendations for alcohol consumption during the middle and later adult years and help clarify of the mechanism of alcohol-related arrhythmogenesis and SCD.

Order of Presentation

The remainder of this dissertation is organized into four additional chapters. Chapter 2 contains a summary of the physiological basis of HRV. This summary introduces the theoretical model of the autonomic consequences of the aging and alcohol that depicts the theoretical framework of the study. The methodology of the study is discussed in Chapter 3. This discussion includes a description of the study design, sample and setting, target population, protocol, data management, and the statistical analysis that was used to answer the research questions. The results of the study and a discussion of the findings are recorded in Chapter 4. Finally, a project summary and a discussion of the limitations and implications of the study are presented in Chapter 5.
CHAPTER 2

BACKGROUND OF THE PROJECT

Overview and Purpose

The discontinuity in the relationship between alcohol consumption level and cardiovascular morbidity and the lack of knowledge regarding the risk/benefit ratio of moderate alcohol consumption and the mechanisms of arrhythmic morbidity and mortality among older adults provokes to two fundamental queries:

1. What is known about the relationships among aging, alcohol consumption, and autonomic tone?

2. What are the consequences of these relationships for older adults and for the health care system?

To answer these questions, a theoretical model of the autonomic consequences of aging and alcohol consumption is developed in this chapter. Initially, the physiological mechanisms of autonomic tone are summarized. This summary is foundational to the subsequent elaboration of the model. Next, the relationships among the other model concepts is elucidated by a review of the literature relating perturbed autonomic tone to cardiac arrhythmogenesis as well as all-cause mortality. This review incorporates the relevant aging and alcohol literature as well as an evaluation of the following possibly confounding variables: gender, concurrent
cardiovascular medication use, clinical state, nicotine use, caffeine use, and activity/and exercise.

Autonomic Tone and Heart Rate Variability

Autonomic tone, the tonic or static state of the autonomic nervous system (ANS), results from the combined influences of the parasympathetic nervous system (PNS) and sympathetic nervous systems (SNS). Autonomic tone is the end point of interactions among the ANS, CNS, and neurohormonal mechanisms at multiple levels of biological organization (Maltzman & Marinkovic, 1996). The autonomic influences on heart rate, integrated at the SAN, illustrate the numerous antecedents of that aspect of autonomic tone that is operationalized by HRV. Heart rate (HR) and HRV are determined by intrinsic cardiac mechanisms, medullary cardiovascular reflexes, supramedullary factors, and hormonal influences (Berne & Levy, 1993; McDonald, 1980) (see Figure 1).

Intrinsic Cardiac Mechanisms

The SAN, a small structure located at the junction of the superior vena cava and the right atrium, is characterized by greater automaticity and rhythmicity that other potential pacemaker cites. Automaticity, the result of phase 4 depolarization in slow response cardiac fibers, is directly regulated by parasympathetic (vagal) and sympathetic influences at SAN. The slope of phase 4 depolarization determines the discharge rate of the SAN and both heart rate and HRV (McDonald, 1980).
Parasympathetic Input

Post-ganglionic parasympathetic neurons release acetylcholine (Ach) at the SAN. Ach binds to muscarinic (M₂) receptors, activating K⁺ channels, increasing K⁺ efflux, and hyperpolarizing the pacemaker fibers. This decreases the slope of phase 4 depolarization and slows heart rate. Because of the quick response time of these ligand gated K⁺ channels, and the rapid degradation of Ach by cholinesterase at the SAN, parasympathetic influences exert beat-by-beat (high frequency) control over heart rate (Berne & Levy, 1993). The effect of vagal stimulation peaks at 0.5
seconds and returns to baseline by 1.0 second (Task Force, 1997). Parasympathetic input dominates at the SAN (Berne & Levy, 1993).

**Sympathetic Input**

Sympathetic post-ganglionic nerve terminals release norepinephrine (NE) at the SAN. NE binds to $\beta_1$ adrenergic receptors and increases the slope of phase 4 depolarization by differentially increasing the conductance of $\text{Na}^+$, $\text{Ca}^{++}$, and $\text{K}^+$. This increases the rate of depolarization of the SAN. NE is slowly inactivated by monoamine oxidase (MAO) in the nerve terminal, by catechol-O-methyltransferase (COMT) after reuptake into the presynaptic neuron, and by dilution in the bloodstream. As a result, NE exerts slow (low frequency) effects that emerge and dissipate gradually (Berne & Levy, 1993). Increases in heart rate lag behind sympathetic stimulation by at least 1.0 seconds, peak at 4.0 seconds, and do not return to baseline for twenty seconds (Task Force, 1997).

**Accentuated Antagonism**

Autonomic influences also affect depolarization of the SAN indirectly by accentuated antagonism. The release of NE at the SAN is attenuated by the release of Ach into the synapse. The inhibitory effect of vagal stimulation on automaticity and conductivity is greater when sympathetic input is high. Inversely, when vagal tone is high, sympathetic stimulation has less of an excitatory effect on automaticity and conductivity. The phenomenon of accentuated antagonism helps define the complex relationship between parasympathetic (vagal) and sympathetic stimulation at the SAN. The relationship is not merely reciprocal. (Randell & Wurster, 1994; Urthaler, Neely,
Hageman, et al, 1986; Warner & Levy, 1989). Increases in heart rate (HR) and decreases in HRV may be the result of increased sympathetic tone, decreased parasympathetic tone or an interaction of both divisions of the autonomic nervous system (Maltzman & Marinkovic, 1996).

**Medullary Cardiovascular Reflexes**

A number of peripheral receptors in the cardiovascular and respiratory systems have input to the medullary nuclei that regulate the autonomic nervous system. Parasympathetic afferents from chemo- and mechanoreceptors in the heart, great vessels, and airways pass cranially over CN IX and X and terminate in the medullary nucleus tractus solitarius (NTS). Sympathetic afferents arise in the dorsal root ganglion of T1-T5, travel caudally to peripheral receptors and cranially to the NTS. The NTS is the primary integration site for the peripheral afferents and the higher central areas influencing heart rate. The NTS affects parasympathetic activity through projections to the dorsal vagal nucleus (DVN) and the nucleus ambiguus of the lateral medulla (NA). Both glutamnergic and GABAergic transmission are operative in medullary cardiovascular reflexes (Kunos & Varga, 1996).

Efferent PNS fibers of CN X join sympathetic fibers to form the vagosympathetic trunk which innervates the SAN. Sympathetic fibers from the rostral ventrolateral medulla travel down the intermediolateral columns of the spinal cord (T1-T5) and exit the cord through the thoracic rami communicantes. Sympathetic fibers then and enter the vagosympathetic trunk which synapses on the SAN (Flores & Sheridan, 1990; Talman & Kelkar, 1993).
Supramedullary Factors

Supramedullary control of heart rate and rhythm depends on integration of input from the cortex (insula, anterior cingulate, and prefrontal cortices), diencephalon (thalamic nuclei), and limbic system (central nucleus of the amygdala and periaqueductal grey). Areas of the cortex and diencephalon initiate changes in HR that are associated with emotional states (Benarroch, 1997; Berne & Levey, 1993). In general, ascending NTS projections carry visceral input to multiple forebrain and brain stem nuclei. This input is integrated and triggers neuroendocrine and autonomic motor responses. This network has connections to the cortex and limbic system and is essential in maintaining homeostasis (Lowery, 1990). Supramedullary influences contribute to individual differences in arousal, cardiovascular reactivity, and neuroendocrine responses to psychological and physiological stimuli (Lane, Adcock, & Burnett, 1992).

Hormonal Influences

The adrenal medulla is innervated by descending pathways from the hypothalamus, midbrain, pons, medulla, and intermediolateral columns of the spinal cord and is an integral component of the autonomic nervous system. Activation of the sympathetic nervous system increases release of epinephrine from the adrenal medulla. However, the release of catecholamines from the adrenal medulla probably plays a secondary role in modulation of HR compared to NE released at cardiac sympathetic nerve endings (Berne & Levey, 1993).
The efficient integration of these mechanisms is fundamental for adaptation to stress and maintenance of the dynamic homeostasis that characterizes healthy organisms. The diversity of physiological mechanisms that contribute to autonomic tone at the SAN may explain the utility of heart rate variability (HRV) for risk stratification in numerous clinical states.

**Heart Rate Variability**

HRV can be expressed by geometric, time-domain and frequency-domain measures. All of these methods begin with a surface ECG recording from which abnormal atrial and ventricular beats have been deleted and the RR signal corrected. R-R variability contains information about the integrity of components influencing the ANS and the health of the SAN.

**Geometric Measures**

Geometric measures estimate HRV by constructing a geometric form from the sequence of RR or NN intervals. Usually, geometric measures are based on the sample density histogram (i.e., HRVI and TINN) or plots of the duration of each RR or NN interval against the preceding RR or NN interval (i.e. Lorenz or Poincare plots). These methods are useful when HRV is estimated from imperfect, long-term recordings (Malik, 1995).

**Time-domain**

Time-domain analysis uses a variety of algorithms to analyze sinus rate over time (Ori, Monir, Weiss, Sayhouni, & Singer, 1993) The basic signal used in time-
domain analysis is a series of intervals labeled as $T_1$, $T_2$, etc. and derived from measuring normal R-R intervals. This series characterizes variability in sinoatrial node firing (Pieper & Hammell, 1995). Time-domain measures can be divided into those measures derived directly from R to R intervals and those measures based on the difference among successive cycle lengths.

Measures based on the differences between consecutive cycles (eg. RMSSD, pNN50, pNN6.25%) capture very short term variability which, due to the pharmacological properties of acetylcholine, can only be attributable to vagal activity. Measures derived from beat-to-beat intervals (eg. SDNN, SDANN, SDANNindex) reflect variability due to both parasympathetic and sympathetic influences.

**Frequency-Domain Measures**

The basic signal for frequency domain analysis in a wave form that can be described as an "interval function". This wave form has an amplitude and frequency that varies according to the values of a time series derived from an electrocardiograph. Frequency-domain analysis assumes that this interval function is the sum of several sinusoidal component signals of various amplitudes and frequencies. Fourier transformation converts an RR interval trend plot (interval length plotted against time) from the time-domain to the frequency-domain and produces a graphic depiction of the component signals that make up the interval function called the power spectrum. These component signals are modulated by autonomic nervous system input. The three most commonly discussed peaks in the power spectrum correspond to the peaks described by Akselrod (1981) and
encompasses the power spectrum from 0 to .5 Hz. The high-frequency component is almost completely the product of parasympathetic tone and is strongly affected by respiration. The mid- and low-frequency components are products of both the parasympathetic and sympathetic system (Pieper & Hammill, 1995). Baroreceptor reflexes and renin-angiotensin system influence mid- and low-frequency components (Akselrod, Gordon, Ubel, Shannon, Barger, and Cohen, 1981).

The ratio of low to high to low frequency power (LF/HF) is generally accepted as representing sympathetic/vagal balance. The power of individual components of the power spectrum is usually computed as the area under the appropriate curves (Ori, Monir, Weiss, Sayhouni, & Singer, 1993).

Perturbed Autonomic Tone

Over recent years two possibly complementary perspectives have developed on the interpretation of perturbations in autonomic tone (diminished HRV). A strong argument for a mechanistically relevant link between perturbed autonomic tone and arrhythmogenesis and sudden cardiac death (SCD) has been make. This interpretation holds that perturbed autonomic tone can be understood as a functional factor that increases electrical instability and triggers both atrial and ventricular arrhythmias in susceptible hearts (Myerburg, Kessler, Bassett, & Castellanos, 1989). Conserved vagal tone produces an antiarrhythmic, antifibrillatory effect. Relative and absolute increases in sympathetic activity support arrhythmogenesis, fibrillation, and sudden cardiac death (Schwartz, Rovere, & Vanoli, 1992). In the past 15 years
methodological advances have supported the measurement of baroreflex sensitivity (BRS), heart rate variability, and adrenergic activity. The results of this research contain evidence for a strong link between perturbed autonomic tone and arrhythmogenesis (Myerburg, Kessler, & Castelannos, 1992).

However, the relationship between the ratio of parasympathetic and sympathetic input and arrhythmia is not as clear cut as this argument implies. Maximally depressed vagal tone has been inconsistently documented prior to fibrillation in monitored patients in the clinical setting (Singer, et al., 1988; Vybiral, et al, 1993). Bigger et al., (1992) reported that diminished power in the VLF and ULF bandwidths was a better predictor of arrhythmic death than either HF or LF power. Although all frequency-domain measures of HRV were significantly related to arrhythmogenesis, Bigger’s findings suggest that the relationship between reduced HRV and arrhythmogenesis is not completely defined by vagal withdrawal (Malik & Camm, 1993).

Therefore, some authors have suggested that HRV may be a serendipitous marker for risk of all-cause mortality (Malik & Camm, 1993). Heart rate variability may index the functional state of the CNS and/or the integrity of the cerebral cardioregulatory centers (Waddington, Macculloch, & Sambrooks, 1978), the presence of coronary artery disease, or a fortunate combination of these and several other factors (Malik & Camm, 1993). Psychological factors associated with various clinical states and manifested as reduced cardiovascular reactivity, may complement physiological mechanisms and enhance alterations in HRV.
Depressed HRV may be a mechanistically relevant risk factor for arrhythmogenesis or it may be a marker not only for arrhythmic risk but also for all-cause mortality. This possibility does not diminish the clinical utility of estimates of HRV as a marker of increased arrhythmogenic risk. Further studies specifying the psychological as well as physiological components of heart rate variability will hone the prognostic significance of estimates of HRV (Malik & Camm, 1993). The commonality in these two perspectives is the conclusion that perturbed autonomic tone, operationalized as altered HRV, is a clinically useful index for risk stratification.

Linking Perturbed Autonomic Tone, Supraventricular Arrhythmia, Ventricular Arrhythmia, and All-Cause Mortality

The published literature linking perturbed autonomic tone to both arrhythmia and all-cause mortality will be summarized in the following section. The linkage among these concepts will be substantiated by a discussion of the relationship of perturbed autonomic tone and supraventricular arrhythmias and ventricular arrhythmias (including ventricular fibrillation and SCD). This discussion will summarize the laboratory and clinical data supporting this relationship. Finally, the utility of HRV as a non-specific marker of all-cause mortality will be reviewed (see Figure 2).
Supraventricular Arrhythmia

Alterations in autonomic tone, specifically increased sympathetic tone, have been associated with inappropriate sinus tachycardia (Morillo, et al., 1994) and paroxysmal atrial fibrillation (Coumel, Maison-Blanche, & Catuli, 1994). Atrial disease is characterized by initial vagal withdrawal and an increased sensitivity to adrenergic stimulation and increased sympathetic drive. In young populations, with healthy hearts, increased vagal tone may precipitate macro-reentrant atrial fibrillation. In the older populations, with hearts compromised by normal aging or pathological changes, micro-reentry, automatic, and triggered activity are facilitated by increased
sympathetic tone. The failure to consider autonomic tone in treating atrial fibrillation may explain cases that are resistant to traditional treatment with vagomemetic drugs (Coumel, 1994).

**Ventricular Arrhythmia/Sudden Cardiac Death**

A significant body of literature has evolved over the past 15 years linking perturbed autonomic tone and ventricular arrhythmias. It is well accepted that perturbations in autonomic influences can function as a triggering event for potentially lethal arrhythmias.

**Laboratory Data**

A well established canine model of sudden cardiac death has been used in a series of experiments that indicate that perturbed autonomic tone is a significant risk factor for ventricular arrhythmia and sudden cardiac death. This ecologically valid model superimposes transient sympathetic stimulation (submaximal exercise and transient ischemia) on an arrhythmogenic substrate (healed anterior myocardial infarction). Dogs resistant to ventricular fibrillation have demonstrated a decreased heart rate in response to sympathetic stimulation. Conversely, dogs susceptible to fibrillation have demonstrated a distinct increase in heart rate in response to sympathetic stimulation. Differences in heart rate between susceptible and resistant dogs were eliminated by atropine, indicating that the essential difference between the dogs was mediated by a powerful vagal reflex (Billman, Schwartz, Gangnol, & Stone, 1985; Schwartz, Billman, & Stone, 1984).
Assessment of the phasic component of autonomic tone (i.e. baroreceptor sensitivity) indicates that the vagal contribution to the baroreflex is lower in dogs susceptible to ventricular fibrillation compared to resistant dogs. This is true both before and after experimentally induced myocardial infarction. Thus, the ability to reflexively increase vagal tone is diminished in susceptible dogs prior to infarction (Schwartz, Billman, & Stone, 1988).

Assessment of the tonic vagal component of autonomic tone (i.e. HRV) does not permit discrimination between resistant and susceptible animals prior to infarction but, postinfarction relatively greater decreases in HRV and slow HRV recovery times do differentiate dogs at risk for fibrillation and arrhythmic death from resistant dogs (Adamson et al., 1994; Hull, Evans, Vanoli, et al., 1990; Schwartz, Billman, & Stone, 1984).

Recent studies have demonstrated that dogs resistant to fibrillation during transient ischemia induced during submaximal exercise in the postinfarction condition, had notably greater preinfarction increases in HRV in response to β-blockade than dogs fated to be susceptible to fibrillation postinfarction. Therefore, β-blockade may reveal individual differences in tonic vagal activity rather than produce an actual increase in vagal activity in dogs resistant to the fibrillatory affect of increased sympathetic input (Adamson et al., 1994). In the same study, postinfarction β-blockade hindered the development of fibrillation but did not ameliorate decreased HRV in either group of dogs (Adamson et al., 1994).
Augmentation of vagal tone by electrical stimulation of the right vagus dramatically decreased the incidence of fibrillation in susceptible dogs during an exercise and ischemia trial (Vanoli, et al., 1991). Similar effects have been attributed to the muscarinic agonist oxotremorine after stimulation of feline stellate ganglion (De Ferari, Vanoli, Curcuruto, Tommasini, & Schwartz, 1992). Endrophonium, an acetylcholinesterase inhibitor, has an antifibrillatory effect in canines with a confirmed susceptibility to fibrillation (De Ferrari et al., 1989). Vagal tone recovered faster in resistant dogs compared to susceptible dogs post infarction and returned to baseline within 10 days (Adamson et al., 1994).

Clinical Data

An association between perturbed autonomic tone, operationalized as altered HRV, and arrhythmic mortality has been reported in numerous clinical studies. Several large-scale Holter EKG projects have demonstrated that altered HRV is an independent predictor of sudden cardiac death, is associated with total cardiac mortality, and is a valuable tool for risk stratification among survivors of acute myocardial infarction (Bigger, et al., 1992; Farrell, et al., 1991; Kleiger, Miller, Bigger, Moss, et al., 1987; Odemuyiwa, Poloniecki, Malik, et al., 1994).

The seminal article in this series documented that a 24 hour SDNN of less than 50 ms was associated with a relative risk of death over 5 times greater than that associated with a SDNN greater than 100 ms (Kleiger, Miller, Bigger, Moss, et al., 1987). A secondary analysis of these tapes revealed that frequency domain estimates of HRV also had significant univariate associations with both arrhythmic death and
total cardiac death. These associations were stronger in the low frequency bandwidth and
the total power and VLF power was uniquely associated with sudden death (Bigger, et al., 1992).

Overall, these electrophysiological, pharmacological, and clinical studies make
a strong argument that perturbed autonomic tone is a functional factor that increases
cardiac electrical instability and decreases the threshold for arrhythmia development
and that parasympathetic influences are a major factor protecting some hearts from
arrhythmogenesis.

All-Cause Mortality

The findings from several large population based projects provide evidence of
a link between alterations in HRV and all-cause, as well as arrhythmic, mortality,
(Dekker, et al., 1997; Tsuiji, et al., 1994). Clinical studies also provide evidence
that HRV is a sensitive predictor of both arrhythmic and all-cause mortality (Bouwer,

The autonomic nervous system innervates every organ in the body.
Researchers have suggested that HRV may be useful in monitoring the development
of autonomic neuropathy and stratifying risk in a variety of clinical states including,
stroke, diabetes, cancer, glaucoma, multiple sclerosis, obesity, and sleep apnea
(Kleiger, Stein, Bosner, & Rottman, 1992). Therefore, as suggested by Malik &
Camm (1993), perturbed autonomic tone, indexed as HRV, may be a non-specific
indicator of poor health in community and clinical populations and be a valuable
indicator not only of arrhythmic mortality but all-cause mortality.
Linking Aging and Other Concepts of the Model

The state of knowledge development linking aging and the other model components will be summarized in the following sections. Initially, aging will be defined and the contemporary theoretical perspective on aging will be presented. Then, the linkages among aging and the other concepts of the model will be substantiated by a discussion of the effect of aging on autonomic tone and HRV and the possible consequences of these perturbations of autonomic tone: increased frequency and complexity of supraventricular arrhythmias and increased frequency of ventricular arrhythmias (including ventricular fibrillation and SCD) (see Figure 3).

Terminology

Aging

The unique essence of individual aging is determined by the interaction of biological, psychological and sociological components (Birren & Schroots, 1984). Biological aging is defined as the process of decline that begins after reproductive maturity and can be conceptualized as the gradual loss of the range and
complexity of the physiological function that supports adaptation to stress (Kaplan, Furman, Pincus, Ryan, Lipsitz, & Goldberger, 1991; Strehler, 1959).

True aging changes have been traditionally characterized as universal, intrinsic, deleterious, and progressive (Strehler, 1959). Aging is distinguished from disease and environmentally determined change. Aging increases the probability of death over time (Comfort, 1979; Strehler, 1959) and is commonly operationalized as years since birth.
Theories of Aging

Historically biological theories of aging have targeted specific age-related changes at isolated levels of organismic organization. The implicit presumption unifying these theories is that the structural and functional changes associated with aging result in a decreased ability to adapt to stress (see Table 1).

<table>
<thead>
<tr>
<th>Level</th>
<th>Theory</th>
<th>Theorist (Year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecule</td>
<td>Error Theory</td>
<td>Menvedev (1961)</td>
</tr>
<tr>
<td>Organelle</td>
<td>Mitochondria</td>
<td>Harmen (1983)</td>
</tr>
<tr>
<td>Cell</td>
<td>Finite Cellular Replication</td>
<td>Hayflick (1961)</td>
</tr>
<tr>
<td>Tissue</td>
<td>Collagen Theory</td>
<td>Verzar (1957)</td>
</tr>
<tr>
<td>Organ</td>
<td>Cardiac Aging</td>
<td>Multiple</td>
</tr>
<tr>
<td>Organ System</td>
<td>Neuroendocrine Theory</td>
<td>Frolkis (1968)</td>
</tr>
<tr>
<td>Organism</td>
<td>Evolutionary Aging</td>
<td>Weisemann (1881)</td>
</tr>
</tbody>
</table>

Table 1: Organizational Level and Biological Theory of Aging

More recently the multifactorial etiology of aging has been recognized and aging has been acknowledged to be a gradual process that is the total expression of multiple causes in multiple subsystems (Olson, 1987). The traditional view that aging is a compendium of universal, intrinsic, deleterious, and progressive changes (Strehler, 1959) has been extended to emphasize that these changes increase the probability of death by damaging the organism’s complex hierarchy of control mechanisms. These changes limit the individual’s range and repertoire (i.e. the
variability) of physiologic responses to stress and the ability to effectively co-ordinate and integrate these physiologic responses to achieve dynamic homeostasis (Lipsitz & Goldberger, 1992).

**Aging, Autonomic Tone, and Heart Rate Variability**

Aging is associated with significant central and peripheral alterations in both sympathetic and parasympathetic function at every level of the autonomic nervous system (Besse et al., 1997; Roberts, Snyder, Johnston, & Horiwitz, 1993) Normal age-related ANS changes alter temperature, blood pressure, and heart rate regulation as well as urinary function, bowel motility, and carbohydrate metabolism and are associated with significant increases in morbidity and mortality among older adults (Schmidt, Choe, Parvin, & Roth, 1990). Functional age-related impairment in autonomic control is frequently superimposed on the significant age-related and pathological structural changes at all levels of the myocardium (Besse et al., 1993; Josephson & Fannin, 1994).

**Intrinsic Cardiac Mechanisms**

A gradual increase in the amount of fibrous tissue and a coincident progressive decrease in the in the volume myocytes in the SAN occurs during aging. At age 70 only about 10%-40% of the SAN is functional tissue. The number of normal atrial fibers in the perinodal area decrease and the there are increases in amyloid and fat deposits. Non-athlersclerotic fibrosis of the sinus node artery intima and medial fibrosis narrow the arterial lumen. Although age related changes are not necessarily associated with electrocardiographic or pathological abnormalities (Titus & Edwards,
1993), neurochemical transmission may be impaired by the binding of the neurotransmitters to collagen (Roberts, Johnson, Snyder & Horiqitz, 1993).

Age-related changes in membrane proteins in the SAN have a direct role in autonomic control of sinus node function. Both adrenergic and vagal influences are attenuated in the senescent heart. The density of both $\beta_1$ and $M_2$ receptors is reduced in aging. A disproportionate number of $M_2$ receptors are lost and the effect is an increase in the ratio of beta-adrenergic to muscarinic receptors (Besse, et al., 1994).

Attenuated HR and HRV responses to exercise and autonomic blockade in elderly males have been reported and double autonomic blockade has shown that age-related changes in HRV in the supine position may be totally attributable to autonomic influences at the SAN (Craft & Schwartz, 1995). Age-related changes in intrinsic cardiac mechanisms are compatible with the finding that aging is accompanied by an overall diminution in HRV as well as an increase in the HF/LF ratio.

**Medullary Cardiovascular Reflexes**

Arterial baroreceptor sensitivity is diminished with age. Heart rate responses to phenylephrine and nitroprusside as well as postural tilt are attenuated in the elderly. This attenuation contributes to the sympathetically mediated compensatory increase in blood pressure and a concomitant increase in serum norepinephrine that is associated with aging (Pfeifer, et al., 1983). Age-related impairment of cardiopulmonary reflexes has also been documented (Giannattasio, Ferrai, & Mancia, 1994). The mechanism of age-related decreased baroreceptor and cardiopulmonary function is not well defined but, may occur at any level of the reflex arc.
Afferently, age-related decrease in arterial compliance may result in reduced efficiency of carotid and aortic stretch receptors (Giannatasio, Ferrari, Mancia, 1993). Age-related decreases in arteriolar compliance are associated with reduced cardiopulmonary reflex influence on the SAN (Meersman, 1993). The number, size, and conduction velocity of peripheral sympathetic nervous system neurons decreases with aging. Scattered axonopathy has also been documented in both sensory and effector neurons (Schmidt, Chae, Parvin, & Roth, 1990).

Centrally, aging is associated with a degeneration in the intermediolateral preganglionic neurons (Schroer, Plurad, & Schmidt, 1992). Excitation delays (Johnson & Felder, 1993), and decreases in both serotonergic inhibition of sympathetic tone and serotonergic enhancement of vagal tone have been documented in the NTS of aged rats (Itoh & Bunag, 1992). Impairment of GABA-ergic regulation of cardiovascular function has also been documented in NTS of aged rats (Mollace, De Francesco, Fersini, Nistico, 1991).

Efferently, both adrenergic and muscarinic receptor response undergo age-related changes. β-receptor density, affinity for agonists, and post-receptor functions are all decreased by aging and probably contribute to the decreased sensitivity to adrenergic stimulation and to the decreased effects of β-adrenergic antagonists such as propranolol. The mechanism of diminished muscarinic response is not well defined (Roberts, Snyder, Johnson, & Horowitz, 1993).

Supramedullary Factors

Older adults demonstrate less heart rate reactivity (i.e. change from baseline heart rates) in response to stress than other age groups (Turner, 1994). Studies in the rat model have demonstrated that marked HR slowing and immobility are the characteristic responses of young animals exposed to stimuli previously associated
with painful experiences. Older animals also respond with immobility but frequent extrasystoles, not bradycardia, are commonly recorded. This age-associated diminished bradycardic response to emotional stress associated with aging in the rat may be related to a central or efferent impairment of vagal tone (Nyakas, Prins, Bohus, 1990).

In humans, psychological and physical stress is associated with increases in heart rate. In a recent study of cardiovascular responses to the Stroop task, elderly male subjects had higher absolute heart rates but smaller relative responses during and immediately after the task. Thus, the authors speculated that aging may be characterized by diminished HR reactivity and augmented cardiovascular stress (Boutcher, & Stocker, 1996).

Hormonal Influences

Increased NE synthesis and decreased renal NE clearance produce higher serum NE levels in older adults. However, the effects of this increase may be balanced by the decreases in β-adrenergic responsiveness that accompanies aging (Johnson, 1993).

Aging and Heart Rate Variability

The relationship between aging and HRV is well defined up to the young adult years. HRV increases through the prenatal period, infancy and early childhood. HRV begins a linear decline somewhere between the ages of 5 and 10 that continues to early adulthood. After age 30 to 35 the shape and direction of the relationship between aging and HRV is incompletely defined. Physiological aging changes are
consistent with the hypotheses that all estimates of HRV decrease with aging and that the shift toward sympathetic predominance and the concomitant withdrawal of vagal tone that accompanies aging augments the HF/LF ratio. Overall, these hypotheses are supported by the literature.

Early studies identified an inverse relationship between aging and HRV (Hellman & Stacey, 1976; Hirish & Bishop, 1981; O'Brien, O'Hare, Corrall, 1986; Schloka, 1937; Waddington, MacCulloch, & Sambrooks, 1978). However, attempts to integrate these studies are hampered by inconsistencies in sample composition, length or R-R recording, measures of HR, and research design that produce seemingly conflicting results. While the direction of the relationship is reported as grossly inverse, the shape of the relationship has been variously described as linear (Hellman & Stacey, 1976), curvilinear (Waddington, MacCulloch, & Sambrooks, 1978), and non-linear (O'Brien, et al., 1986).

A closer look at sample composition may explain some of these inconsistencies. Older subjects in several of these studies were recruited from hospitals or adult day care centers. Younger subjects were described as factory workers or volunteers. Since many clinical states are associated with decreased HR, sample selection may have artificially increased supposed age-associated decrements in HRV among older subjects. Similarly several studies report samples composed of males and females but do not report possible gender effects on HR. O'Brien notes that women comprised the preponderance of subjects in the 70-85 year range and that this precluded an analysis of gender effects for subjects over 70.
In general lower estimates of LF HRV and total power and higher estimates of HF HRV have been documented in women compared to men (O'Brien, et al., 1986; Ryan, Goldberger, Pincus, Mietus, Lipsitz, 1994). A disproportionate distribution of women among the oldest subjects could also alter the shape of the relationship between aging and HRV and produce paradoxical results when compared to all male samples. Bruggerman, et al., (1992) reported that gender had a significantly greater influence on HRV than age.

At least one study failed to describe a significant association between age and HRV after age 35 (Hirsch & Bishop, 1981). The sensitivity of the idiosyncratic RSA measure used in the study may explain this failure. Shannon, Carley, and Benson (1992) reported that age was not significantly associated with the high frequency component of the power spectrum (i.e. the respiratory frequency) after age 28 while the low frequency component (sympathetic and non-respiratory parasympathetic) continued to decline linearly to age 62. The authors suggest that there may be differential aging rates among the various components of heart rate variability. However, current studies using spectral analysis have reported declines in total power, high frequency, and low frequency power across all age groups (Lipsitz, Mietus, Moody, Goldberger, 1990; Piccirrilo, Fimognari, Viola, Marigliano, 1995; Ryan, Goldberger, Pincus, Mietus, & Lipsitz, 1994; Schwartz, Gibb, & Tran, T., 1991).
The changes in HRV that accompany aging have significant prognostic significance. Standard estimates of HRV obtained by ambulatory Holter EKG as part of the Framingham study indicate that VLF power, LF power, HF power, and standard deviation of the RR intervals (SDRR) were all associated with increased risk of all-cause mortality in elderly subjects. LF power had the strongest association with all-cause mortality with a 1 standard deviation decrease in LF carrying a 1.70 greater hazard for death (Tsuji, et al., 1994).

**Aging, Supraventricular Arrhythmia, and Ventricular Arrhythmia, and All-Cause Mortality**

The prevalence, incidence, frequency, and complexity of arrhythmias at all levels of the myocardium are increased in older adults. These increases are amplified by the clinical and subclinical heart disease that is associated with aging. The relationship between aging and both supraventricular and ventricular arrhythmias will be described in the next two sections.

**Supraventricular Arrhythmias**

Several Holter EKG studies focusing on healthy community-dwelling subjects have documented an increased prevalence of all classes of supraventricular arrhythmias among normal older adults (Frishman et al., 1996; Furberg, et al, 1992; Manyari, et al., 1990). Atrial premature beats have been were documented in up to 93% of subjects (Frishman et al., 1996). Major abnormalities were documented in 19% of non-hypertensive subjects free of CAD and in 37% of functional, free living elders with documented history of CAD and/or hypertension (Furberg et al., 1992).
The frequency and complexity of these arrhythmias increased with age. Non-sustained atrial tachycardia, atrial fibrillation, and atrioventricular blocks were detected most commonly (Frishman, et al., 1996). A large population based study of healthy older adults detected atrial fibrillation in 1.6% of subjects who were free of clinical or subclinical cardiovascular disease. Baseline atrial fibrillation rates of 4.6% for subjects with subclinical cardiovascular disease only and, 9.1% for subjects with clinical heart disease were also reported. Within the sample, age was independently associated with increases in prevalence of atrial fibrillation (Furberg, 1994). These rates are consistent with the overall literature (Podrid, 1997).

Individuals with chronic atrial fibrillation have a 5-7 times greater risk of stroke than comparable individuals in normal sinus rhythm (Peterson, 1990). Thirty years of data from the Framingham Heart Study indicates that atrial fibrillation makes a significant contribution to the incidence of stroke in the elderly. The percentage of strokes attributable to atrial fibrillation increased from 6.7% for subjects in the fifth decade of life to 36.2% for subjects during the eighth decade of life. During this age span, the association of heart failure, CAD, and hypertension with stroke declined (Wolf, Abbott, & Kannel, 1987).

Atrial fibrillation is also indirectly associated with increased morbidity and mortality among the elderly. Both animal and human studies have demonstrated that atrial fibrillation causes decreased cerebral perfusion, especially during diastole (Friedman, O'Connor, Kottmeier, Shaugnessey, & McGuire, 1987; Gomez, McLaughlin, Njemanze, Nashed, 1992; Peterson, 1990). This diminished capacity to
sustain cerebral blood flow may explain the increased infarction size documented in atrial fibrillation associated strokes (de Falco, et al., 1991) and contribute to the incidence of fall related injury and cognitive impairment among older adults.

**Ventricular Arrhythmia/Sudden Cardiac Death**

The prevalence of premature ventricular depolarizations is also increased with aging and premature ventricular depolarizations are commonly detected during Holter EKG monitoring. The Cardiovascular Health Study documented non-sustained ventricular tachyarrhythmias in 10.3% of older male and 4.3% of older female subjects (Manolio, et al., 1994). While the prognostic significance of ventricular arrhythmia in the elderly is uncertain (Podrid, 1997), one study estimated that non-sustained ventricular tachycardia in the older adult carried relative risk for death of 2.8. (Frishman, et al., 1996). The rates of SCD increase dramatically with age (Manolio et al., 1994).

Similar to other populations, a circadian variation in cardiac mortality (Nicolau, Haus, Popescu, Sackett-Launste, & Petrescu, 1991) and arrhythmia occurrence have been documented in the elderly (Kupari, Koskinen, Leinonen, 1990). For example, supraventricular reentry tachycardia, atrial flutter, and ectopic atrial tachycardia exhibit a bimodal distribution with incidence between 600 and 1200 and 1800 and 2400. These peaks were modified in subjects treated with β-blockers (Kupari, Koskinen, Leinonen, 1990) and are coincident with previously reported circadian peaks in LF power of HRV. The lowest level of arrhythmia onset was between 2400 and 600 and coincident with the peak for HF power (Kupari, Koskinen,
Leinonen, 1990). These facts infer that the autonomic component of arrhythmogenesis is significant in the elderly.

Aging and All-Cause Mortality

Aging, by definition, is associated with an increased likelihood of death and increased incidence of most causes of mortality (Comfort, 1979; Strehler, 1959). As discussed above, perturbed HRV may be a mechanistically irrelevant marker for risk of all-cause mortality as well as arrhythmic risk.

Proportional hazards regression analyses of two-hour recordings obtained on 736 elderly Framingham study subjects (mean age 72) revealed that a one standard deviation decrease in LF power carried significantly increased risk of all-cause mortality. All frequency domain measures and the SDNN were significantly correlated with all-cause mortality (Tsuiji, et al., 1994). Cancer was the most common cause of death during the 3.9 year follow up period. These findings are similar to those of the Zutphen Study where a decreased SDNN carried between a 1.4 relative risk of death for middle-aged men and a 2.1 relative risk for elderly men. Again cancer was a significant cause of mortality among the sample (Dekker, et al., 1997). The complex genesis of HRV may explain its utility as a non-specific marker of poor health. Aging is characterized by an increase in chronic disease rates, increased incidence of acute illness, and a decrease in the range and repertoire of responses to these challenges. Therefore, for the elderly, altered HRV may be an especially valuable prognostic indicator of risk.
Linking Alcohol and Other Components of the Model

The next section will initially define alcohol and the behaviors: alcohol abuse, dependence, and misuse. Then the concept of alcohol consumption will be integrated with the other concepts of the model (see Figure 4).

**Terminology**

An alcohol is a compound that contains a hydroxyl functional group (-OH) bonded to a saturated carbon chain. Ethanol is an alcohol comprised of a two carbon backbone and a hydroxyl group (Ouellette, 1990). Because of its low molecular weight and its amphiphilic characteristics, alcohol can easily diffuse through any biological membrane, is miscible with both water and lipids, and equilibrates rapidly through the body (Kalant & Khanna, 1989).

Alcohol is oxidized to its major metabolites, aldehyde and then acetate, via one or more of the following metabolic pathways: (1) Alcohol dehydrogenase (ADH) found in the gastric mucosa and liver, (2) the microsomal ethanol-oxidizing system (MEOS) located in the endoplasmic reticulum, (3) catalase found in the cytosol (Kalant & Kahanna, 1989). Although there are between and within individual
differences in the metabolism of ethanol, on average, a 70 kg man can absorb and
distribute a dose of ethanol so that a peak blood ethanol concentration (BEC) is
achieved in 30 to 90 minutes. This dose can be oxidized at a rate of about 10 g, or
about 1 standard drink per hour and results in a constant decline about 15 - 20
mg/dL/hour from peak BEC. Similar figures for women are not available.

A plot of BEC over time yields a non-linear blood alcohol curve (BAC) with
an ascending and descending arm. Any BEC can be associated with two time values
on this curve. Both BEC and the its location on the BAC affect alcohol-related events. For example, intoxication is greatest on the ascending limb of the BAC (Kalant & Kahana, 1989). Functional tolerance, decreased performance impairment at a given BEC during one episode of alcohol use, occurs on the descending limb of the BAC.

Definitions

The inconsistent and imprecise use of the terms alcohol abuse, alcohol dependence, alcohol misuse, and alcoholism hamper attempts to integrate findings across studies and develop new hypotheses related to alcohol consumption. The use of certain terms (eg., "alcoholism") no longer reflect prevailing political or social values. While use of these terms is no longer considered appropriate, several were formerly pervasive in the literature and, for the purposes of this review will be reported as used in cited research. Otherwise, the following definitions will be used:

(1) **Alcohol Dependence** is evidenced by continued drinking despite the knowledge that significant alcohol-related problems have occurred. The salient features of substance dependence are tolerance, withdrawal, and/or compulsive drinking behavior. **Alcoholism** and alcohol dependence are considered to be equivalent terms.

(2) **Alcohol Abuse** is identified by continued drinking despite knowledge that significant alcohol-related problems have occurred but, without tolerance, withdrawal, or compulsive drinking behavior. Alcohol abuse and alcohol dependence are mutually exclusive behaviors.
Alcohol Misuse is defined by the inadvertent or unknowing use of alcohol in a way that is currently causing or may cause alcohol-related problems in the future. These definitions incorporate the significant commonalities of the Diagnostic and Statistical Manual of Mental Disorders (DSM) from DSM-III (1980) through DSM-IV (1994). The exception to this generalization is mutual exclusivity of the terms alcohol abuse and alcohol dependence. Alcohol abuse did not become a default diagnosis until DSM-III-R (1987). These definitions are purposefully broader than the DSM-IV (1994). Current DSM-IV criteria have evolved over decades and, while diagnostically precise, do not accurately reflect the use of these terms prior to 1994.

Theories of Alcohol Abuse and Alcohol Dependence

Contemporary theories of alcohol abuse and dependence speculate that alcohol reinforces its own use via stress reduction or mood enhancement (Reid & Carpenter, 1990). In the absence of identifiable "alcohol" receptors in the brain, it has been assumed that the neurobiology of alcohol involves multiple mechanisms that affect nearly all neurons (Hunt, 1993). The effect of alcohol on the brain's opioid peptide system has a pivotal role in explaining why some people continue drinking despite the knowledge that significant alcohol-related problems have occurred.

The relationship between reward and behavior is the foundation of learning theory and is assumed to play a pivotal role in the use and abuse of alcohol (Koob, Rassnick, Heinrichs & Weiss, 1994). Olds and Milner's (1954) classic work with intercranial self-stimulation in rats suggested that specific areas in the brain are involved in pleasure. This work laid the foundation for contemporary research related
to opioids and brain reward (Izenwasser & Kornetsky, 1992). Although the relationship between alcohol and the brain's opioid peptide reward system is not completely understood (Terenuis, 1994), it has been convincingly argued that opioid peptides play a role in the reinforcing effects of alcohol and the development of dependence (Koob, Rassnick, Heinrichs, & Weiss, 1994).

The development of two genetically distinct strains of rats, P rats (preferring alcohol) and NP rats (non-preferring alcohol), support the hypothesis that the reward associated with alcohol is mediated, at least in part, by the opioid peptide system. P and NP rats vary in tonic and phasic enkephalin levels in the mesolimbic region (Li, Li, & Froehlich, 1993). P rats have higher levels of hypothalamic and striatal Met-enkephalin than NP rats (Froehlich, Harts, Lumeng, & Li, 1987).

Pharmacological manipulations also support the hypothesis that endogenous opioids maintain alcohol consumption. The opioid receptor antagonists naloxone and naltrexone decrease alcohol intake in rats (Froehlich, Harts, Lumeng, & Li, 1987), monkeys (van Ree, Kornet, Goosen C., 1994), and humans (Volpicelli, Obrien, Alterman, & Hayashida, 1990). The delta opioid receptor antagonist ICI 174864 also reduces alcohol intake (Froelich, Zweifel, Harts, Lumeng, & Li, 1991). Subjects who consumed alcohol after taking the opioid antagonist naloxone reported that they experienced a diminished "high" compared to routine experiences after alcohol consumption without naloxone (Volpicelli, personal communication, as cited in Froehlich & Li, 1992).
The Opiod Deficiency Hypothesis and the Opiod Surfeit Hypothesis represent current perspectives on the role of the opioid system in the reinforcement of alcohol consumption. The former theory asserts that alcohol misuse is motivated by a deficiency in basal endogenous opioid levels and that alcohol consumption mediates an increase in the activity of opioid receptors. This hypothesis supports the disease model of alcohol abuse and drive reduction models of motivation (Reid et al., 1991). The latter perspective asserts that the compulsive drinking behavior that characterized alcohol dependence is a result of surplus opioid activity (Reid et al., 1991). While both theories are driven by a putative alteration in endogenous opioid activity, they have diametrically opposing implications for prevention and pharmacological intervention.

Recent findings support the Opiod Surfeit Hypothesis (Reid et al., 1991). However, these two hypotheses do not exhaust all of the possible relationships between the opioid system and alcohol consumption. A deficit in dynorphin modulation of mu receptors, rather than a deficit or excess of endogenous opioids may drive the relationship between alcohol and reward (Terinius, 1994). Additionally, the impact of other neurotransmitter systems (e.g. the serotonergic system) must be considered in any explanation of alcohol's reinforcement effects. Alcohol abuse/dependence is biopsychosocial phenomenon with multiple determinants. Increased reactivity of the opioid system in response to alcohol consumption may support a broader theory of opioid related consummatory behavior (Koob & Weiss, 1990). However, it is clear that answers to the question "Why do some people
continue drinking despite the knowledge that significant alcohol-related problems have occurred?", must recognize the importance of the opioid system.

Measurement of Alcohol Consumption

The only certain marker of alcohol consumption is the detection of ethanol in body fluids (e.g., serum, saliva, or urine) or expired air, but such measures are only useful for a few hours after alcohol consumption and do not accurately reflect chronic consumption patterns (Rommelspacher & Muller, 1995; Sobell & Sobell, 1992). Although several biological indicators for chronic alcohol consumption have been examined, no one value has sufficient precision to be useful for screening ambulatory populations for alcohol abuse or dependence (Roseman & Lieber, 1992).

In the absence of any practical "gold standard" for chronic alcohol consumption, self-report has become the most common research and clinical method of measuring long-term alcohol consumption. It is frequently the only measure of alcohol consumption in the epidemiological and clinical literature (Fuller, Lee, & Gordis, 1988).

Self-report measures can be categorized as either: 1) the product of quantity and frequency (QF) or 2) standardized questionnaires. Calculation of alcohol consumption rate, the primary independent variable in this dissertation project, was based on a QF measure of self-reported alcohol consumption. Since no single measure of alcohol consumption is considered irreproachable, convergent estimates of consumption are useful in validating self-reported alcohol consumption (Babor, Stevens, & Marlatt, 1987; Sobell, & Sobell, 1980). The concurrent validity of the
aggregate QF measure used in this project was established by comparing alcohol consumption rate with scores on a standardized questionnaire (the T-ACE) and two biological markers, \(\gamma\)-glutamyl transpepsidase (GGT) and mean corpuscular volume (MCV), known to be influenced by alcohol consumption. QF measures require a summary description of drinking pattern and are the primary measures reported. However, QF measures homogenize drinking patterns and mask periodic heavy and binge drinking. Periodic heavy drinking is associated with greater physical and social problems than the steadier consumption of an equal amount of alcohol. Aggregate measures also tend to produce lower levels of reported drinking (Sobell & Sobell, 1992). Therefore, the relationship between MCV and GGT and self-reported levels of alcohol consumption was used to approximate the upper-bounds of alcohol consumption in the sample.

The T-ACE is a standardized questionnaire originally developed to detect "at risk drinking" in pregnant women. The chronicity or pattern of consumption over time is a dimension of alcohol use that is frequently overlooked in investigations of alcohol effects. Typically subjects with drastically different drinking histories are lumped together or subjects are simply described as "alcoholics". This practice obscures intragroup differences and consequently may mask the differential temporal exposure effects of alcohol consumption. Self-report measures should incorporate a valid measure of chronicity. Age at first use, the direction and pattern of any recent changes in consumption, and the use of a standardized questionnaire can support the measurement of chronicity.
Standardized questionnaires complement summary measures of alcohol consumption by appraising the physical, legal, and social consequences of alcohol consumption (Ford, Klag, Whelton, Goldsmith, & Levine, 1994). These tools effectively screen for lifetime prevalence of alcohol abuse and have been considered a surrogate for chronicity.

A standardized questionnaire should assess the consequences of alcohol abuse that characterize the population of interest. Tolerance and withdrawal effects set elderly apart from general population (Beresford, 1994) and are the salient features of alcohol dependence among older adults. Age-related physiological changes result in higher BECs for a given dose of alcohol among older adults when compared to middle-aged and young adults (Tupler, Hege, & Ellinwood, 1995). Moderate alcohol dosing is associated with greater performance impairment and increased subjective report of intoxication in older non-alcoholic subjects compared to young non-alcoholic subjects (Jones & Neri, 1985; Vogel-Sprott & Barrett, 1984; Roehrs, Baere, & Roth, 1992). Elderly alcoholics in withdrawal experience a greater number of withdrawal symptoms than younger alcoholics. These symptoms are more severe and require more medications than withdrawal symptoms experienced by younger alcoholics (Liskow et al, 1989; Bower, et al., 1994). Therefore, an increase in tolerance may be an singularly ominous indicator of early increases in alcohol intake in the elderly. Withdrawal symptoms may also have greater significance among older adults. Theoretically, questions specific to these two phenomena will increase validity of alcohol assessment questionnaires in the elderly.
The T-ACE is a concise screening questionnaire originally developed to detect "at risk drinking" in pregnant women (Sokol, Martier, & Ager, 1989) (see Appendix A). Like older women, pregnant women have a lower threshold for alcohol related harm and a lower point prevalence of alcohol abuse or dependence than other populations. A highly sensitive screening tool is critical for both populations. Although the T-ACE test has not been previously validated in older adults it is believed to measure the salient features of alcohol dependence in the elderly, tolerance (T) and withdrawal symptoms (Eye-opener), along with ramifications of drinking on personal relationships (Annoyed) and attempts to control (Cut down) on drinking.

The T-ACE differs from the CAGE by one question (Guilt). This substitution was made to avoid denial and underestimation of alcohol consumption levels and because discriminant analysis revealed that the Guilt question did not significantly increase the identification of at risk drinking (Russell, et al, 1994). Denial may be a particular problem in the current cohort of elderly who grew up sensing the social stigma associated with alcohol consumption that characterized the prohibition era (Beresford, 1995). While the CAGE has been effective in identifying elderly outpatients with a history of drinking problems (Buchbaum, Buchanan, Welsh, Centor, & Schnoll, 1992) and has proved more useful than the Michigan Alcoholism Screening Test (MAST) in detecting alcohol abuse and dependence in elderly medical outpatients (Jones, Lindsey, Young, Soltys, & Farani-Enayat, 1993), the CAGE has
not established adequate sensitivity to qualify as an independent screening tool for alcohol-related problems in the elderly or in middle aged-women (Adams, Barry, Fleming, 1996; Seppa, Koivula, & Sillanaukee, 1992). The T-ACE, by introducing the construct of Tolerance, should offer improved sensitivity for drinking problems in older adults, especially in older females who are markedly more sensitive to alcohol-effects than older males and prove to be an effective tool to establish concurrent validity of the independent variable, alcohol consumption rate, well as confirm chronicity of alcohol consumption.

**Laboratory Verification of Self-Report**

\[ \gamma \text{-glutamyl transpepsidase (GGT)} \] and mean corpuscular volume (MCV) are indices of biological variables and are routinely ordered during clinical evaluations. The availability of these measures made them attractive as possible screening tools for alcohol abuse/dependence and as potential measures of chronic alcohol consumption. However, these indices lack sufficient sensitivity, specificity, and precision to be used independently for screening or diagnostic purposes. However, these biological indicators have provided independent objective laboratory confirmation of self-report measures in diverse populations including middle-aged women (Seppa, Koivula, & Sillanaukee, 1992).

**GGT.** Alcohol consumption increases GGT synthesis, decreases biliary GGT secretion, and reduces hepatic GGT clearance (Rommelspacher & Muller, 1995). Elevations in GGT levels, above normal limits, are associated with alcohol-related liver damage. Abnormally elevated GGT activity has been detected in abstaining
abusers within 24-48 hours after acute consumption of 1g/kg (Nemesansky, Lott, & Arato, 1988). While acute one time alcohol dosing does not increase GGT levels in non-alcoholics (Cushman, 1992), steady consumption of four to six standard drinks per day will increase GGT values above normal limits in normal drinkers (Magruder-Habib, Durand, & Fry, 1991).

A weak positive correlation between GGT values in the normal range and self reported low-moderate chronic alcohol consumption has been reported (Papoz, et al., 1981). GGT values within the normal range were significantly reduced when non-alcoholic subjects decreased alcohol consumption from 21 standard drinks per week to five standard drinks per week (Puddey, Masarei, Vondongen, & Beilin, 1986). Other investigators have found that three drinks per day was the threshold of consumption for an alcohol-related affect on GGT values. GGT has a half-life of about two weeks and usually returns to normal level during two to six weeks of abstinence (Cushman, 1992). Thus, abnormal increases in GGT activity may be a useful marker for evolving alcohol abuse, relapse, or chronic alcohol-related hepatic damage. Absence of abnormally elevated GGT values corroborate self-report of abstinent to moderate alcohol consumption. Higher normal GGT values may be associated with chronic low level alcohol consumption.

**MCV.** Chronic alcohol consumption is associated with an increase in MCV. Macrocytosis may be a result of the direct toxic effect of alcohol on the cell membrane, the erythroid precursor cells, and/or bone marrow (Lindenbaum, 1992; Whelan, 1992; Wu, Chanarin, & Levi, 1974). Abnormally increased MCV is a
common hematological finding in alcoholics with adequate dietary folate intake (Wu, Chanarin, & Levi, 1974) and alcohol abuse is the most common cause of increased MCV in middle-aged general practice patients (Seppa, Laippala, & Saami, 1991). Although MCV values rarely exceed 110 fl in the macrocytosis of alcoholism (Seppa, Laippala, & Saarni, 1991), MCV is highly correlated with alcohol consumption (National Institute on Alcohol Abuse and Alcoholism, 1990). MCV has been reported to increase by approximately 1.7 fl for every standard drink consumed daily (Whitfield, Hensley, Bryden, & Gallager, 1978) and a direct relationship has been reported between low-moderate alcohol consumption (1-2 standard drinks per day) MCV values within the normal range (Popez et al., 1981). Due to the long half life of red blood cells and the remote effects of alcohol on the developing erythrocyte, the initiation of alcohol-related increases in MCV values is delayed and macrocytosis is sustained the initial months of abstinence (Rosman & Leiber, 1992). Thus, abnormally increased MCV values suggest chronic alcohol abuse. Upward trends in normal values may be associated with chronic low-moderate alcohol consumption and corroborate self-reported alcohol consumption.

Alcohol, Autonomic Tone, and Heart Rate Variability

Alcoholic neuropathy involves both isolated parasympathetic (vagal) and combined parasympathetic/sympathetic damage (Bartner & Tanner, 1987; Duncan, Lambie, Johnston, & Whiteside, 1980). Although the mechanism of alcohol-related perturbations of autonomic tone is not completely defined, any evidence of vagal neuropathy in alcoholics is associated with a markedly decreased (66% vs 91%)
survival rate over five years (Johnson & Robinson, 1988). Alcoholic neuropathy may affect autonomic tone and alter HRV via one or more intrinsic mechanisms, medullary reflexes, and/or supramedullary mechanisms or via hormonal influences. HRV has demonstrated greater sensitivity for alcoholic autonomic neuropathy than traditional autonomic tests (Malpas, Whiteside, & Maling, 1991).

**Intrinsic Cardiac Factors**

Chronic alcohol treatment is associated with early changes in $\alpha$-1 adrenergic and muscarinic cardiac receptor systems. These changes precede the $\beta$-1 and adenylyl cyclase changes associated with alcohol induced heart failure and may affect the intrinsic properties of the SAN (Strasser, Nuchter, Rauch, Marquetant, Seitz, 1996).

**Medullary Cardiovascular Reflexes**

A dose dependent decrease in baroreceptor reflex sensitivity has been documented in healthy young adults after consumption of alcohol at levels that produce insignificant to mild states of intoxication (Abdel-Rahman, Merrill, & Wooles, 1987; Koskinen, Virolainen, & Kupari, 1994). The conduction velocity of cardiac vagal and sympathetic A, B and C fibers is decreased by chronic alcohol consumption. The faster myelinated fibers are affected earlier (Fujimura, Araki, Murata, Yokoyama, & Handa, 1993). The severity of autonomic and peripheral neuropathies associated with long-term alcohol abuse/dependency is also dose dependent (Monforte, et al., 1995). These dose dependent relationships suggest that the relationship between alcohol abuse/dependency and perturbations in autonomic tone may be causal.
High chronic alcohol dosing is associated with central neurophysiological effects that influence HRV. Changes in the murine dorsal vagal complex, a structure involved in vagal control, after chronic alcohol treatment may help explain the changes in HRV that are exhibited in humans after chronic alcohol exposure (Pineda, et al., 1995). Low to moderate alcohol dosing is known to enhance GABAergic inhibition and inhibit the excitatory effect of glutamate. These neurotransmitters are operational in medullary cardiovascular reflexes (Kunos & Varga, 1995). All of these mechanisms may have a detrimental effect on medullary reflexes and, therefore, contribute to alcohol-related effects on autonomic tone and HRV.

Supramedullary Factors

Autonomically mediated cardiovascular reactivity in response to signaled shock is heightened in both male alcoholics and the non-abstinent non-alcoholic sons of male alcoholics when these groups are compared to non-abstinent non-alcoholic males without a family history of alcoholism. This increased cardiac reactivity may be the consequence of altered processing of novel and/or noxious stimuli in limbic and prefrontal centers (Peterson, Pihl, Seguin, Finn, & Stewart, 1993) and may be a supramedullary mechanism contributing to the alterations in HRV associated with both acute and chronic alcohol use. Cardiac hyperactivity is dampened in alcoholics by moderate and high levels of acute alcohol ingestion (Finn, Zeitouni, & Phil, 1990).

Hormonal Influences

Increases in plasma catecholamines have been documented after moderate and high alcohol consumption and during alcohol withdrawal. These findings suggest that
sympathetically mediated hormonal influences contribute to the increases in HR and blood pressure seen after both acute and chronic alcohol ingestion (Ireland, Vandongen, Davidson, Beilin, & Rouse, 1984). Similarly, increased plasma catecholamines may contribute to alterations in HRV.

**Alcohol and Heart Rate Variability**

A modest but evolving body of literature indicates that alcohol consumption has a significant impact on HRV. In general, these projects can be categorized as those examining the effects of acute alcohol consumption and those examining the effects of chronic alcohol dependence. Additionally, a very small number of projects have attempted to examine the relationship of chronic moderate alcohol consumption and HRV.

**Acute alcohol consumption.** Four particularly salient studies of the effects of acute alcohol ingestion on HRV published over the last decade indicate that:

1. Acute alcohol consumption is associated with a quick persistent initial decrease in vagal modulation of heart rate followed by a switch to less sustained period of predominant sympathetic control (Gonzalez, Llorens, Novoa, Valeriano, 1992; Koskinen, Virolainen, & Kupari, 1994; Rossinen, et al., 1997; Weise, Krell, & Brinkholl, 1986).

Rossinen, et al. (1997) found that the HF component of the HRV power spectrum remained depressed for up to 13 hours in a group of CAD patients after very high alcohol dosing (approximately 7.5 standard drinks). This was significantly
longer than alterations in the LF and VLF components. Since the HF component remained depressed for up to three hours after the BAC should have returned to baseline (Rossinen et al., 1997), this finding implies that the extended depression of vagal tone may be due to a sympathomimetic metabolite of ethanol such as aldehyde or another unknown variable. It is not known if the attenuation of the HF component persists after the complete elimination of more moderate alcohol doses that would not recruit the cytochrome p450 metabolic pathway and produce comparable levels of alcohol metabolites.

(2) HRV measures may be differentially sensitive to level of alcohol consumption (see Table 2). While all levels of alcohol dosing depress power in the high- and mid-frequency components of the HRV power spectrum (Gonzalez, Llorens, Novoa, & Valeriana, 1992; Koskinen, Virolainen, & Kupari, 1994; Rossinen et al., 1997), time-domain measures based on both interbeat intervals and the differences between successive beats may be insensitive to moderate non-intoxicating doses of alcohol. Mean Momentary Arrhythmia (MMA), an estimate of vagal tone based on the mean differences between successive beats was unaffected by moderate dose of alcohol (.3g/kg or approximately 1.75 standard drinks) in healthy males (Gonzalez, Llorens, Novoa, Valeriano, 1992), but, was significantly attenuated in a similar group after doses of 1.0g/kg. More global measures based on interbeat intervals were insensitive to alcohol doses of 1.0g/kg (Koskinen, Virolainen, & Kupari, 1994). Therefore, the following descending hierarchy in the sensitivity of HRV measures to the effects of acute alcohol can be hypothesized: HF, MF, LF,
time-domain measures based on successive differences between beats, time domain measures based on interbeat intervals, and finally VLF. Thus, vagal tone measures (measures based on successive differences between beats and the HF spectral component) may be more susceptible to the short term effects of acute alcohol dosing.

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<th>Study</th>
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<td>Weise et al. (1996)</td>
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<td>Koskinen et al. (1994)</td>
<td>1.0g/kg (6 SD)</td>
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<td>Rossinen et al. (1997)</td>
<td>1.25g/kg (7.50 SD)</td>
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SD = standard drink; HR = heart rate; IBI = time domain measures based on interbeat intervals; Δ = time domain measures based on successive differences between beats; VLF = Very low frequency spectral component; LF = Low frequency spectral component; HF = High frequency spectral component; * not reported.

Table 2: Differential Effects of Level of Alcohol Dosing on HRV Measures

Neither the mechanism by which acute alcohol dosing influences HR nor the direction of relationship between alcohol consumption and HRV is well defined. Inconsistent changes in heart rate have been observed in human subjects after parenteral and oral alcohol dosing (Friedman, Korsten, M.A., Alcorta, C., &
Venkatachalapathy, D., 1974). Animal studies have failed to demonstrate an increase in heart rate when alcohol was delivered directly in to the SAN (James and Bear, 1997). Therefore, the slight increase in heart rate that is inconsistently associated with alcohol may be attributable to the attenuation of baroreceptor-mediated vagal slowing of the heart and/or to the alcohol-mediated release of adrenal hormones (Friedman, 1992).

**Chronic alcohol dependence.** Knowledge of the effects of chronic alcohol consumption on the autonomic nervous system and HRV is largely restricted to the consequences of long-term high-dose alcohol abuse in samples of middle-aged male alcohol dependent inpatients. Interpretation of this literature is complicated by inconsistencies in research design. Subjects were studied during various stages of withdrawal and abstinence and reported diverse drinking histories. Comparison groups were frequently drawn from populations described as "non-alcoholic" or "healthy" individuals. Researchers have either failed to report alcohol consumption rates in these comparison groups or have reported rates that exceed the current USDA/USDHHS guidelines for moderate consumption. Comparison across studies is compromised by the lack of standardization of measures of HRV reported and the variety of recording period times used.

Nevertheless, the following statements about the relationship between chronic alcohol dependence and deviations in HRV are supported by the literature:

1. Time domain estimates of vagal tone determined by the successive differences between beats (runs, MMA, and rMSSD) are generally decreased in
alcohol dependent individuals. These estimates of parasympathetic function have demonstrated greater sensitivity for autonomic neuropathy than standard tests of autonomic dysfunction (Malpas, Whiteside, & Maling, 1991).

2. Global time-domain estimates of HRV (SDR-R, SDSD, and mean R-R interval, C-VRR, mean HR) are lower in samples of alcohol dependent individuals compared to normal controls (Malpas, Whiteside, & Mailing, 1991; Melgraad & Somnier, 1981; Matikainen, Juntunen, & Salmi, 1986; Monforte, Valls-Sole, Villalta, & Urbano-Marquez, 1995; Weis, Mueller, & Krell, 1985; Yokoyama, et al., 1991).

3. High- and low-frequency power, coefficient of variation of the (CVRR, C-CVHF, and C-CVLF), computed by spectral analysis, have also been found to be depressed in alcohol dependent subjects compared to age matched controls (Murata, Araki, Yokoyama, Sata, Yamashita, Ono, 1993). The authors report C-CVHF as an estimate of vagal input and C-CVLF as an estimate of sympathetic input.

4. HRV is markedly decreased during alcohol withdrawal and recovers in some individuals over a period of 30 days. However, in a large segment of alcohol dependent individuals, decreases in HRV persist in excess of 60 days and vagal tone may be permanently impaired (Yokoyama, et al., 1991). This supposition is supported by recovery of vagal reflexes in abstaining alcohol dependent individuals (Tan, Johnson, Lambie, & Whiteside, 1984). However, recovery may be limited to alcoholic dependent individuals with relatively short drinking histories and functional as opposed to organic vagal neuropathy (Weise, Muller, Krell, Kielstein, & Koch, 1986).
5. Autonomic neuropathy is an early, dose dependent consequence of alcohol abuse. The dose dependence of this relationship suggests that alcohol or a metabolite of alcohol has a direct toxic effect on the autonomic nervous system (Monforte, Valls-Sole, Villalta, & Urbano-Marquez, 1995). The association between long-term moderate alcohol consumption and the frequency components of the power spectrum, a major focus of this dissertation project, has not been reported.

**Chronic moderate alcohol consumption.** Only two projects have examined the effects of moderate levels of chronic alcohol consumption on measures of HRV in samples of healthy community dwelling subjects. These studies included adequate numbers of both male and female subjects to support the description of gender effects. The earlier of these studies examined alcohol effects in 68 healthy males and females and failed to find a significant relationship between HRV and either gender or alcohol consumption (Murata, Landrigan, & Araki, 1991). In this study the lack of statistical significance may be related to the very low dosing and the narrow range of alcohol consumption in the sample, especially among female subjects. Females reported mean weekly alcohol consumption of 21ml of 100% ethanol (approximately 1.25 standard drinks per week). Although the authors do not report the sample standard deviation, the use of the sample mean as a measure of central tendency implies that alcohol consumption rate was normally distributed and thus at least half of these women drank approximately one standard drink per week and the great majority drank less than two standard drinks per week. Since only one female subject was over 60 years of age, the results of this study can not be generalized to older women.
Subjects in the second study were selected for age homogeneity (39 years) and reported meaningfully higher alcohol consumption rates (0 - 84 g/day; median = 16 g/day). Thus the most common consumption rate was approximately 20ml of 100% ethanol or 1.25 standard drinks per day (seven times the rate reported by Murata, Landrigan, & Araki, 1991). This consumption rate approximates the USDA/USHHS guideline for moderate alcohol consumption in women. Half of the women in this study drank at rates exceeding moderate alcohol consumption. Heart rate recordings were made during spontaneous breathing under laboratory conditions in the supine position over two hours between 8 am. and 12 noon. While estimates of HRV were not significantly related to alcohol consumption in males or in the sample as a whole, separate analysis of the data from female subjects revealed a significant positive correlation between alcohol consumption and both the rMSSD (an estimate of vagal tone) and total power (Kupari, Virolainen, Koskinen, Tikkanen, 1993). This finding is difficult to interpret. It may indicate a differential gender effect of alcohol consumption on autonomic tone and HR. However, if female subjects generally drank at lower rates than males, the results may be dose dependent and the study may be the initial suggestion that the relationship between alcohol dose and HRV is not physiologically neutral. In middle-aged women, moderate levels of alcohol dosing may actually enhance vagal tone and increase the electrical stability of the heart. This relationship has not been examined in older women and the findings may differ significantly.
Alcohol, Supraventricular Arrhythmia, Ventricular Arrhythmia, and All-Cause Mortality

The proarrhythmic effects of alcohol are well documented. Alcohol abuse has been identified as the most common cause of cardiomyopathy (Regan, 1990). Half of the individuals abusing alcohol are at risk for ventricular hypertrophy related to hypertension. In alcoholic heart muscle disease (AHMD) the heart is hypertrophied and the left ventricle is dilated. Myopathic changes in the ventricle include fibrosis, lipid deposits, and inflammation. Chronic alcohol abuse compromises the electrical structure of the heart by damaging the ultrastructure of cardiac fibers. Abnormal mitochondria, and dilation of gap junctions have been described in the hearts of alcoholics (Preedy & Richardson, 1990). Alcohol-induced coronary vasospasm may initiate ischemia/reperfusion (Altura, Altura, & Carella, 1983). Acidosis, hypomagnesia, and hypokalemia associated with acute alcohol misuse may enhance alcohol’s intrinsic proarrhythmic effects (Friedman, 1992). Perturbations in autonomic tone may be a mediating factor between chronic alcohol abuse and lethal arrhythmias (Monforte, et al., 1995).

Alcohol and Arrhythmia

Acute alcohol dosing produces an initial decrease in parasympathetic (vagal) input to the SAN that is followed by increases in sympathetic input (Gonzalez, Llorens, Novoa, & Valeriano, 1992; Rossinen, et al. 1997). This alteration in autonomic state increases oxygen consumption, vasoconstriction, and ischemia (van Boven, Brouwer, Crijs, Haaksma, & Lie, 1995) and potentiates triggers for
ventricular (Huikuri, Valkama, Airaksinen, Seppanen, Kessler, et al., 1993) and supraventricular arrhythmias (Coumel, 1994). These mechanisms may help to explain the high incidence of arrhythmia associated with acute alcohol use.

**Electrocardiographic data.** Several electrocardiographic (EKG) changes have been suggested as non-specific markers of alcohol-related cardiac disease. Narrow shallow, spinus, and blunted T waves have been described in the EKGs of alcoholics. These changes were accompanied by less frequent arrhythmic changes including: atrial fibrillation, multifocal extra systoles, paroxysmal supraventricular tachycardia, ventricular extra systoles, nodal rhythms, and abnormal p waves (Evans, 1958; Brigden & Robinson, 1962).

**Electrophysiological data.** Electrophysiological studies suggest that the relationship between alcohol and arrhythmogenesis may be direct. Alcohol doses well below those associated with binge drinking (60 ml and 120 ml of 86 proof alcohol or approximately 1.3 and 2.6 standard drinks) decreased the atrial extrastimulation arrhythmogenic threshold in both alcohol abusing and control subjects. Although the authors were unable to define a mechanism of arrhythmogenesis in these subjects, they conclude that social drinking may be dangerous in individuals at risk for atrial fibrillation or atrial flutter (Engle, & Luck, 1983).

Greenspon and Schaal (1983) studied the arrhythmogenic potential of moderate alcohol dosing in a sample of 14 subjects with a history of chronic alcohol use. The subjects were classified as heavy drinkers (>100 ml of ethanol/day or greater than 4 standard drinks/day), moderate drinkers (50 - 100 ml of ethanol/day or
approximately 2-4 standard drinks/day), and social drinkers (<50 ml of ethanol/day less that 2 standard drinks/day). Eleven of the subjects had been diagnosed with heart disease. Ventricular dysfunction in these subjects was reported as either absent, moderate, or severe. Although nine of these subjects initially presented in the emergency room with rhythm disturbances and one presented with second degree Mobitz II block, none had significant or sustained arrhythmias during baseline electrophysiological testing. After alcohol dosing the subjects had documented BECs of 50 to 100mg/dL of ethanol. These levels indicate minimal to moderate intoxication. Tachyarrhythmias were recorded during the postabsorptive state in eight of the 14 patients. Atrial fibrillation and atrial flutter were the most common arrhythmias evoked. However, three of the patients experienced paroxysmal ventricular tachycardia and one experienced sustained ventricular tachycardia. These results strengthen the implication of an alcohol related etiology for sudden cardiac death (Greenspon & Schaal, 1983).

Clinical data. Several clinical studies strongly suggest an association between alcohol consumption and arrhythmogenesis. The term "Holiday Heart", was coined as a result of Ettinger, Wu, De La Cruz, Wiese, Ahmed, & Regan's (1978) classic descriptive study of 24 hospital patients, free of overt cardiomyopathy, but with a history or alcohol misuse and a recent episode of acute intoxication. A significant majority of these patients were admitted during the Christmas holidays and the post-weekend days of the month, suggesting a syndrome described as "an acute rhythm and /or conduction disturbance associated with heavy ethanol consumption in a person
without other clinical evidence of heart disease and disappearing without evident residual, with abstinence”. These arrhythmias occurred at multiple levels of the conduction system and included atrial fibrillation, atrial flutter, atrial and junctional tachycardia, PATs, PVCs, and ventricular tachycardia. PRc, QRS, and QTc intervals were prolonged in these patients. These conduction delays may be precursors of re-entrant arrhythmias and herald the onset of alcohol related sudden cardiac death.

Atrial fibrillation is the most frequent arrhythmia associated with alcohol use and alcohol use may be the most common cause of new onset atrial fibrillation (Koskinen, Kupari, Leinonen, & Luomanmaki, 1987; Lowenstein, Gabow, Cramer, Olva, Ratner, 1983; Rich, Siebold, & Campion, 1985). Alcohol-related atrial fibrillation has been reported in both social drinkers and abstainers after an episode of binge drinking as well as in heavier drinkers under similar conditions.

Alcohol and Sudden Cardiac Death

Two distinct lines of research illustrate the relationship between alcohol abuse/dependence and sudden cardiac death. The epidemiological literature supports the argument that alcohol dependence is a significant risk factor for sudden cardiac death. However, epidemiological findings do not resolve the possibility that alcohol dependence may be a surrogate for another more proximate variable such as psychological profile or nutritional state. Electrophysiological studies in the canine model complement epidemiological studies by providing evidence that both acute and chronic alcohol consumption facilitate ventricular fibrillation.
Epidemiological Data. Epidemiological studies provide an indirect link between alcohol and SCD. Although synthesis of epidemiological studies linking alcohol and sudden cardiac death is complicated by the differences in the definitions of SCD death, index of alcohol consumption, populations of interest, outcome variables, and length of the follow up period (FUF) it is clear that alcohol abuse is associated with an increased incidence of SCD. Lithell (1987) relied on registration of the temperance board to operationalize alcohol abuse and identified a 2.43 times increased risk of SCD among individuals who were registered compared to controls. In Sweden, temperance board registration is mandatory for all individuals who have encountered a legal problem as a consequence of alcohol use.

Iso, Kitamura, Shimamoto, Sankai, Naito, Sato, Kiyama, Iida, & Komachi (1995) separated ex-drinkers from abstainers and reported a relative risk of 10 for SCD for individuals in the highest alcohol consumption category. Two studies have identified a dose response relationship between alcohol and SCD (Suhonen, Aromaa, Reunanen, & Knekt, 1987) indicating a possible causal relationship. Wannamethee and Shaper (1992) reported that high alcohol consumption rates discriminated between fatal and non-fatal myocardial infarction. A relative risk for SCD (RR = 1.73) remained after adjustment for increased systolic blood pressure. The authors conclude that the predominance of SCD among heavy drinkers suggests an alcohol related arrhythmogenic etiology. One of the rare studies to examine the relationship of alcohol and sudden death in women estimated the relative risk of sudden death associated with alcohol abuse at be 4.8 (Beard, Griffin, Offord, & Edwards, 1986).
Experimental Data. In order to confirm a causal role for alcohol in the genesis of SCD the mechanism of alcohol-induced ventricular arrythmia must be identified. Patel, McArdle, & Regan (1991) used a canine model of chronic alcohol consumption to demonstrate that both chronic and acute alcohol consumption can increase cardiac vulnerability to ventricular fibrillation and link alcohol causally to SCD. A significant decrease in ventricular fibrillation threshold both at base line and after alcohol treatment was identified in the alcoholic canine group compared to control canines. The ventricular fibrillation threshold was also decreased in nonalcoholic dogs after acute alcohol dosing. These findings support an etiological role for alcohol in ventricular arrhythmogenesis and sudden cardiac death. It is arguable that alcohol constitutes a functional toxic influence that can act independently or in conjunction with other functional influences on an altered anatomical substrate to precipitate ventricular fibrillation and SCD. The anatomical substrate for alcohol induced arrhythmogenesis may be conduction delays related to dilatation of the intercalated disc and alterations in the action potential which leave the myocardium vulnerable to circus rhythms resulting from a random premature contraction (Ettinger, Lyons, Oldewurtel, & Regan, 1976) or the substrate may exist independent of alcohol misuse.

Alcohol and All-Cause Mortality

Alcohol affects all body systems and the harmful influences of high levels of alcohol consumption are manifested in such diverse pathologies as: liver and pancreatic disease, CNS and PNS damage, degenerative heart and muscle disease,
hypertension, diabetes, and carcinomas (Dufour & Fe Caces, 1993). All of these effects can be expected to alter autonomic tone. Although no prospective HRV studies of arrhythmic and all-cause mortality in alcohol dependent populations have been published, the pervasive biological effects of high level alcohol consumption and the documented autonomic neuropathy associated with alcohol dependence, support the hypothesis that HRV would be a useful predictor of arrhythmic and all-cause mortality associated with alcohol dependence.

Linking Aging and Alcohol

The elderly have a heightened vulnerability to alcohol-related harm. Some theorists have speculated that alcohol accelerates aging (Cermak & Peck, 1982) while others have proposed that aging and alcohol independently create deficits that are merely additive (Riege, Tomaszewski, Lantos, & Metter, 1984). Recently quantitative analysis of computerized tomography (CT) and magnetic resonance imaging (MRI) studies have provided evidence for an interaction between alcohol and aging and suggested that the aged brain may be particularly vulnerable to the toxic effects of alcohol (see Figure 5).

Greater loss of both cortical grey and white matter along with increased ventricular volume has been documented in older alcoholics compared to age-matched controls. Younger alcoholics, matched for alcohol use history with the older alcoholics did not demonstrate similar losses when compared to age-matched non-alcoholic controls (Pfefferbaum, Lim, Sipursky, Mathalon, Rosenbloom, Lane, Ha, & Sullivan, 1992). The appeal of the increased vulnerability in aging theory is
enhanced by its compatibility with the theme of overall increased vulnerability to stress that characterizes the gerontology literature.

The pharmacokinetics and pharmacodynamics of alcohol, performance testing after acute alcohol dosing, and the subjective reports of acute alcohol dosing by older adults also point toward a synergistic relationship between aging and alcohol
consumption. The next section will summarize these age-related effects and substantiate the position that aging and alcohol consumption are interrelated.

**Pharmacokinetics of Alcohol in Aging**

**Absorption**

Age-related changes in absorption do not, in general, have a significant impact on the passive diffusion of alcohol across gastrointestinal membranes. However, age differences in absorption may increase the area under the blood alcohol curve in the fasting state for elderly subjects of both genders (Beresford & Lucy, 1995). Several non-prescription drugs that are frequently used by older adults (e.g. antacids) prolong age-associated increases in gastric emptying times, and may increase passive diffusion of alcohol across the gastric membrane (Katzung, 1995).

**Clearance**

Although the hepatic structural and functional changes associated with aging are moderate, the predominant role that the liver plays in the metabolism of alcohol indicates that these changes may significantly influence alcohol metabolism in older persons. Cell loss is responsible for a decrease in the liver weight and size that begins about the age of 60. Additionally hepatic blood flow is decreased by 40% in individuals over age 60 (Timaris, 1994).

**Gastric Metabolism.** Recent research examining the effect of gender and age on gastric alcohol dehydrogenase production (ADH) and first pass metabolism found that while no significant differences in gastric ADH were found between young and old female subjects, old male subjects demonstrated a precipitous decline compared to
young subjects. This decline coupled with increased gastric mucosal atrophy among males may reduce first pass metabolism among older males to a level below that found in older females and reverse the relationship characteristic of younger subjects (Pozzato, et al., 1995). Some prescription and over-the-counter drugs commonly used by the elderly, such as cimetadine (Tagamet), ranitidine (Zantac), and aspirin all reduce the production of gastric ADH (U. S. Department of Health and Human Safety, 1993). Administration of cimetadine elevates the BEC in individuals who drink low or moderate amounts of alcohol but not in individuals consuming very large amounts of alcohol which entirely overwhelm gastric ADH.

Microsomal ethanol-oxidizing system. The greatest changes in drug metabolism during aging are related to a decline in the microsomal mixed function oxidase system (MEOS). MEOS is part of the mixed function oxidase system and is critical in the metabolism of certain drugs (barbiturates, diazepam, tolbutamide, theophylline) as well as high doses of ethanol (Katsung, 1995). These age related reductions in the MEOS may cause disproportionate increases in BECs in the elderly at higher levels of alcohol consumption.

The average person over the age of 65 takes between two and seven prescription drugs a day in addition to over-the-counter medications (Korrapati & Vestal, 1995). The probability of adverse drug reactions increases as the number of drugs consumed and number of medical diagnoses increase (Katung, 1995). Aging-related changes in the MEOS coupled with polypharmacy augment the potential for
adverse alcohol-drug reactions at all levels of alcohol consumption and may intensify alcohol-related autonomic effects.

**Volume of Distribution**

As noted above, alcohol is a very small amphophilic molecule that easily diffuses across all biological membranes and equilibrates rapidly in body water. Aging-related reductions in total body mass, loss of lean body mass, absolute and relative decreases in total body water (18%-25%), and increases in adipose tissue as a percentage of body mass (10%-50%) increase the BECs for any given dose of alcohol in older compared to younger adults (Katzung, 1995). The smaller volume of distribution found in females across all age groups, combined with low levels of gastric ADH, and low hemoglobin levels explain why women of all ages have a heightened vulnerability to alcohol-related harm compared to males. For example, men incur a significant risk for cirrhosis at consumption levels of 80g/day. Women incur a similar risk at 20 g/day. Alcoholic cardiomyopathy is more common among alcohol-dependent women than alcohol-dependent men and occurs in women compared to men at lower levels of use over shorter periods of time. Alcohol dependent women score lower on the New York Heart Association (NYHA) functional classification system than alcohol dependent men with higher levels of alcohol consumption (U.S. Department of Health and Human Services, 1993).

**Half-Life**

Analysis of the area under the blood alcohol curve (AUC) for older and younger adults revealed that moderate (.3g/kg) acute alcohol dosing (i.e.
approximately one-standard drink) yielded earlier and higher peak BECs and greater total exposure in older subjects than in younger subjects (Beresford & Lucey, 1995). Since age does not affect rate of alcohol elimination (Vestal, McGurie, Tobin et al., 1977) the half-life ($t_{1/2}$) of ethanol is similar across age groups. The AUC differences between older versus younger women was three times greater (39%) than the AUC differences for older versus younger men (Beresford & Lucey, 1995).

**Pharmacodynamics of Alcohol in Aging**

**Maximum Effect**

The maximum effect ($E_{\text{max}}$) dose of a drug is that dose of a drug at which no further increment in response can be achieved. For alcohol the maximum response can be considered death. Animal studies contain evidence that the LD (lethal dose) of alcohol (and therefore the $E_{\text{max}}$) declines with increasing age (Wiberg, 1970).

**Sensitivity**

The sensitivity of an organ to a drug is defined as the concentration of the drug needed to produce 50% of the maximum effect ($ED_{50}$). It is reasonable to speculate that the $ED_{50}$ decreases with aging from changes in motor performance and from subjective reports of acute alcohol dosing by older adults. BEC values of 0.69 +/- 6.8 mg/dL produced greater decrements in large and small motor performance in older versus younger subjects (Vogel-Sprott & Barnett, 1984). Older subjects self-report greater subjective feelings of intoxication for a given dose of alcohol (Beresford & Lucey, 1995; Jones & Neri, 1985), for a longer duration than younger
subjects (Beresford & Lucey, 1995). These findings suggest an age-related increased sensitivity to alcohol (Jones & Neri, 1985).

The heightened sensitivity to alcohol-related affects among older adults is also evidenced by the increased salience of the tolerance and withdrawal as symptoms of alcohol dependence in older adults. The increased susceptibility to alcohol-related harm and the altered metabolism of alcohol associated with aging make and increases in tolerance especially ominous sign of dependence for older adults. Both animal and human studies indicate that withdrawal symptoms are prolonged, require more medication, and are more complicated in older verses younger alcohol-dependent subjects (Beresford, 1995).

The Effects of Aging on Alcohol Consumption

The prevalence of alcohol abuse/dependence in the elderly has been estimated at between four and ten percent (Berefsord & Lacey, 1995). The consensus of cross sectional studies is that alcohol consumption declines with age (Bucholtz, Sheline, & Helzer, 1995). However, these studies are compromised by a generational bias and a differential mortality among alcohol dependent individuals that over simplifies the effect of age on alcohol consumption (Liberto, Oslin, & Ruskin, 1992).

Some longitudinal studies indicate that while heavy alcohol consumption may decrease with age (Fillmore, 1987), moderate alcohol consumption increases with age and that this increase is driven by a rise in consumption among older women (Gorden & Kannel, 1983). Other longitudinal studies have indicated that drinking behaviors
tend to remain stable over time and that early drinking behavior is the best predictor of drinking behavior in old age (Adams, 1990).

In any case, the highest rates of alcohol abuse/dependence have been described in women during mid-life (35-49) and the number of women in this age-group experiencing alcohol abuse/dependence increased from 1.5 million to 2.0 million during the last decade. This increase was the result of the passage the baby-boom generation into this middle-age (Chatham, 1990). Thus, a substantial increase can be expected in alcohol related problems among older adults, and especially older women, in the near future. Eleven thousand baby-boomers turn 50 every day.

A significant subgroup of older adults experience initial alcohol-related problems after age 49 (Beresford, 1995). These late-onset drinkers frequently report higher rates of life stressors than older adults who do not experience late-onset problem drinking (Finlayson, 1988). These late-onset drinkers make up about one-third of all older adults experiencing problem drinking (Liberto, Oslin, & Ruskin, 1992). Rates of late-onset alcohol dependence seem to be higher among the affluent and women (Beresford, 1995).

In summary, research linking aging and alcohol consumption show that:

1. Aging is associated with an increased alcohol exposure for any given dose of alcohol, and that this exposure is greater for women than for men. Hence, the definitions of "a standard drink" and "moderate" alcohol consumption must be reinterpreted for older adults in general and women in particular. Both aging and gender must be considered as possible confounders in alcohol studies.
2. Cross-sectional studies indicate that alcohol misuse, abuse, and dependence decrease in prevalence during aging. Longitudinal studies have identified a sustained incidence of problem drinking behaviors during aging. Late-onset drinking, heightened physiological vulnerability of older adults (especially women) to alcohol related harm, and the increased risks of alcohol-medication interactions in this population insure that alcohol consumption among older adults will present an increasingly important public health challenge through at least the year 2030.

3. The high incidence of late-onset problem drinking among older adults mandates the expeditious development of rational guidelines for both safe and optimal alcohol consumption in this population.

Circadian Influences on Autonomic Tone and Heart Rate Variability

Circadian rhythms refer to phenomena that are characterized by a time series with a principal frequency of 24 to 26 hours (see Figure 6). The cardiovascular system exhibits well known circadian periodocities. The typical nighttime decreases and abrupt early morning increases in heart rate and blood pressure have been recognized since the 18th century (Kraft & Martin, 1995). More recently a nighttime rise in vagal tone has been reported between midnight and early morning (Malpas & Purdie, 1990). Spectral analysis (Furlan, et al., 1990) and batteries of time domain measures (Molgaard, Sorensen, & Bjerregaard, 1991) have been used to specify the circadian pattern of autonomic control of the heart in normal subjects and to quantitatively delineate the timing of contributions of the parasympathetic and sympathetic nervous systems to HRV. It is now accepted that increases in vagal tone
drive the night time changes in HRV (Furlan et al., 1990). Vagal estimates peak about 4:00 a.m., coincident with trough serum catecholamine levels (Fallen, & Kameth, 1995). High nighttime vagal tone is succeeded by a daytime predominance of LF power (sympathetic and non-respiratory vagal tone). The dynamics of this reorganization of autonomic tone that begins about 5:00 a.m. are not completely defined. Some researchers argue that, in normal adults, the early morning hours (6:00 a.m. to 12:00 n.) are distinguished by a rapid increase in sympathetic tone (approximately .1 Hz) and a simultaneous decrease in vagal input (approximately .25 Hz) (Furlan et al., 1990; Huikuri, et al., 1994; Sapoznikov, Luria, Mahler, & Gotsman, 1992). But other researchers have argued that both HF and LF tone increase during the hours of sleep (Adamopoulous, et al., 1995) or have not identified a circadian variation in LF power (Casolo, et al., 1991; Niemelda, Airaksinen, & Huikuri, 1994). If LF power is stable over 24 hours, early morning vagal withdrawal must drive the relative increase in LF activity that begins 6:00 a.m. and lasts throughout the daytime hours (Casolo, et al., 1991). The common inference that can be drawn from these two positions is that the circadian pattern of HRV results from complex interactions between both branches of the autonomic nervous system. Vagal tone (HF power) predominates at night and is abruptly succeeded by predominantly LF power during the daytime hours.
Figure 6: Circadian Influences on the Concepts of the Model

Analysis of the circadian variation in estimates of HRV is of interest for four reasons:

1. The circadian pattern of time-related events can provide insights regarding the pathophysiological mechanisms. Myocardial infarction, stroke, angina, and SCD are profound cardiovascular events with a peak frequency of onset between 6:00 a.m. and 12:00 noon. A second smaller peak occurs in early evening (Muller, Tofler, & Stone, 1989). Similarities between the circadian pattern of cardiovascular events and
the circadian pattern of autonomic tone suggest that the abrupt rearrangement in autonomic tone that begins around 6:00 a.m. may trigger adverse cardiovascular events.

2. Changes in the circadian pattern are the sentinel indicators of perturbed autonomic tone in many disease states and have prognostic significance (Malik, Farrell, & Camm, 1990; Spallone & Menzinger, 1997). While global measures of HRV based on 24 hour averages have failed to distinguish between normal subjects and subjects with various cardiovascular conditions, circadian analysis of HRV has shown that subjects with uncomplicated CAD patients have lower nighttime HF tone and attenuated changes in the LF/HF ratio between 6:00 a.m. and noon compared to normal subjects (Huikuri, et al., 1994). Similar results have been reported for post-MI patients (Klingenhaben et al., 1995; Malik, Farrell, & Camm, 1990).

3. Nighttime offers the best time to evaluate baseline tonic autonomic vagal tone. HRV estimates calculated from recordings made during the hours of sleep are the most independent of exogenous influences such as activity, upright posture, mental stress, or the use of nicotine or alcohol. The hours of sleep is also the period least likely to be affected by individual differences in reactivity.

4. Measures of vagal tone are more sensitive to neurotoxins than other components of HRV (Murata & Araki, 1991; Murata, Araki, Yokoyama, & Maeda, 1991) and therefore nighttime vagal tone could be the most sensitive index of alcohol-related alteration of autonomic function. The absence of circadian analysis in
previous studies examining the relationship between moderate alcohol consumption and HRV may explain the lack of significant findings.

**Gender, Age, and the Circadian Pattern of HRV**

Estimates of vagal tone are known to decline with advancing age (Molgraad, Sorensen, & Bjerregraad, 1991). A global reduction in all HRV indices is mediated by a night time increase in HR in older males compared to younger males. These changes may drive the gender-specific loss of circadian variability in older males.

Aging is not associated with an increase in night time HR in females and circadian rhythms are preserved. SDNN, SDANN, log [total power], and log [ULF power], long term indices that estimate circadian rhythms remain stable in women through old age (Stein, Kleiger, & Rottman, J.N., 1997).

The failure to consider gender effects on circadian rhythms in HRV studies can lead to misinterpretation of findings. The circadian rhythm of HRV, calculated from 24 hour Holter EKG monitor recordings in community-dwelling young healthy males and females is reproducible over periods from one day to one week (Huikuri, et al. 1990).

**The Influence of Possible Confounding Variables on Heart Rate Variability**

A number of factors influence heart rate variability in healthy subjects. These factors will be considered in the following section. Methods for controlling these factors will be discussed in Chapter 3 (see Figure 7).
Gender

The greater longevity of females (Ryan, Goldberger, Pincus, Mietus, & Lipsitz, 1994) and poorer outcome for females after MI (Huikuri, Pikkujamsa et al., 1996) have stimulated attempts to define the relationship between gender and HRV.

Early studies reported only suggestions of gender-related differences in autonomic tone and tendencies toward increased blood pressure reactivity to sustained hand grip in males (a test of sympathetic responsiveness) (Gautschy, Weidmann, & Gnadinger, 1986; O'Brien, O'Hare, & Corrall, 1986). These equivocal findings may have resulted from inability of clinical tests of autonomic function and moment estimates to evoke gender differences.

Figure 7: Possible Confounding Variables Influencing Autonomic Tone (HRV)
More recent studies have used a battery of time-domain and frequency-domain indices to evaluate gender differences in autonomic tone. These investigations generally report higher time-domain indices of high frequency modulation of autonomic tone in females compared to males. Female gender is also associated with greater HF power, lower LF/HF ratios, lower LF power, and decreased HF responsiveness to tilt when compared to males (Bigger, Fleiss, et al., 1997; Hanyo, Muaiki, et al., 1991; Huikuri, et al., 1996; Liao, et al., 1995; Ryan, Goldberger, Pincus, Meitus, & Lipsitz, 1994).

Middle-aged female subjects display a lower BRS than age-matched male subjects and a concomitant impairment in reflex HR responses to blood pressure changes. These changes may explain poorer outcomes for females after MI. As noted above, experimental studies have revealed that BRS is lower in dogs susceptible to ventricular fibrillation than in dogs resistant to fibrillation. This attenuation has been documented both before and after experimentally induced myocardial infarction (Schwartz, et al. 1988). Attenuation of LF power has a greater association with post-infarction arrhythmogenesis than HF power. Therefore, the lower LF power commonly documented in females coupled with attenuated BRS may render females more vulnerable to the initial infarction and subsequent arrhythmic complications (Huikuri, et al., 1996).

Some inconsistencies in gender by age effects have been reported. Both a converge (Stein, Kleiger, & Rottman, 1997) and a divergence (Ryan, Goldberger, Pincus, Meitus, & Lipsitz, 1994) of gender-related differences in HRV indices have
been reported among the oldest subjects studied (65-90 years) and a final specification of the relationship between gender and HRV requires further research. However, on the whole, this line of research has identified significant HRV effects of gender. Failure to consider gender-effects during a study can mask true treatment effects and lead to inaccurate conclusions.

Clinical State

Every organ in the body is innervated by the autonomic nervous system, so it is logical that diverse clinical states are associated with perturbations in HRV and must be considered as confounders in HRV studies. The associations among peripheral neuropathy, diabetes mellitus, and altered HRV is well known. Perturbed autonomic tone has also been documented in chronic renal disease, liver disease, chemotherapy (eg. vincristine, barbiturates, phenothiazines, tricyclics), rheumatoid arthritis, and Parkinson’s (Bannister & Mathias, 1993; Edmonds, Jones, Saunderes, Sturrock, 1979; Barendregt, van der Heijede, Breedveld, Markusse, 1996). Myocardial Infarction (Kleiger, Miller, Bigger, Moss, et al., 1987), stroke (Naver, H.K., Blomstrand, C., Wallin, B.G., 1996; Oppenheimer, Kedem, & Martin, 1996), hypertension and risk of hypertension (Liao et al., 1996; Piccirillo, Munizzi, Fimognari, Marigliano, 1996) have well known autonomic components.

Body Mass Index

Obesity is associated with increased morbidity and mortality (including SCD). Autonomic nervous system function may contribute to an explanation of this association. Lower LF power and higher HF power have been reported in middle-
aged obese (BMI > 33kg/m²) subjects compared to comparable non-obese (BMI < 26kg/m²) subjects in the supine position and after tilt. Obese subjects also had markedly higher NE levels than controls. Thus, obese subjects had blunted responses to increased sympatho-adrenal activity and position change (Piccirillo, Vetta, et al., 1996). An indirect relationship between BMI and both the expiratory to inspiratory ratio (E/I ratio) and the difference between maximum and minimum heart rates (HR max-min) have been reported (Freeman, Weiss, Roberts, Zbikowski, & Sparrow, 1995). Obesity appears to partially mediate the overall lower levels of HRV and attenuated BRS reported in hypertensives (Huikuri, Ylitalo, et al., 1996). However, no consistent association between age-related changes in BMI and HRV have been found. Thus, the changes in body composition that accompany normal aging do not appear to mediate age-related alterations in HRV (Byrne, Fleg, Vaitkexicius, Wright, Porges, 1996).

**Medications**

Cardiovascular medications frequently have autonomic effects. The direction and magnitude of these effects on HRV depend on the level and duration of dosage, the basal state of the autonomic nervous system, and the condition of the cardiovascular system (Fie, 1995). These qualifications may explain the inconsistent findings that characterize the literature describing medication effects on HRV and are especially critical in specifying the effects of cardiovascular effects on the elderly. For example, significant improvement in HRV measures have been reported in laboratory animals and clinical populations after treatment with cardiovascular
<table>
<thead>
<tr>
<th>Drug</th>
<th>Effect</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propranolol</td>
<td>Increase HRV in MI patients</td>
<td>$\beta_1$-blockade</td>
</tr>
<tr>
<td>Metroprolol</td>
<td>Increase rMSSD, pNN50%, and log[VLF] and log[HF] after MI (Keeley et al., 1997) Decrease SD in rats</td>
<td>$\beta_1$-blockade</td>
</tr>
<tr>
<td>Acebutolol</td>
<td>Decrease in LF/HF ratio in cardiac hypertrophy (Coumel et al., 1991)</td>
<td>Possible altered SAN response to sympathetic input</td>
</tr>
<tr>
<td>Diltiazem</td>
<td>Decreases LF/HF ratio No effect (Cook et al., 1991)</td>
<td>Negative chronotropic</td>
</tr>
<tr>
<td>Nefidepine</td>
<td>Decreased HF power Increased LF/HF ratio in acute hypertension (Wolk, et al., 1996)</td>
<td>Mechanism Unknown</td>
</tr>
<tr>
<td>ACE Inhibitors</td>
<td>Increase in HF and Decrease LF/HF ratio (Binkley et al., 1993) No change in HF but increase in ULF and VLF after MI (Bonaduce et al., 1994)</td>
<td>Increased BRS and vagal output Attenuate RAAS cascade</td>
</tr>
</tbody>
</table>

ACE = angiotensin-converting enzyme; CV = coefficient of variation; RAAS = renin-angiotensin-aldosterone system.


Table 3: Antihypertensive Medication Effects on HRV
medications (Pieper & Hammell, 1995). However, other reports have failed to discern a significant relationship between calcium channel blockers, beta-blockers, and antihypertensive medications in survivors of sudden cardiac death (Cowan, Pike, Burr, Cain, & Narayanan, 1993).

HRV studies indicate that autonomic tone in general, and vagal tone specifically, is lower in the elderly compared to other adults. If cardiovascular medications reveal rather than enhance vagal tone, it can be speculated that autonomic effects of these medications will be attenuated in the elderly. Still, many cardiovascular medications have autonomic effects and must be considered as a possible confounding variable in HRV studies. Current findings related to pharmacological effects of some common antihypertensive medications on HRV are summarized in Table 3.

**Nicotine**

Nicotine directly affects intrinsic cardiac mechanisms (liberates catecholamine stores in the heart), medullary cardiovascular reflexes (excites peripheral chemoreceptors and baroreceptors), supramedullary influences and hormonal factors (triggers release of epinephrine via the HPA axis) (Hanyo, Yamada, et al., 1990). Thus, cigarette smoking causes alterations in measures of both short-term and long-term HRV that may explain the association between smoking and SCD (Hanyo, Yamada, et al., 1990; Tsuiji, Venditti, et al., 1996).

The effects of nicotine on HRV have not been examined in older adults. However, Hanyo's classic studies on the acute and chronic effects of nicotine on...
HRV measures demonstrated that smoking one cigarette a day decreased the respiratory component of spectral power (0.25 Hz.) in males aged 24-30 for approximately seven minutes. This increase is followed by a 14 minute increase in the Mayer wave component (0.1 Hz.) produced by increases in vasomotor tone the sympathetic component of the baroreceptor reflex.

A second study revealed that neither chronic moderate nor heavy cigarette smoking affected the Mayer wave component in either young males (19-30) or older males (30-52) in the supine position. The Mayer wave component increased on standing only in the nonsmokers. The respiratory component was significantly reduced only in young heavy smokers in the supine position. HRV estimates of vagal tone decline more rapidly before age 30 than after and vagal reserve may be lower after the age of 30 (Hanyo, Yamada, et al, 1990). These findings document a smoking related reduction in vagal outflow to the SAN and attenuation of BRS and establish nicotine use as a possible confounding variable in HRV studies.

Caffeine

Published reports describing the effects of caffeine on HRV are sparse. However, altered HRV measures have been reported for individuals consuming three or more cups of coffee per day (Tsuji, Venditti, et al, 1996) and immediate increases in HR and increased night time HR have been reported after acute caffeine ingestion (Green & Suls, 1996; Stubbs & MacDonald, 1995). Standard clinical tests of cardiovascular autonomic function uncovered decreased HR response to standing after ingestion of caffeine, and increased blood pressure responses to sustained handgrip in
healthy subjects after acute caffeine ingestion (Piha, 1994). These responses may be
attenuated by multiple-dosing (James, 1994; Mosqueda-Garcia, Tseng, Biaggioni,
Robertson, & Robertson, 1990). Caffeine produced greater skin conductance, and
skin temperature responses in healthy subjects with low caffeine consumption histories
than in comparable subjects with high consumption histories (Zahn, & Rapoport,
1987). The findings indicate that acute caffeine consumption produces baroreceptor
and sympathetic nervous system mediated effects on autonomic function that may vary
as a function of intake history. Thus, caffeine use is an associated factor that requires
consideration in HRV studies.

**Exercise/Activity Level**

The direct relation ship between endurance training and heart rate is well
know. The bradycardia commonly found in trained athletes results, at least partially,
from altered neurocardiac mechanisms (i.e. enhanced vagal tone, decreased β-
adrenergic activity, and baroreceptor changes) coupled with a decrease in intrinsic HR
(Dixon, Kamath, McCartney, & Fallen, 1992; Fallen & Kameth, 1993). Differences
in regular exercise may partially explain the differences in HRV between older and
younger subjects (Hellman & Stacey, 1976) and age-related rates of decline in HRV
may be modified by regular exercise (Odemuyja, 1995). Similar values for
respiratory sinus arrhythmia have been documented in young (38 years) and old (78
years) subjects who engaged in comparable levels of regular exercise (Hirsh &
Bishop, 1981). Higher HF and LF power as well as BRS has been reported for
physically active postmenopausal female subjects compared to age-matched more
sedentary controls (Davey, Miniclier, Taylor, Stevenson, & Seals, 1996). Thus, exercise/activity level is an associated factor that may attenuate age-related differences in HRV and requires consideration in HRV studies.

Excess Morbidity and Mortality

The profound medical consequences of alcohol dependence for all age groups is well known. Even low levels of alcohol consumption have widespread effects on human physiology and high levels of alcohol consumption are associated with every known cause of cardiovascular and non-cardiovascular mortality (Zakhari, 1991). This is especially true for the elderly. Serious medical conditions are significantly increased among elderly alcohol-dependent patients in inpatient treatment facilities. Elderly alcohol-dependent individuals experience inflated mortality rates after discharge compared to non-alcohol-dependent elderly (Hurt, et al. 1988). High level alcohol consumption directly contributes to high rates of disease and accidental injury and may indirectly increase normal declines in physiological function (Gambert & Katsoyannis, 1995). The association of high levels of alcohol consumption and excess morbidity and mortality in the elderly is undeniable and even more moderate levels of alcohol use have been associated with higher rates of medical admissions (Mangion, Platt, & Syam, 1992). In the cardiovascular system, as in other systems, these effects may be superficially indistinguishable from the effects of aging (Gupta, 1993).

A recent study of almost 4 million Medicare medical patients showed that less than 1% of the elderly were discharged with a diagnosis of alcohol dependence. However, this diagnosis was associated a 51% increase in annual hospital days and
almost $175 million in excess hospital charges (Ingster & Cartwright, 1995). Given
the low rate of diagnosis of alcohol dependence in the elderly this figure is probably
very conservative.

The threshold for alcohol related harm is undefined for older adults. But, this
threshold is very likely particularly low for older women compared to other groups.
The biology of aging, the increased vulnerability to alcohol effects associated with
female gender, and the pervasive age-related alterations in autonomic function all
lower the threshold for alcohol related harm among older women. Since both high
and moderate levels of alcohol consumption are know to be arrhythmogenic and all
classes of arrhythmia are known to increase in frequency and complexity in aging, it
is reasonable to speculate that alcohol related arrhythmogenesis may define the
threshold for alcohol related harm among older adults (especially women).
Additionally, arrhythmias at all levels of the myocardium are a significant source of
excess morbidity and mortality among the elderly.

Thus the theoretical model of the autonomic consequences of aging and alcohol
use presented in Figure 8 graphically summarizes the theoretical framework of the
study and answers the questions posed at the beginning of this chapter:

1. What is known about the relationships among aging, alcohol consumption,
and autonomic tone?

2. What are the consequences of these relationships for older adults and for the
health care system?
The intersection of the circles representing the concepts of aging and alcohol consumption represents the phenomenon of interest of this study - alcohol consumption during aging. The question mark placed on the arrow connecting this intersection and the rectangle representing the autonomic nervous system (and HRV) locates the focus of this study. The threshold level for the effects of alcohol on the autonomic nervous system are unknown for older adults. The purpose of this study is to initiate the specification of these relationships by describing the relationship between HRV and moderate alcohol consumption in a healthy community dwelling sample of older women.

Figure 8: Theoretical Model of Autonomic Consequences of Aging and Alcohol Consumption
CHAPTER 3

METHODS

Research Design

A descriptive cross-sectional design was used to determine:

1. the direction and strength of the relationship between alcohol consumption rate and heart rate variability in older women.

2. the unique contribution of alcohol consumption rate to HRV for older women.

3. the relationship between alcohol consumption rate and the circadian rhythm of heart rate variability in older women.

Sample and Setting

This sample consists of the 52 women enrolled in a larger parent project who satisfied the inclusion/exclusion criteria of this study.

Criteria for Subject Selection

Subjects were recruited from three senior citizen centers and their surrounding neighborhoods between May, 1995 and May, 1997 by announcements in newsletters and word of mouth. Respondents were given additional information about the project and screened during a telephone interview. Inclusion criteria for the parent project were: female, at least 60 years of age, community dwelling, in good general health,
and moderately active. Exclusion criteria for the parent project were history of: atrial fibrillation, myocardial infarction, pacemaker, stroke, chemotherapy, renal disease, electrolyte imbalance (documented by Chem7 and Mg+), diabetes mellitus, or an indication of clinical heart disease during a screening examination by a board certified cardiologist. These exclusion criteria define conditions that have known neurocardiac effects and so are appropriate for any investigation of HRV. Known alcohol abuse/dependence, and hepatic disease were specified as exclusion criteria for the parent project. These exclusion criteria were also appropriate for this dissertation project as they excluded subjects who were likely to have a history of immoderate alcohol use.

The criteria introduced for this project extended the exclusion criteria of the parent project by eliminating subjects who reported a history of alcohol abuse or conditions known to affect the autonomic nervous system (e.g., Parkinson’s disease, rheumatoid arthritis), transient ischemic attack (TIA), use of an alpha-1 agonist or cholinergic antagonist during the recording period, a change in alcohol consumption pattern within the last six months, or who revealed exclusion criteria after initial screening. Women with a history of controlled hypertension were admitted to the study. The incidence of hypertension and antihypertensive therapy increases with aging and these conditions can influence HRV. However, there is a direct association between alcohol and hypertension. For women, consumption of 2 drinks a day increases the risk of hypertension by 40%. Excluding women treated for hypertension would reduce the sample’s comparability to the target population.
Criteria for ECG Recordings

Only tapes in which at least 75% of the 288 mutually exclusive 5 minute epochs calculated for each 24 hour contained at least 80% normal artifact free R-R intervals were retained for analysis.

Eighty-three subjects in the parent project had 24-hour tapes available for HRV estimation. A total of twenty-six tapes were eliminated prior to scanning for the following reasons: Transient Ischemic Attack (TIA) within one month after Holter monitoring (1), stroke unreported on initial screening (3), Myocardial Infarction (MI) unreported during initial screening (1), Na+ below normal limits (1), atrial fibrillation (revealed by Holter monitoring) (2), history of alcohol dependence (1), artifact (8), use of an alpha-1 agonist during recording (1), use of cholinergic antagonist during the recording (1), change in drinking pattern with in the last 6 months (1), uncontrolled hypertension (SBP > 200 on two occasions) (2), and rheumatoid arthritis (2). Additionally, two subjects were lost due to lab error. No subjects were eliminated because of clinically apparent heart disease or amount of ectopy. A total of 57 tapes were rescanned for HRV analysis.

The data files generated from five of these 57 tapes failed to meet the predetermined standards for HRV analysis (i.e. 75% of 288 5-minute epochs free of artifact). Estimates of HRV were calculated for the remaining 52 subjects. The percent of ectopy per 24 hour recording, the overall percent of splined beats per 24 hour recording (% ectopy + % artifact), and the number of 5-minute epochs that were retained over the twenty-four hour record are presented in Appendix B.
Research estimating the strength of the relationship between chronic moderate alcohol consumption and HRV in women during the later adult years is nonexistent. Therefore, an effect size of .57 was empirically estimated, a posteriori, from the $R^2 (.25)$ for the correlation between alcohol consumption rate and SDNN for the subjects who met the inclusion criteria for this dissertation project. A total of 6 independent variables (alcohol consumption rate, age, caffeine, tobacco, cardiovascular medications, and activity level) were theoretically specified. A power analysis indicated that a multiple regression procedure with a sample size of 43 would yield a power of .80 with an effect size of this magnitude when $\alpha$ is set at 0.05 (Faul & Erdfelder, 1992). Since complete Holter data was available on 52 subjects the study was judged to be adequately powered.

Target Population

The target population is healthy community dwelling women 60+ years old.

Protocol

1. All subjects enrolled in the study were screened through a telephone interview for atrial fibrillation/heart disease, prior chemotherapy, diabetes mellitus, and stroke. Appointments were scheduled for subjects who answered the screening questions negatively and expressed a desire to participate in the study.

2. A health history and physical examination were performed at the time of the appointment. The interview included a detailed alcohol history and specific questions about current and past alcohol consumption, activity level, and medication history including prescribed and over-the-counter drugs.
Blood pressures were measured once after 10 minutes of quiet conversation, via auscultation of the left brachial artery with the patient in the seated position.

3. Five electrodes were placed according to the Zymed preferred placement after the placement cites were cleaned with alcohol swabs. Holter EKG monitoring was then initiated. Twenty-four hour Holter recordings were obtained with Zymed model 1100-010 channel multitrack recorders.

Blood samples were obtained by inserting a 23 gauge butterfly into the antecubital vein. Three 7-ml samples were drawn into three Vacutainers (two containing ethylenediaminetetraacetic acid (EDTA) and one containing heparin). Samples were then collected and prepared for analysis. When appropriate, samples were centrifuged with in one hour of phlebotomy. Blood chemistries, hematological indices, GGT, and a lipid profile were obtained on each subject. Arrangements were made with each subject for retrieval of the Holter monitor and tape.

4. All subjects were screened by a board certified cardiologist for clinical cardiac disease.

Instrumentation

Alcohol Consumption Rate

Alcohol Consumption Rate is reported in the pharmacological gold standard of grams of ethanol per square centimeter of body surface per day (g/cm²/day). Body surface in cm² = 71.8 x weight⁰.⁴²₅ x height⁰.⁷₂₅ (Dubois & Dubois, 1916).

Alcohol consumption was reported by subjects as quantity (drinks per occasion) and frequency (number of drinking days per month). Chronicity was
estimated by age at first drink and report of recent change in current drinking habits. The number of standard drinks per month (Quantity x Frequency) was calculated for each subject.

The following equivalencies were used to convert the product of quantity x frequency into grams: A **standard drink** = 12 grams of ethanol (Forty-five milliliters of 80 proof distilled spirits, 5 ounces of wine, or 12 ounces of beer = 12 grams of ethanol).

**Self-Reported Data**

In this study calculation of alcohol consumption rate, the primary independent variable, was based on self-reported Quantity x Frequency of alcohol consumption. The concurrent validity of this aggregate measure was established by comparing alcohol consumption rate, T-ACE scores, and GGT and MCV values.

**Validity of Self-Report Measures of Alcohol Consumption**

Self-report data is reported in approximately 90% of studies in alcohol dependent clinical populations and is the dominant method of collecting consumption data in alcohol studies. Self-report data is considered to be valid and reliable when specific caveats for data collection are respected. The validity of self-report measures is enhanced when data is collected from sober, intelligent, motivated subjects, in a research setting, after confidentiality has been assured by an interviewer with whom the subject has established a rapport (Babor & Del Boca, 1995; Babor, Stephens, & Marlatt, 1987). Self-reported alcohol consumption is more accurate when actual levels of alcohol consumption are low to moderate (Sobell, & Sobell, 1992).
In this study data about moderate alcohol consumption were collected from cognitively intact individuals all of whom reported abstaining from alcohol consumption on the day of the interview. The significance of the research and the importance of accurate answers was explained to the subjects prior to the initiation of the interview. Confidentiality was assured. Alcohol consumption questions were asked about one hour into the interview after a rapport had been established between the interviewer and the subject.

**QF measures.** Aggregate QF measures have exhibited satisfactory criterion validity in diverse populations when compared to collateral reports and diary entries (Babor, Stephens, & Marlatt, 1992).

**Reliability of Self-Reported Alcohol Consumption**

Overall, self-report measures of alcohol consumption have demonstrated adequate test-retest reliability in diverse populations. While the findings of reliability studies have varied by population and by between test interval length. Test-retest QF measures have consistently high correlations across populations (.85 to .90) (Armor, et al. 1978; Blumhagen & Little, 1985).

**Heart Rate Variability**

The following time-domain and frequency-domain measures if HRV were reported as group data:

1. **HRV Triangular Index (HRVI)** is the integral (area) of the density distribution divided by the maximum (modal value) of the density distribution.
2. **Triangular Interpolation of the NN (TINN)** is the length of the base of a triangle derived from a minimum square difference interpolation. The base of the triangle is equal to the horizontal axis of the density histogram of a triangle and a height equal to the peak of the maximum point (highest peak) of the sample density histogram.

3. **SDNN** is calculated as the standard deviation of all the inter-beat interval between normal beats (NN intervals) over a 24 hour period. The SDNN includes all frequency components in a 24 period.

4. **SDANN** is calculated as the average standard deviation of the NN intervals contained in 5 minute epochs and measures the variability of oscillations greater than 5 minutes in duration.

5. **ASDNN5** is calculated as the standard deviation of the average of all 5 minute epochs of NN intervals in a 24 hour period and assesses the variability of oscillations less than 5 minutes in duration.

6. **RMSSD** is calculated as the square root of the mean of the squared differences between successive NN intervals and is an estimate of high frequency (vagal) oscillations in HRV.

7. **High Frequency (HF)** the variance (power) in all NN intervals in the frequency range 0.15-0.4 Hz and reported as AUC in ms².

8. **Low Frequency (LF)** the variance (power) in all NN intervals in the frequency range between 0.04 - 0.15 HZ reported as AUC in ms².
9. **Very Low Frequency (VLF)** the variance (power) in all NN intervals in the frequency range 0.003-0.04 Hz and reported as AUC in ms².

These indices are consistent with the standards developed by The Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (Task Force, 1996) in an effort to develop standard vocabulary, definition of terms, and battery of measures for HRV research.

**Validity of Time-Domain and Frequency-Domain Measures**

Animal and human and experiments using pharmacological blockade of autonomic input to the SAN have established the validity of frequency-domain measures as indices of autonomic influences. In a seminal study, conscious, unanesthetized dogs were treated with glycopyrrolate, propranolol, and an angiotensin converting enzyme inhibitor in order to pharmacologically dissociate the effects of the parasympathetic, sympathetic, and renin-angiotensin systems on HRV.

Parasympathetic blockade with glycopyrrolate eradicated the mid- and high-frequency peaks and reduced the amplitude of the low-frequency peak. Sympathetic blockade produced a tendency for the amplitude of the low-frequency peak to decrease. However, this decrease is inconsistent in the canine model due to the inherently low level of tonic sympathetic activity. Further manipulations indicated that both parasympathetic and sympathetic activity contribute to the low frequency peaks.

When arterial pressure was increased by infusion of the vasoconstrictor methoxamine during β-sympathetic blockade, parasympathetic input was reflexively increased as predicted by the baroreceptor reflex, heart rate fell and the area under
low-frequency peak increased. When arterial pressure was decreased by continuous infusion of the vasodilator nitroprusside during parasympathetic blockade, sympathetic input reflexively increased as predicted by the baroreceptor reflex, heart rate increased and the area under the low-frequency peak again increased. Blockade of the renin-angiotensin system increased the area under the low-frequency peak by a factor of 2 to 4.5. This finding has been used to argue that tonic input from the renin-angiotensin system attenuates the amplitude of perturbations in peripheral vasomotor tone that lead to fluctuations in central arterial and venous pressures and stabilizes the cardiovascular system (Akselrod, Gorden, Ubel, Shannon, Barger, & Cohen, 1981).

These findings have been replicated in human studies combining autonomic blockade and postural change. Baseline HR was recorded from subjects in the supine and standing position and after treatment with atropine and propranolol on two consecutive days. In the preabsorptive state, standing resulted in a ten-fold increased the LF peak compared to supine values. Deep breathing increased HF peak values and the peak HF value was altered by changes in respiratory rate. The HF spectra was almost completely eradicated by atropine in both the supine and standing position. This reduction was not augmented by adding propranolol indicating that the HF component in humans is solely mediated by parasympathetic influences. Similar manipulations demonstrated that in the supine position the LF peak parasympathetic influences are dominant but sympathetic influences mediate LF variability in the standing position. This reversal reflects baroreceptor mediated responses to blood
pressure oscillation during position change (Pomeranz, MacAulay, Caudill, Kurtz, Adam, Gordon, Kilborn, Barger, Shannon, Cohen, & Benson, 1985).

Craft & Swartz (1995) expanded on previous research and validated the use of heart rate power spectra in older adults by describing the effects of aging, autonomic influence blockade, and position change on HRV. The power spectra of healthy young (20-40) and older adults (aged 60-81) were shown to be qualitatively similar. However, in older adults the components of the spectral density curve and the effects of position change are quantitatively diminished. Various combinations of autonomic blockade and position change certified that in older adults LF power is mediated by both sympathetic and parasympathetic input and that HF power is mediated solely by parasympathetic influences. However, atropine had less of an effect on HF power in older subjects than in younger subjects. Total pharmacological blockade eliminated heart rate variability in both age groups.

Pharmacological research directly validating time-domain measures as indices of autonomic influences is scare. Two studies have examined the direct effects of autonomic blockade on a limited number of time-domain measures. Hagerman, Berglund, Lorin, Nowak, & Sylven (1996) report a study of 10 healthy middle-aged men and women designed to estimate the effects of rest and exercise on multiple measures of HRV. Autonomic blockade decreased a time-domain index calculated from an interval histogram (Cripps, Malik, Farell, & Camm, 1991). Unfortunately, the differential effects of parasympathetic and sympathetic blockade were not reported and this measure is very rarely cited in the literature. Although the finding implies
that the measure is affected by autonomic blockade, this affect is difficult to interpret. Craft & Schwartz (1995) related that the SD of R-R was decreased by atropine and that this decrease was greater in younger (20-40) compared to older (60-81) subjects. The SD of R-R was not affected by propranolol dosing. Total autonomic blockade did decrease SD of R-R. These results imply that the decrease was mediated by parasympathetic input. The major focus of these studies was not the effect of autonomic blockade on HRV. As a result this effect is either explored haphazardly or incompletely reported.

Concurrent validity of time-domain measures of HRV has been established by correlational studies where frequency-domain measures served as the "gold standard". The correlation of three time-domain measures calculated from ECG recordings of 15 healthy male subjects obtained during autonomic blockade and frequency-domain measures calculated from the same recordings provide evidence of concurrent validity for SD, coefficient of variation (SD/mean R-R), and mean successive difference. Vagal tone was defined as the decrement in spectral power when atropine was added to sympathetic blockade with propranolol. SD, coefficient of variation, and mean successive differences all correlated significantly (p < .00026) with HF power. The significance of these correlations validates all three measures as indices of vagal tone (Hayano, Sakadibara, Yamada, Yamada, Mukai, Fujinami, Yokayoma, Watanabee, & Takata, 1991). The time-domain measures of SDNN, SDNN index, r-MSSD, and pNN50 calculated from 24-hour Holter recordings in post-infarction patients correlated with the components of the power spectrum. Time-domain and frequency-
domain measures were considered to be equivalent if they achieved a correlation ≥ 0.90 (Bigger, Fleiss, Steinman, Rohnitzky, Kleiger, & Rottman, 1992).

**Stability of Time-Domain and Frequency-Domain Measures**

Time-domain measures of HRV (day/night ratio, mean day heart period, mean night heart period, SDNN, and mean and standard deviation of all 5-minute normal epochs, r-MSSD and pNN50) and the frequency-domain measures of LF power and HF power calculated from 24 hour ambulatory EKG Holter recordings from normal adults remain stable over periods from three to 65 days (Klieger, Bigger, Bosner, Chung, Cook, Rohnitzky, Steinman, & Fleiss, 1991).

**Possible Confounding Variables**

As discussed in Chapter 2, clinical state, exercise/activity level, age, gender, BMI, cardiovascular medications, nicotine, caffeine, are variables that may affect HRV. The influence of these possible confounders were controlled for in the current study by the following research design and statistical methods:

1. Clinical State: the extensive exclusion criteria were selected to control for clinical states that are known to affect cardiac status and autonomic tone.

2. Activity/Exercise level was entered into the regression equation as an independent variable and coded as:

   1 = almost no activity- most of time in bed, on chair or couch
   2 = basic activity - housework, job, shopping, visiting
   3 = mild exercise - walking, bowling, golf, isometrics
   4 = strenuous exercise - tennis, swimming, exercise bike, etc.
3. Age was indexed as years since birth and entered into the regression equation as an independent variable.

4. Gender: effects of gender were be controlled by limiting the sample to female subjects.

5. The influences of BMI on alcohol dosing and measures of HRV were controlled by dividing g/day by body surface. Body surface was determined by the following formula: \( \text{cm}^2 = 71.8 \times \text{weight}^{0.425} \times \text{height}^{0.725} \) (Dubois & Dubois, 1916).

6. Current use of cardiovascular medications was dummy coded as:
   - 0 = no current use of cardiovascular medications
   - 1 = current use of cardiovascular medications (i.e., ace inhibitors, beta blockers, and/or calcium channel blockers).

   Current use of cardiovascular medications was entered into the regression equation as an independent variable.

7. Nicotine use was dummy coded as:
   - 0 = current non-smoker
   - 1 = current smoker.

   Nicotine use was entered into the regression model as an independent variable.

8. Caffeine use was indexed as total caffeine intake from coffee, tea, soft drinks, and chocolate per day. This index was entered into the regression equation as an independent variable.
Data Management

Twenty-four hours of Holter recordings were obtained using a Zymed model 1100-010 channel multitrack recorder. The recordings were digitalized by a Zymed 1500 scanner and submitted to the standard algorithms for QRS labeling and editing. The tapes were iteratively reviewed by a technician blind to the alcohol consumption history of the subjects until all sinus beats were correctly labeled, non-sinus beats eliminated, and all normal to normal, normal to premature and normal to late atrial, and normal to premature and late ventricular beats were properly classified. When ectopic beats or noise caused gaps in the R-R stream the preceding and succeeding RR interval were excluded and the instantaneous heart period function was be estimated by a linear interpolation. Linear splines can cause a slight attenuation of high frequency power but, other components are robust to splining effects (Albracht & Cohen, 1988). The annotated data files produced by this method were transferred to a PC for further analysis. The 24 hour files were divided into 288 mutually exclusive, consecutive, 5-minute segments. As described by Rottman, et al. (1990), the Zymed Software samples an instantaneous heart period function at 292-ms intervals, and uses a 584-ms wide boxcar filter to provide 1,024 data points per 5-minute segment. The mean NN interval is then subtracted from the heart period data and a Hanning window is applied before a Fast Fourier Transformation is
calculated. The 5-minute power spectra that result from this process are corrected for the effects of the Hanning window and sampling and then averaged. This method produces a power spectra with a frequency range of 0.003 to 0.800 Hz.

Time-domain measures were calculated from the original annotated data files and based on continuous, non-overlapping, 5-minute segments. Only segments with at least 75% normal R-R noise free intervals were included in the analysis. This criterion was chosen to minimize subject rejection while providing maximally consistent results and closely approximates the criterion of 80% suggested by Rottman (1990).

The results of these calculations were entered into EXCEL and SPSS/PC for statistical analysis.

Statistical Analysis

Summary statistics (mean and standard deviation or frequencies and percents) were calculated for the following sample characteristics: age, years of education, yearly income, marital status, employment status, antihypertensive medication use, caffeine intake, nicotine use, exercise and activity level, alcohol consumption rate, QF, T-ACE, and HRV indices.

The direction and strength of the full relationships between alcohol consumption rate and HRV measures were determined by bivariate correlations and standard univariate regression. Standard multiple regression analysis was used to determine the unique contribution of moderate alcohol consumption and each of the other independent variables to estimates of HRV. The unique contributions of alcohol
consumption rate and each of the five other independent variables in the regression model to the variability in HRV measures were assessed by the squared semipartial correlation coefficient (sr^2) derived from standard multiple regression analysis. In calculating the sr^2 the contributions of IVs other than the IV of interest are removed from the IV of interest and thus, the sr^2 is a more useful measure of the unique relationship between an IV and the DV than the standardized beta coefficient (β). In standard multiple regression the sr^2 represents the decrease in R^2 that would result from the IV of interest being omitted from the regression equation (Tabachnick & Fidel, 1996).

Repeated measures analysis of variance was used to determine the relationship between alcohol consumption rate and the circadian rhythm of heart rate variability in older women. The relationship between moderate alcohol consumption and the circadian pattern of HRV was assessed by the following methods:

1. the entire sample was partitioned into three groups:

   **Group 1 or "Abstainers"** = subjects who reported abstinence (n = 15)

   **Group 2 or "Drinkers"** = subjects who reported drinking alcohol at an average rate of at least 1.9g/cm²/day (approximately one standard drink every other day). The highest consumption rate reported in this group was 4.3g/cm²/day (approximately three drinks per day) (n=19)

   **Group 3** = women who reported drinking alcohol at an average rate less that 1.9g/cm²/day but not abstinence (n = 18).

   Group 3 was eliminated from further analysis.
3) a total of 24 hourly averages were calculated for each frequency domain measure (HF, LF, VLF) and for the LF/HF ratio for each individual Abstainer or Drinker. These values were averaged across subjects to calculate hourly means for each group. The hourly group means, plotted against the appropriate hour of the recording period (midnight = hour 1, 1:00 a.m. = hour 2, etc.) serve to describe the 24 hour pattern of HRV for specific components.

4) the stream of 24 hourly averages calculated for each group were divided into four periods of 6 hours each: 12:00 a.m.-6:00 a.m.; 6:00 a.m.-12:00 p.m.; 12:00 p.m.-6:00 p.m., and 6:00 p.m.-12:00 a.m. and averaged. Repeated measures analysis of variance was used to estimate group differences across these time periods.

Protection of Human Rights

Prior to enrollment in the parent project written informed consent was obtained from each subject. As part of the informed consent procedure the protocol, risks, and time requirements were explained to the subjects. Each subject was given ample time to read the consent form and ask questions. All questions were answered. Additionally, the concept of informed consent, the role of an internal review board (IRB), and federal protection for human subjects was discussed. The subject was given a second chance to withdraw after this discussion. None of the subjects who consented to interview after telephone screening withdrew. After informed consent was obtained, the subject was given a copy of the consent form that included the name and phone number of the principle investigator of the parent study as well as the phone number of the IRB offices.
Confidentially

All data were coded with a subject number and entered without other identification. Blood samples were similarly coded. Interview and laboratory data were kept in a locked cabinet in an secured research office.

Risks and Benefits

Risks involved in the data collection were limited to: (1) infection or hematoma at the blood draw cite, (2) skin irritation from electrode patches, and (3) inconvenience associated with Holter EKG monitoring. Benefits included the option of access to the hematological and cardiac electrophysiological data collected during the study. These reports were sent to each subject's personal physician with the subject's permission.
CHAPTER 4

RESULTS AND DISCUSSION

Data Screening

Before the primary analysis, all independent, dependent, and descriptive variables were checked by scatterplots and descriptive statistics for plausibility and possible outliers. Possible outliers were checked for coding errors and corrected when a coding error was detected. The single missing variable, one blood pressure reading, was replaced with the mean of the sample.

Histograms and normal probability plots were used to visually screen continuous variables for normality. Skewedness and kurtosis indices as well as the Kolmogrov-Smirnov Goodness of Fit (K-S) statistics were calculated to quantitatively check these findings. All frequency-domain measures and the rMSSD were found to be right skewed. Scatterplots of IVs and HRV measures were characterized by decreasing dispersion at higher alcohol consumption rates indicating a possible non-linear element in the relationship between alcohol consumption rate and HRV. Receptor mediated dose response relationships are frequently characterized by a logarithmic curve. Therefore, a logarithmic transform of right skewed variables was justified on both an empirical and theoretical grounds. Logarithmic transformation of the right skewed variables improved both skewness and kurtosis indices as well as
the normal probability plots. The normality of all log transformed continuous measures was satisfactory as verified by the K-S statistic.

Alcohol consumption rate was noted to have an L-shaped distribution. The modal consumption rate was 0 (15 subjects). The remaining 38 subjects reported average consumption between 0 and 3 standard drinks per day. Since some cases reported 0 as a consumption rate, a square root transform was used as a proxy of the logarithmic transform. The square root transform of alcohol consumption rate data improved p value of the K-S statistic from .0037 to .2109. Overall, the transformations linearized the bivariate scatterplots that included the transformed variables and improved the residual plots.

Independent Variables

Six independent variables were selected for consideration in the primary analysis: alcohol consumption rate (ACR), exercise/activity level (ACT), age (AGE), caffeine (CAFF), cardiovascular medications (MEDS), and smoking (SMK). ACR and AGE were considered continuous ratio level variables, ACT was considered an interval level variable, and CAFF, MEDS, and SMK were dummy coded as dichotomous variables (0 = characteristic absent; 1 = characteristic present).

Inspection of the correlation matrix revealed that correlations among the independent variables ranged from -.19 to .34 (see Table 4). Only one of these correlations reached statistical significance (smoking and alcohol consumption rate, \( r = .34, p = .01 \)). Tolerance and Variance Proportions were calculated and the data was found to be free of multicolinearity problems.
<table>
<thead>
<tr>
<th></th>
<th>ACR</th>
<th>ACT</th>
<th>AGE</th>
<th>CAFF</th>
<th>MEDS</th>
<th>SMK</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACR</td>
<td>1.00 (52)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p = .</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACT</td>
<td>-.0152 (52)</td>
<td>1.00 (52)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p = .915</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AGE</td>
<td>-.0745 (52)</td>
<td>-.2005 (52)</td>
<td>1.00 (52)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p = .600</td>
<td></td>
<td></td>
<td>p = .154</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAFF</td>
<td>-.2047 (52)</td>
<td>-.0203 (52)</td>
<td>-.0929 (52)</td>
<td>1.00 (52)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p = .145</td>
<td></td>
<td></td>
<td>p = .887</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEDS</td>
<td>-.1130 (52)</td>
<td>-.0320 (52)</td>
<td>-.0338 (52)</td>
<td>-.1441 (52)</td>
<td>1.00 (52)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p = .425</td>
<td></td>
<td></td>
<td>p = .822</td>
<td>p = .512</td>
<td></td>
</tr>
<tr>
<td>SMK</td>
<td>.3426 (52)</td>
<td>-.1921 (52)</td>
<td>.1443 (52)</td>
<td>.0681 (52)</td>
<td>.0652 (52)</td>
<td>1.00 (52)</td>
</tr>
<tr>
<td></td>
<td>p = .013</td>
<td></td>
<td></td>
<td>p = .172</td>
<td>p = .308</td>
<td>p = .646</td>
</tr>
</tbody>
</table>

ACR = alcohol consumption rate; ACT = exercise/activity level; AGE = age; CAFF = total caffeine intake; MEDS = cardiovascular meds; SMK = smoking.

Table 4: Correlation Matrix of the Independent Variables
Sample Characteristics

Overall, sample subjects when compared to the general population of women over 65, were more likely to be married (66% vs 41%) or divorced (17% vs 5%) and less likely to be widowed (15% vs 48%). Subjects were more likely to be in the labor force (13% vs 9%) and to have completed both high school (98% vs 58%) and four or more years of college (25% vs 12%). About 75% of all subjects reported incomes above the national median for older adults (American Association of Retired Persons, 1992). Approximately 8% (4 subjects) smoked cigarettes. All smokers consumed alcohol.

For part of the analysis, two groups of subjects were extracted from the total sample. The first group, "Abstainers", was defined as the 15 study subjects considered abstainers (less than one standard drink per month). The second group, "Drinkers", was defined as the 19 non-abstainers who reported consuming at least 1.9g/cm³/day (slightly less than one standard drink every other day). Drinkers used more cardiac drugs (ace inhibitors, β-blockers, & calcium channel blockers) and estrogen replacement therapy than Abstainers but these differences did not reach statistical significance. The abstainer and drinking groups did not differ on caffeine scores or the exercise/activity index. Systolic and diastolic blood pressures were similar across groups.

Subjects who drank approximately 15 or more standard drinks per month when compared to abstainers were more likely to be married (63% vs 47%), less likely to be divorced (16% vs 27%) and equally likely to be widowed (21% vs 20%).
Drinkers were more likely to be currently employed (37% vs 27%) and less likely to describe their primary career as a "housewife" (58% vs 73%). Educational and income levels were comparable between groups.

On average, the total sample subjects drank a little more than one standard drink per drinking occasion and consumed alcohol on 11 days per month. Approximately 80% of the study subjects reported ever drinking 2 or more drinks on any one day and about half reported ever drinking more than 3 drinks on any one day. As a group, the subjects reported that they began using alcohol in young adulthood (21.5 years (6.3). Roughly 20% of the study subjects reported a change (decrease only) in their drinking pattern (range 6 months to 20 years).

Demographic, life style, clinical, and alcohol history characteristics of the study sample are reported as ungrouped (total sample) and grouped data (see Tables 5 to 7). All subjects were caucasian.
## Table 5: Ungrouped and Grouped Demographic Characteristics of the Study Sample

<table>
<thead>
<tr>
<th></th>
<th>Grouped Data (n=52)</th>
<th>Abstainers (n=15)</th>
<th>Drinkers (&gt;1.9g/cm^2/d) (n=19)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>69 (5.2)</td>
<td>68 (4.2)</td>
<td>69 (5.5)</td>
<td>.548</td>
</tr>
<tr>
<td><strong>Marital Status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>34 (65%)</td>
<td>7 (47%)</td>
<td>12 (63%)</td>
<td></td>
</tr>
<tr>
<td>Divorced</td>
<td>9 (17%)</td>
<td>4 (27%)</td>
<td>3 (16%)</td>
<td></td>
</tr>
<tr>
<td>Widowed</td>
<td>8 (15%)</td>
<td>3 (20%)</td>
<td>4 (21%)</td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>1 (1.9%)</td>
<td>1 (07%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><strong>Employment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Housewife</td>
<td>4 (7.6%)</td>
<td>11 (73%)</td>
<td>11 (58%)</td>
<td></td>
</tr>
<tr>
<td>Retired</td>
<td>35 (67%)</td>
<td>0</td>
<td>1 (06%)</td>
<td></td>
</tr>
<tr>
<td>Employed Full-Time</td>
<td>11 (21%)</td>
<td>3 (20%)</td>
<td>6 (32%)</td>
<td></td>
</tr>
<tr>
<td>Employed Part-Time</td>
<td>2 (3.8%)</td>
<td>1 (07%)</td>
<td>1 (05%)</td>
<td></td>
</tr>
<tr>
<td><strong>Educational Level</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grammar School</td>
<td>1 (1.9%)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>High School</td>
<td>9 (17%)</td>
<td>2 (13%)</td>
<td>2 (11%)</td>
<td></td>
</tr>
<tr>
<td>Technical School</td>
<td>4 (7.5%)</td>
<td>0</td>
<td>4 (21%)</td>
<td></td>
</tr>
<tr>
<td>Associate Degree</td>
<td>2 (3.8%)</td>
<td>1 (07%)</td>
<td>1 (05%)</td>
<td></td>
</tr>
<tr>
<td>College (no degree)</td>
<td>18 (34%)</td>
<td>6 (40%)</td>
<td>7 (37%)</td>
<td></td>
</tr>
<tr>
<td>College Degree</td>
<td>11 (21%)</td>
<td>4 (26%)</td>
<td>3 (16%)</td>
<td></td>
</tr>
<tr>
<td>Graduate Degree</td>
<td>7 (13.2%)</td>
<td>2 (13%)</td>
<td>2 (11%)</td>
<td></td>
</tr>
<tr>
<td><strong>Yearly Income</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Below 10,000</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$10,000 to $19,999</td>
<td>5 (9.6%)</td>
<td>2 (13%)</td>
<td>3 (16%)</td>
<td></td>
</tr>
<tr>
<td>$20,000 to $29,999</td>
<td>4 (7.6%)</td>
<td>1 (07%)</td>
<td>2 (11%)</td>
<td></td>
</tr>
<tr>
<td>$30,000 to $39,999</td>
<td>6 (12%)</td>
<td>1 (07%)</td>
<td>3 (16%)</td>
<td></td>
</tr>
<tr>
<td>$40,000 to $49,000</td>
<td>22 (42%)</td>
<td>3 (20%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>More than $50,000</td>
<td>5 (9.6%)</td>
<td>5 (33%)</td>
<td>8 (42%)</td>
<td></td>
</tr>
<tr>
<td>No Response</td>
<td>5 (9.6%)</td>
<td>3 (20%)</td>
<td>3 (16%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 5: Ungrouped and Grouped Demographic Characteristics of the Study Sample
<table>
<thead>
<tr>
<th></th>
<th>Grouped Data (n=52)</th>
<th>Abstainers (n=15)</th>
<th>Drinkers (&gt;1.9g/cm²/d) (n=19)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>alcohol consumption rate (g/cm²/d)</td>
<td>1.54 (1.44)</td>
<td>0</td>
<td>3.19 (.79)</td>
<td>.000</td>
</tr>
<tr>
<td>nicotine use</td>
<td>4 (7.6%)</td>
<td>0 (0%)</td>
<td>4 (21%)</td>
<td>.037</td>
</tr>
<tr>
<td>caffeine use</td>
<td>4.02 (1.84)</td>
<td>3.6 (2.3)</td>
<td>4.4 (1.5)</td>
<td>.264</td>
</tr>
<tr>
<td>exercise/activity level</td>
<td>3.15 (.61)</td>
<td>3.0 (.6)</td>
<td>3.2 (.5)</td>
<td>.464</td>
</tr>
</tbody>
</table>

Table 6. Ungrouped and Grouped Life Style Characteristics of the Study Sample
<table>
<thead>
<tr>
<th></th>
<th>Grouped Data</th>
<th>Abstainers (n=15)</th>
<th>Drinkers (&gt;1.9g/cm³/d) (n=19)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drinks/Occasion</td>
<td>1.27 (1.34)</td>
<td>.27 (.4)</td>
<td>1.8 (1.0)</td>
<td>.000</td>
</tr>
<tr>
<td>Occasions/Month</td>
<td>11.13 (12.37)</td>
<td>0</td>
<td>26 (7)</td>
<td></td>
</tr>
<tr>
<td>Ever &gt;2</td>
<td>42 (81%)</td>
<td>53% (.5%)</td>
<td>95% (22%)</td>
<td>.003</td>
</tr>
<tr>
<td>Ever &gt;3</td>
<td>28 (54%)</td>
<td>46% (.5%)</td>
<td>80% (.4%)</td>
<td>.041</td>
</tr>
<tr>
<td>Age at 1st Drink</td>
<td>21 (6.3)</td>
<td>20.7 (8.1)</td>
<td>20.7 (5.9)</td>
<td>.998</td>
</tr>
<tr>
<td>Change In Drinking Pattern</td>
<td>9 (17%)</td>
<td>1 (06%)</td>
<td>2 (11%)</td>
<td></td>
</tr>
<tr>
<td>Years Since Change</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;.5 - &lt;1 y</td>
<td>2 (3.8)</td>
<td>0</td>
<td>1 (05%)</td>
<td></td>
</tr>
<tr>
<td>1 - 4 yrs</td>
<td>3 (5.7%)</td>
<td>1 (06%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>5 - 9 yrs</td>
<td>2 (3.8%)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>10 -19 yrs</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>20 yrs +</td>
<td>2 (3.8%)</td>
<td>0</td>
<td>1 (05%)</td>
<td></td>
</tr>
<tr>
<td>T-ACE</td>
<td>.75 (1.17)</td>
<td>.07 (.26)</td>
<td>1.5 (1.5)</td>
<td>.001</td>
</tr>
<tr>
<td>Tolerance (standard drinks)</td>
<td>1.2 (.57)</td>
<td>.9 (.35)</td>
<td>1.6 (.60)</td>
<td>.000</td>
</tr>
</tbody>
</table>

Table 7: Ungrouped and Grouped Alcohol Use of the Study Sample
### Table 8: Ungrouped and Grouped Clinical Characteristics of the Study Sample

<table>
<thead>
<tr>
<th></th>
<th>Grouped Data (n=52)</th>
<th>Abstainers (n=15)</th>
<th>Drinkers (&gt;1.9g/cm²/d) (n=19)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac Drugs</td>
<td>31%</td>
<td>26% (.5%)</td>
<td>42% (.5%)</td>
<td>.365</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>135 (16)</td>
<td>137 (16)</td>
<td>133 (16)</td>
<td>.491</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>77 (9)</td>
<td>77 (9)</td>
<td>78 (9)</td>
<td>.867</td>
</tr>
<tr>
<td>MCV</td>
<td>90.83 (4.74)</td>
<td>89.4 (5.6)</td>
<td>92.8 (4.9)</td>
<td>.145</td>
</tr>
<tr>
<td>GGT</td>
<td>27.14 (14.79)</td>
<td>23.2 (6.9)</td>
<td>35.5 (20.2)</td>
<td>.025</td>
</tr>
<tr>
<td>Estrogen</td>
<td>51% (.5%)</td>
<td>40% (.5%)</td>
<td>63% (.5%)</td>
<td>.190</td>
</tr>
</tbody>
</table>
Concurrent Validity of Self-Reported Alcohol Consumption

Standardized Questionnaire

T-ACE

Standardized assessment tools appraise long term consequences of alcohol use and are considered valid indicators of chronic alcohol consumption. T-ACE scores correlated positively with alcohol consumption rate (Spearmann correlation coefficient = .53, p < .00001) and were significantly lower in subjects reporting abstinence compared to subjects reporting alcohol consumption rates of ≥ 1.9g.cm³/day (15 standard drinks per month) (p = .0006). As anticipated, scores on the Tolerance question ("How many drinks does it take to make you feel high?") made a large contribution to the difference among groups on T-ACE scores (p = .0004). The relatively low mean response to this question (.9 standard drinks SD = .35) is consistent with low to moderate alcohol consumption.

Laboratory Verification

GGT

The mean value of GGT for the total sample was 27.14U/L with a range of 12U/L to 91U/L (normal range 5U/L-85U/L). Only one value outside the normal range (91U/L) was noted. Steady consumption of four standard drinks per day has been shown to increase GGT levels above normal limits (Magruder-Habib, Kurand, & Fry, 1991) and three drinks per day is considered to be the threshold for an abnormal alcohol-related increase in GGT (Cushman, 1992). Therefore, the GGT values of the study subjects validate the range of alcohol consumption reported by the subjects (0 -
3 standard drinks per day) and indicate that the exclusion criteria successfully eliminated subjects currently drinking immoderately.

The correlation of alcohol consumption rate and GGT for the sample was .37 (p = .006). Weak positive correlations between GGT values in the normal range and low-moderate alcohol consumption have been reported (Papoz et al., 1981). Since GGT has a half-life of two weeks and returns to normal after two to four weeks of abstinence, these findings also confirm that the chronic moderate alcohol consumption levels reported by the sample subjects describe current drinking patterns. GGT values in Abstainers were significantly different than GGT values Drinkers (p = .025).

**MCV**

The mean MCV value for the sample was 90.94 fL (range 77 fL to 101 fL). Three values fell below the normal range of 82 fL to 99 fL and two values were slightly above. The correlation between alcohol consumption rate and MCV for the sample as a whole was .26 (p = .059). MCV values were not significantly different in the abstaining and drinking group. A direct relationship between consumption of 1-2 drinks per day and MCV has been reported (Popez et al., 1981). Although MCV values rarely exceed 110 fL in the macrocytosis of alcoholism, MCV values within the normal range are highly correlated with alcohol consumption (National Institute of Alcohol Abuse and Alcoholism, 1990). MCV has been reported to increase 1.7 fL per standard drink per day (Whitfeild, Hensley, Bryden, & Gallager, 1978).

No subject had an MCV value at the level associated with alcohol abuse or dependence verifying the absence of alcohol abuse in the sample, and again
confirming the effectiveness of the exclusion criteria in eliminating women drinking at higher than moderate levels. In the absence of abnormally high MCV values, the moderate correlation of alcohol consumption rate and MCV validates the report of moderate alcohol consumption in the sample. The fact that the MCV failed to separate the two groups defined on the basis of alcohol use may be related to the low sensitivity of MCV for alcohol abuse, sample size, or the constrained variability in MCV values.

In summary, scores on the T-ACE verify both the chronicity and validity of self-reported moderate alcohol consumption rates in the sample. Scores on the Tolerance question are consistent with moderate alcohol consumption. MCV and GGT values were, on the whole, within normal ranges and below those associated with high levels of alcohol consumption. These findings substantiate self-reported moderate alcohol consumption and establish a ceiling for level of alcohol consumption in the sample. The modest but significant relationship between alcohol consumption rate and GGT and the trend toward significance in the correlation between MCV suggest that alcohol was consumed at the levels reported. These indicators provide independent, objective confirmation of the self-reported alcohol consumption in the sample and provide concurrent validation of self-reported alcohol consumption rate.

Dependent Variables

A total of eleven measures of HRV were calculated from the ambulatory Holter ECG recordings: two geometric measures (Heart Rate Variability Index (HRVI) and Triangular Index of NN (TINN)); four time-domain measures (SDNN,
<table>
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<th>Mean</th>
<th>SD</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
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<tr>
<td><strong>Geometric Measures</strong></td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
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<td>230.00</td>
<td>830.00</td>
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<td></td>
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<tr>
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<td>28.78</td>
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<td>192.70</td>
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<tr>
<td>SDANN (ms)</td>
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</tr>
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<td>ASDNN (ms)</td>
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<td>10.49</td>
<td>20.83</td>
<td>67.32</td>
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<tr>
<td>rMSSD (ms)</td>
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<td>9.29</td>
<td>7.75</td>
<td>46.60</td>
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<tr>
<td>HR bpm</td>
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<td>9.21</td>
<td>57.86</td>
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<td></td>
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<td>4.74</td>
<td>141.40</td>
<td>620.71</td>
</tr>
<tr>
<td>[log] HF</td>
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<td>.94</td>
<td>2.50</td>
<td>6.43</td>
</tr>
<tr>
<td>LF (ms²)</td>
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<td>1320.77</td>
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<td>VLF (ms²)</td>
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<td>491.49</td>
<td>2736.45</td>
</tr>
<tr>
<td>[log] VLF</td>
<td>278.36</td>
<td>.47</td>
<td>5.53</td>
<td>7.91</td>
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</table>

Table 9: Descriptive Heart Rate Variability Statistics for the Entire Sample (n=52)
SDANN, ASDNN, and [log] rMSSD; three frequency-domain measures ([log]HF, [log]LF, [log]VLF), and average heart rate (HR). Overall, these values are comparable to or slightly lower than values reported for older women in other studies (Bruggerman, et al., 1984; Piccirillo, Fimognari, Viola, Margliano, 1995; Ryan, Goldberger, Pincus, Mietus, & Lipsitz, 1994; Stein, Kleiger, & Rottman, 1997). Differences may be related to differences in sample composition, processing techniques, or HRV algorithms (see Table 9).

While HRV measures were all significantly correlated (p < .002), many assess different aspects of HRV and the pattern of interrelationship of these measures can provide important insights into the association of the alcohol consumption rate and HRV. The dependent variable correlation matrix reveals that highest correlations among HRV estimates fall into three clusters (see Appendix C).

The first cluster is produced by the correlations between the geometric (HRVI and TINN) and time-domain measures of overall HRV (SNNN). SDANN, an intermediate measure of HRV that estimates the variability in cycles longer than 5 minutes also correlates strongly with the global measures. These global measures estimate the influences of long-term oscillations (circadian patterns, day-night differences in activity) as well as shorter term oscillations (vagal and sympathetic input) and can be considered general indices of ANS flexibility (Coumel, Maison-Blanche, & Catuli, 1994).

The findings of frequency-domain analysis in other samples indicates that shorter term oscillations (HF and LF) account for only 10% of the power in the total
bandwidth (Kautzner & Hnatkova, 1995). In the sample considered in this project, HF and LF accounted for only a relatively small portion of the total variance (see table 10).

<table>
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<th>Measure</th>
<th>Variance</th>
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</thead>
<tbody>
<tr>
<td>HF (ms²)</td>
<td>167</td>
</tr>
<tr>
<td>LF (ms²)</td>
<td>382</td>
</tr>
<tr>
<td>VLF (ms²)</td>
<td>1127</td>
</tr>
</tbody>
</table>

Table 10: Distribution of Power Spectrum For the Sample

Thus, it was predictable that the correlations between global measures of HRV and measures that estimate components of total the variability (HF, LF, rMSSD) are lower than the correlations among the global measures themselves.

The second cluster illustrates the association between intermediate length oscillatory components identified by the ASDNN (variability in cycle lengths less than 5 minutes) and all frequency domain measures as well as rMSSD the time-domain equivalent of HF. This pattern is consistent with the findings of other HRV studies. ASDNN is highly correlated with all frequency-domain estimates and is considered a surrogate for VLF power (Krautzner & Knatkova, 1995).
The third cluster reveals the relationship among the frequency-domain values. Total frequency contains the variability of the entire bandwidth and is strongly correlated with all of its component frequencies. While vagal tone is accepted as the origin of HF input, other components are blends of HF and several influences. Thus, the high correlations among all the components of the power spectrum were anticipated.

Correlations between HR and all HRV measures were significant but among the lowest correlations in the table. This is to be expected. HR and HRV are subject to common autonomic influences but are not surrogates. In general, lower heart rates are associated with greater variability.
Question One

What is the strength and direction of the relationship between moderate alcohol consumption rate and heart rate variability in older women?

The Full Relationship Between Alcohol Consumption Rate and Heart Rate Variability

Correlation analysis and univariate standard regression were used to assess the full nature of the relationship between alcohol consumption and HRV in the sample. The total relationship of alcohol consumption rate, and each of the other variables in the study, was assessed by examining bivariate correlations between alcohol consumption rate and the HRV measures (see Tables 11 through 13).

<table>
<thead>
<tr>
<th></th>
<th>ACR</th>
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<th>MEDS</th>
<th>SMOK</th>
<th>CAFF</th>
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<td>-.482</td>
<td>-.0326</td>
<td>.0220</td>
<td>-.0726</td>
<td>-.4180</td>
</tr>
<tr>
<td></td>
<td>R²</td>
<td>.232</td>
<td>.0001</td>
<td>.0005</td>
<td>.0053</td>
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<tr>
<td></td>
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<td>.8768</td>
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<td>.0020</td>
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<td>TINN</td>
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<td>.0481</td>
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<td>-.4139</td>
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<td></td>
<td>R²</td>
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<td>p</td>
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<td>.7349</td>
<td>-.5962</td>
<td>-.0023</td>
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</table>

Table 11: Full Correlations of Geometric Measures and Independent Variables

131
Of the six variables specified for entry into the regression model, only alcohol consumption rate and smoking had significant correlations with the global geometric measures of HRV. Although these negative correlations were of moderate strength, the associations were significant and p values of at least .0005 were noted. The associations between alcohol and both HRVI and TINN were slightly stronger than the association between smoking and alcohol (see Table 11).

Among the time domain measures, significant negative associations were documented between both alcohol and smoking and SDNN, SDANN, and ASDNN. SDNN is a global measure of HRV, equivalent in scope to the geometric measures. SDANN and ASDNN are more focused measures. SDANN estimates variability related to oscillations greater than 5 minutes in duration. ASDNN estimates the variability in oscillations of less than five minutes. The rMSSD estimates the variability in the highest frequency oscillations contributing to HRV and has the narrowest focus of the time domain measures. The relationship between alcohol consumption rate and [log] rMSSD was not significant at $\alpha = .05$ ($p = .10$). The relationship between smoking and [log] rMSSD also failed to achieve significance. Smoking was the only independent variable to have a significant effect on HR (see Table 12). The finding that alcohol consumption rate was not significantly correlated with HR argues that the relationship between alcohol and HRV is not mediated by increased HR.
<table>
<thead>
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<td></td>
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<td>-.4541</td>
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</tr>
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</tr>
<tr>
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<tr>
<td>r</td>
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<tr>
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<td>.7752</td>
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</tr>
<tr>
<td>r</td>
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<td>-.1082</td>
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</tr>
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<td>.0002</td>
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<td>.0392</td>
</tr>
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<td>p</td>
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</table>

Table 12: Full Correlations of Time-Domain Measures and Independent Variables
The full correlations between alcohol consumption rate and $\log$ VLF, and $\log$ LF were moderate and significant. The correlation between alcohol consumption rate and $\log$ HF approached significance ($p = .065$).

Among the other independent variables only the correlations between smoking and $\log$ VLF reached significance ($p = .029$ respectively).

However, the correlation between smoking and $\log$ HF and $\log$ LF approached significance ($p = .092$ and $p = .089$ respectively) (see Table 13).

<table>
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<tr>
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<th>CAFF</th>
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<td></td>
<td>$R^2$</td>
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<td>.0034</td>
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<td>.0570</td>
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<tr>
<td></td>
<td>$p$</td>
<td>.0648</td>
<td>.6549</td>
<td>.6802</td>
<td>.3002</td>
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<td>Log LF</td>
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<td>-.0370</td>
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<tr>
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<td>Log VLF</td>
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<td>.5093</td>
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</table>

Table 13. Full Correlations of Frequency-Domain Measures and Independent Variables

Taken as a whole these full correlations suggest that moderate alcohol consumption has an inverse relationship with measures of HRV that is of moderate
strength. The relationship is strongest between moderate alcohol consumption and long-term global measures of HRV and is attenuated when measures of shorter-term significance are considered. The relationship between alcohol consumption rate and HR was not significant. However, bivariate correlations do not isolate the unique portion of the variance in HRV measures attributable to alcohol consumption rate from the variance shared by alcohol and the other independent variables in the study; this issue will be addressed next.
Question 2

What is the unique contribution of alcohol consumption rate to HRV for older women?

The Unique Relationship Between Alcohol Consumption Rate and Heart Rate Variability

The results of the standard multiple regression analysis indicated that the unique contribution of alcohol to estimated HRV was greatest when HRV was operationalized by broad based global measures. Alcohol consumption rate accounted for approximately one third of the variability in HRVI ($r^2 = -.34$, $p = .0086$), TINN ($r^2 = -.32$, $p = .0122$), and SDNN ($r^2 = -.32$, $p = .0086$). A similar relationship is documented for alcohol and SDANN ($r^2 = -.31$, $p = .0190$). However, the unique contributions of alcohol to shorter-term indices were found to be smaller and did not achieve statistical significance. The unique contributions of alcohol consumption rate to ASDNN and $[\log] rMSSD$ were -.22 ($p = .11$) and -.12 ($p = .37$) respectively (see Tables 14 through 19).
### Table 14: Standard Multiple Regression of Independent Variables on HRVI

(R = .57; R² = .32; F = 3.66121, p = .0058)

<table>
<thead>
<tr>
<th>IV</th>
<th>B</th>
<th>β</th>
<th>sr²</th>
<th>T</th>
<th>p</th>
</tr>
</thead>
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</tr>
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</table>

### Table 15: Standard Multiple Regression of Independent Variables on TINN

(R = .56; R² = .34; F = 3.4, p = .0075)

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<th>IV</th>
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<th>β</th>
<th>sr²</th>
<th>T</th>
<th>p</th>
</tr>
</thead>
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Table 14: Standard Multiple Regression of Independent Variables on HRVI
(R = .57; R² = .32; F = 3.66121, p = .0058)

Table 15: Standard Multiple Regression of Independent Variables on TINN
(R = .56; R² = .34; F = 3.4, p = .0075)
### Table 16: Standard Multiple Regression of Independent Variables on SDNN

(R = .61; R² = .38; F = 4.47; p = .0012)

<table>
<thead>
<tr>
<th>IV</th>
<th>B</th>
<th>β</th>
<th>sr²</th>
<th>T</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACR</td>
<td>-7.129083</td>
<td>-.356123</td>
<td>.324111</td>
<td>-2.747</td>
<td>.0086</td>
</tr>
<tr>
<td>ACT</td>
<td>-3.422721</td>
<td>-.072126</td>
<td>.069420</td>
<td>-.588</td>
<td>.5592</td>
</tr>
<tr>
<td>AGE</td>
<td>.238525</td>
<td>.042858</td>
<td>.041306</td>
<td>.350</td>
<td>.7279</td>
</tr>
<tr>
<td>CAF</td>
<td>-1.309031</td>
<td>-.083754</td>
<td>.080077</td>
<td>-.679</td>
<td>.5008</td>
</tr>
<tr>
<td>MED</td>
<td>-9.030503</td>
<td>-.146211</td>
<td>.142603</td>
<td>-1.209</td>
<td>.2331</td>
</tr>
<tr>
<td>SMK</td>
<td>-32.576397</td>
<td>-.336894</td>
<td>.308772</td>
<td>-2.617</td>
<td>.0120</td>
</tr>
<tr>
<td>(Constant)</td>
<td>134.82355</td>
<td></td>
<td></td>
<td>2.444</td>
<td>.0185</td>
</tr>
</tbody>
</table>

### Table 17: Standard Multiple Regression of Independent Variables on SDANN

(R = .61; R² = .38; F = 4.54, p = .0011)

<table>
<thead>
<tr>
<th>IV</th>
<th>B</th>
<th>β</th>
<th>sr²</th>
<th>T</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACR</td>
<td>-6.37759</td>
<td>-.343397</td>
<td>.312529</td>
<td>-2.657</td>
<td>.0109</td>
</tr>
<tr>
<td>ACT</td>
<td>-4.761252</td>
<td>-.108162</td>
<td>.104090</td>
<td>-.885</td>
<td>.3810</td>
</tr>
<tr>
<td>AGE</td>
<td>.072164</td>
<td>.013976</td>
<td>.013470</td>
<td>.114</td>
<td>.9094</td>
</tr>
<tr>
<td>CAF</td>
<td>-.570124</td>
<td>-.039319</td>
<td>.037592</td>
<td>-.320</td>
<td>.7508</td>
</tr>
<tr>
<td>MED</td>
<td>-10.648786</td>
<td>-.185841</td>
<td>.181255</td>
<td>-1.541</td>
<td>.1304</td>
</tr>
<tr>
<td>SMK</td>
<td>-31.070413</td>
<td>-.346347</td>
<td>.317435</td>
<td>-2.698</td>
<td>.0098</td>
</tr>
<tr>
<td>(Constant)</td>
<td>122.945110</td>
<td></td>
<td></td>
<td>2.617</td>
<td>.0120</td>
</tr>
</tbody>
</table>

Table 16: Standard Multiple Regression of Independent Variables on SDNN

Table 17: Standard Multiple Regression of Independent Variables on SDANN

(R = .61; R² = .38; F = 4.54, p = .0011)
<table>
<thead>
<tr>
<th>IV</th>
<th>B</th>
<th>$\beta$</th>
<th>$sr^2$</th>
<th>T</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACR</td>
<td>-.1592851</td>
<td>-.218291</td>
<td>.219954</td>
<td>-1.628</td>
<td>.1103</td>
</tr>
<tr>
<td>ACT</td>
<td>-.1382505</td>
<td>-.076928</td>
<td>.058032</td>
<td>-.430</td>
<td>.6695</td>
</tr>
<tr>
<td>AGE</td>
<td>.030061</td>
<td>.014282</td>
<td>.031002</td>
<td>.230</td>
<td>.8195</td>
</tr>
<tr>
<td>CAF</td>
<td>-.235704</td>
<td>-.041374</td>
<td>.034889</td>
<td>-.258</td>
<td>.7973</td>
</tr>
<tr>
<td>MED</td>
<td>1.664177</td>
<td>-.073922</td>
<td>.035249</td>
<td>.261</td>
<td>.7953</td>
</tr>
<tr>
<td>SMK</td>
<td>-9.556875</td>
<td>-.271151</td>
<td>.227865</td>
<td>-1.687</td>
<td>.0984</td>
</tr>
<tr>
<td>(Constant)</td>
<td>51.050166</td>
<td></td>
<td></td>
<td></td>
<td>.0651</td>
</tr>
</tbody>
</table>

Table 18: Standard Multiple Regression of Independent Variables on ASDNN
($R = .41; R^2 = .17; F = 1.51; p = .1957$)

<table>
<thead>
<tr>
<th>IV</th>
<th>B</th>
<th>$\beta$</th>
<th>$sr^2$</th>
<th>T</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACR</td>
<td>-.039145</td>
<td>-.136273</td>
<td>.124023</td>
<td>-.903</td>
<td>.3715</td>
</tr>
<tr>
<td>ACT</td>
<td>-.105451</td>
<td>-.154882</td>
<td>.149050</td>
<td>-1.085</td>
<td>.2838</td>
</tr>
<tr>
<td>AGE</td>
<td>-7.80E-04</td>
<td>.009779</td>
<td>.009425</td>
<td>.069</td>
<td>.9456</td>
</tr>
<tr>
<td>CAF</td>
<td>-.031155</td>
<td>-.138918</td>
<td>.132818</td>
<td>-.967</td>
<td>.3389</td>
</tr>
<tr>
<td>MED</td>
<td>.120549</td>
<td>.136018</td>
<td>.132662</td>
<td>.966</td>
<td>.3395</td>
</tr>
<tr>
<td>SMK</td>
<td>-.319420</td>
<td>-.230208</td>
<td>.210991</td>
<td>-1.536</td>
<td>.1316</td>
</tr>
<tr>
<td>(Constant)</td>
<td>3.616248</td>
<td></td>
<td></td>
<td>3.923</td>
<td>.0003</td>
</tr>
</tbody>
</table>

Table 19: Standard Multiple Regression of Independent Variables on [Log] rMSSD
($R = .39; R^2 = .15; F = 1.32, p = .2646$)
Frequency-domain analysis produced a similar pattern of results in that the unique contribution of alcohol consumption rate to a measure of HRV was the smallest for the vagal tone estimate, [log] HF ($sr^2 = .18$, $p = .2381$). Among the remaining frequency-domain measures, the unique contribution of alcohol was largest to [log] LF ($sr^2 = -.27$, $p = .0559$). The contribution of alcohol consumption rate to [log] VLF ($sr^2 = -.23$, $p = .1028$) showed only a trend towards significance. (see Tables 20 through 22).
### Table 20: Standard Multiple Regression of Independent Variables on [Log] VLF
(R = 40; R^2 = .16; F = 1.4, p = .22)

<table>
<thead>
<tr>
<th>IV</th>
<th>B</th>
<th>( \beta )</th>
<th>( sr^2 )</th>
<th>T</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACR</td>
<td>-.081963</td>
<td>-.249789</td>
<td>.227335</td>
<td>-1.665</td>
<td>.1028</td>
</tr>
<tr>
<td>ACT</td>
<td>-.063522</td>
<td>-.081676</td>
<td>.078601</td>
<td>-.576</td>
<td>.5676</td>
</tr>
<tr>
<td>AGE</td>
<td>-.005149</td>
<td>-.054396</td>
<td>.054396</td>
<td>-.398</td>
<td>.6922</td>
</tr>
<tr>
<td>CAF</td>
<td>-.002109</td>
<td>-.008234</td>
<td>.007872</td>
<td>-.058</td>
<td>.9543</td>
</tr>
<tr>
<td>MED</td>
<td>.080147</td>
<td>.079167</td>
<td>.077213</td>
<td>.566</td>
<td>.5745</td>
</tr>
<tr>
<td>SMK</td>
<td>-.362646</td>
<td>-.228804</td>
<td>.209704</td>
<td>-1.536</td>
<td>.1315</td>
</tr>
<tr>
<td>(Constant)</td>
<td>7.628654</td>
<td></td>
<td></td>
<td>7.291</td>
<td>.0000</td>
</tr>
</tbody>
</table>

### Table 21: Standard Multiple Regression of Independent Variables on [Log] LF
(R = .38; R^2 = 15; F = 1.29, p = .2802)

<table>
<thead>
<tr>
<th>IV</th>
<th>B</th>
<th>( \beta )</th>
<th>( sr^2 )</th>
<th>T</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACR</td>
<td>-.148732</td>
<td>-.303815</td>
<td>.270215</td>
<td>-1.963</td>
<td>.0559</td>
</tr>
<tr>
<td>ACT</td>
<td>.041254</td>
<td>.038814</td>
<td>.033436</td>
<td>.243</td>
<td>.8092</td>
</tr>
<tr>
<td>AGE</td>
<td>-3.315E-04</td>
<td>.008395</td>
<td>.002294</td>
<td>.017</td>
<td>.9868</td>
</tr>
<tr>
<td>CAF</td>
<td>-.027099</td>
<td>-.068167</td>
<td>.066246</td>
<td>-.481</td>
<td>.6327</td>
</tr>
<tr>
<td>MED</td>
<td>-.006150</td>
<td>-.012661</td>
<td>.003881</td>
<td>-.028</td>
<td>.9776</td>
</tr>
<tr>
<td>SMK</td>
<td>-.300437</td>
<td>-.119971</td>
<td>.113798</td>
<td>-.827</td>
<td>.4129</td>
</tr>
<tr>
<td>(Constant)</td>
<td>5.986945</td>
<td></td>
<td></td>
<td>3.716</td>
<td>.0006</td>
</tr>
</tbody>
</table>

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The full regression models using HRVI, TINN, SDNN, and SDANN as dependent variables were all significant. The full models using ASDNN, [log] rMSSD, or any of the frequency domain measures did not reach statistical significance. When HR was entered into the regression model as the dependent variable the model reached statistical significance. Smoking was the only significant independent variable ($sr^2 = .31, p = .02$) while alcohol consumption rate did not approach significance ($sr^2 = .07, p = .58$). Again this argues that the relationship between moderate alcohol consumption and HRV is not mediated by changes in HR (see Table 23).
<table>
<thead>
<tr>
<th>IV</th>
<th>B</th>
<th>β</th>
<th>sr²</th>
<th>T</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACR</td>
<td>.460369</td>
<td>.081520</td>
<td>.065425</td>
<td>.503</td>
<td>.6173</td>
</tr>
<tr>
<td>ACT</td>
<td>-.509798</td>
<td>-.39825</td>
<td>.032321</td>
<td>-.249</td>
<td>.8048</td>
</tr>
<tr>
<td>AGE</td>
<td>-.209433</td>
<td>-.136560</td>
<td>.113366</td>
<td>-.872</td>
<td>.3879</td>
</tr>
<tr>
<td>CAF</td>
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<td>.155593</td>
<td>.150231</td>
<td>1.155</td>
<td>.2541</td>
</tr>
<tr>
<td>MED</td>
<td>-4.734915</td>
<td>-.231444</td>
<td>.233724</td>
<td>-1.797</td>
<td>.0790</td>
</tr>
<tr>
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<td>.341035</td>
<td>.318020</td>
<td>2.446</td>
<td>.0184</td>
</tr>
<tr>
<td>(Constant)</td>
<td>87.781467</td>
<td></td>
<td></td>
<td>4.513</td>
<td>.0000</td>
</tr>
</tbody>
</table>

Table 23: Standard Multiple Regression of Independent Variables on HR
(R = .49; R^2 = .24; F = 2.36, p = .0460)
Question 3

What is the relationship between moderate alcohol consumption rate and the circadian rhythm of heart rate variability in older women?

Group Differences in 24-Hour Average Measures of HRV

For this analysis two groups were extracted from the total sample:

"Abstainers" = subjects who reported an ACR of 0 (n = 15)

"Drinkers" = subjects reporting an ACR of at least 1.9g/cm²/day (approximately one standard drink every other day (n=19).

Independent t-tests revealed that the pattern of group differences across HRV measures was similar to the pattern of relationships produced by regression analysis for all 52 subjects. The largest and most significant differences were found for the geometric measures and the global time-domain measures. The differences across groups for ASDNN, and [log] VLF power approached significance (p = .06) and no significant differences were found between groups for measures of vagal tone or for [log] LF power (see Table 24).
HF, LF, and VLF variance, in absolute terms, were also lower in Drinkers than in Abstainers (see Table 25).

<table>
<thead>
<tr>
<th>Measure</th>
<th>Groups</th>
<th>Mean (SD)</th>
<th>T</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRVI</td>
<td>Abstainers</td>
<td>27 (7)</td>
<td>3.39</td>
<td>.002</td>
</tr>
<tr>
<td></td>
<td>Drinkers</td>
<td>20 (5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TINN</td>
<td>Abstainers</td>
<td>545 (147)</td>
<td>3.14</td>
<td>.002</td>
</tr>
<tr>
<td></td>
<td>Drinkers</td>
<td>404 (114)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDNN</td>
<td>Abstainers</td>
<td>129 (32)</td>
<td>3.32</td>
<td>.002</td>
</tr>
<tr>
<td></td>
<td>Drinkers</td>
<td>98 (21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDANN</td>
<td>Abstainers</td>
<td>115 (30)</td>
<td>3.18</td>
<td>.003</td>
</tr>
<tr>
<td></td>
<td>Drinkers</td>
<td>87 (21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASDNN</td>
<td>Abstainers</td>
<td>42 (45)</td>
<td>1.92</td>
<td>.064</td>
</tr>
<tr>
<td></td>
<td>Drinkers</td>
<td>36 (10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[log]rMSSD</td>
<td>Abstainers</td>
<td>3.09 (.40)</td>
<td>1.46</td>
<td>.153</td>
</tr>
<tr>
<td></td>
<td>Drinkers</td>
<td>2.89 (.38)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[log]HF</td>
<td>Abstainers</td>
<td>4.86 (.99)</td>
<td>1.41</td>
<td>.167</td>
</tr>
<tr>
<td></td>
<td>Drinkers</td>
<td>4.40 (.89)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[log]LF</td>
<td>Abstainers</td>
<td>5.82 (.64)</td>
<td>1.60</td>
<td>.120</td>
</tr>
<tr>
<td></td>
<td>Drinkers</td>
<td>5.29 (.84)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[log]VLF</td>
<td>Abstainers</td>
<td>7.02 (.42)</td>
<td>1.95</td>
<td>.06</td>
</tr>
<tr>
<td></td>
<td>Drinkers</td>
<td>6.70 (.52)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 24: Group Differences for 24 Hour Average HRV Measures
Table 25: Power Distribution of Spectral Components for Abstainers and Drinkers

<table>
<thead>
<tr>
<th>Measure</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Abstainers (n=15)</td>
</tr>
<tr>
<td>HF (ms²)</td>
<td>186</td>
</tr>
<tr>
<td>LF (ms²)</td>
<td>412</td>
</tr>
<tr>
<td>VLF (ms²)</td>
<td>1214</td>
</tr>
</tbody>
</table>

**Group Differences in the Circadian Pattern of Frequency-Domain Measures**

**HF Power.** In the subset of 34 subjects selected for this analysis [log] HF power was significantly higher during the night time hours and lower during the day. Multivariate tests identified a circadian pattern in vagal activity for the group as a whole. Mean [log] HF power values were significantly higher during the 12:00 a.m. - 6:00 a.m. period than any other time period (see Table 26).

Table 26: Circadian Variation in [log] HF for Abstainers and Drinkers Combined

<table>
<thead>
<tr>
<th>Pairwise Comparison</th>
<th>Mean Difference</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>12MN-6AM vs 6AM-12N</td>
<td>.2568</td>
<td>.039</td>
</tr>
<tr>
<td>12MN-6AM vs 12N-6PM</td>
<td>.4525</td>
<td>.000</td>
</tr>
<tr>
<td>12MN-6AM vs 6PM-12MN</td>
<td>.4223</td>
<td>.001</td>
</tr>
</tbody>
</table>
This circadian pattern is similar to that reported for older women in other studies (Stein, Kleiger, & Rottman, 1997).

The analysis produced three major findings that describe group differences in the circadian pattern of HF power:

1. Drinkers had lower values for HF variability than Abstainers at all hours of the day except one (9:00 a.m.) (see Figure 9). Differences between groups reached statistical significance during the time period from 12:00 a.m. - 6:00 a.m. (p = .024) (see Table 27).

Figure 9: 24-Hour Plot of Group Means for [log] HF for Abstainers and Drinkers
<table>
<thead>
<tr>
<th>Time Period</th>
<th>Groups</th>
<th>Mean (SD)</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>12MN - 6AM</td>
<td>Abstainers Drinkers</td>
<td>5.23 (.97) 4.45 (.94)</td>
<td>2.371</td>
<td>.024</td>
</tr>
<tr>
<td>6AM - 12N</td>
<td>Abstainers Drinkers</td>
<td>4.69 (.92) 4.51 (1.00)</td>
<td>.555</td>
<td>.555</td>
</tr>
<tr>
<td>12N - 6 PM</td>
<td>Abstainers Drinkers</td>
<td>4.56 (1.18) 4.22 (.88)</td>
<td>.942</td>
<td>.353</td>
</tr>
<tr>
<td>6PM - 12MN</td>
<td>Abstainers Drinkers</td>
<td>4.62 (1.14) 4.22 (.90)</td>
<td>1.128</td>
<td>.268</td>
</tr>
</tbody>
</table>

Table 27: Differences in Twenty-Four Hour Spectral Analysis of [log]HF Variability Between Abstainers and Drinkers

2. Spectral analysis of the HF component revealed that Abstainers experienced a steep decline in HF tone that began at 7:00 a.m. and reached its nadir at 9:00 a.m. A similar decline, beginning at 7:00 a.m. was noted among Drinkers. However, since this decline was initiated at a lower level of HF input and did not reach its nadir until 11:00, the rate of decline was significantly less abrupt (see Figure 9).

3. Chronic moderate alcohol consumption is associated with a two hour delay in the circadian pattern of HF tone. In addition to the prolonged decline in nighttime
vagal tone, Drinkers had a delayed late evening rise in HF power that began two hours later than the late evening rise in HF power noted in Abstainers (see Figure 9).

**LF Power.** Multivariate tests identified a circadian pattern in LF power for the group as a whole. Mean [log] LF power values were highest during the 12:00 a.m.-6:00 a.m. period (see Table 28).

<table>
<thead>
<tr>
<th>Pairwise Comparison</th>
<th>Mean Difference</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>12MN-6AM vs 6AM-12N</td>
<td>9.751E-02</td>
<td>.359</td>
</tr>
<tr>
<td>12MN-6AM vs 12N-6PM</td>
<td>.2186</td>
<td>.046</td>
</tr>
<tr>
<td>12MN-6AM vs 6PM-12MN</td>
<td>.2825</td>
<td>.008</td>
</tr>
</tbody>
</table>

Table 28: Circadian Variation in [log] LF for Abstainers and Drinkers Combined

However, these differences were greater than those seen in [log] HF tone and the decline in [log] LF tone throughout the day was more gradual so that differences between the succeeding six-hour periods and the 12:00 a.m.-6:00 a.m. high did not reach significance until 12:00 p.m.-6:00 p.m. Drinkers had lower values for LF variability than Abstainers at all hours of the day except one (7:00 p.m.) (see Figure 10). Again, differences between groups reached statistical significance during the time period from 12:00 a.m.-6:00 a.m. (p = .03) (see Table 29).
The analysis produced three major findings that describe group differences in the circadian pattern of LF power:

1) Visual inspection of the 24 time plots indicated that Drinkers had lower values for LF variability than Abstainers at all hours of the day except one (7:00 p.m.) (see Figure 10). Differences in LF power between groups reached statistical significance during the time period from 12:00 a.m. - 6:00 a.m. (p = .03) (see Table 29).

Figure 10: 24-Hour Plot of Group Means of [log] LF Power for Abstainers and Drinkers
<table>
<thead>
<tr>
<th>Time Period</th>
<th>Groups</th>
<th>Mean (SD)</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>12MN - 6AM</td>
<td>Abstainers Drinkers</td>
<td>6.03 (.73) 5.38 (.90)</td>
<td>2.27</td>
<td>.030</td>
</tr>
<tr>
<td>6AM - 12N</td>
<td>Abstainers Drinkers</td>
<td>5.77 (.44) 5.44 (.92)</td>
<td>1.25</td>
<td>.221</td>
</tr>
<tr>
<td>12N - 6 PM</td>
<td>Abstainers Drinkers</td>
<td>5.69 (.81) 5.30 (.89)</td>
<td>1.37</td>
<td>.180</td>
</tr>
<tr>
<td>6PM - 12MN</td>
<td>Abstainers Drinkers</td>
<td>5.59 (.81) 5.24 (.92)</td>
<td>1.14</td>
<td>.265</td>
</tr>
</tbody>
</table>

Table 29: Differences in Twenty-Four Hour Spectral Analysis of [log]LF Variability Between Abstainers and Drinkers

2. Spectral analysis of the LF component revealed Abstainers experienced a gradual decline in LF power that began about 5:00 a.m. and continued until approximately 10:00 p.m. A similar decline, beginning at 7:00 a.m. and continuing until approximately 11:00 p.m. was noted in Drinkers (see Figure 10).

3. Chronic moderate alcohol consumption is associated with a two hour delay in the circadian pattern of LF tone (see Figure 10).

VLF Power. For the subset of 34 subjects as a whole, [log] VLF power was highest during the 6:00 a.m. - 12:00 p.m. period. VLF power progressively declined during the 12:00 p.m. - 6:00 p.m. and 6:00 p.m. - 12:00 a.m. periods and subsequently increased during the 12:00 a.m. - 6:00 a.m. period (see Table 30 and Figure 11).
**Table 30: Circadian Variation in [log] VLF for Abstainers and Drinkers Combined**

<table>
<thead>
<tr>
<th>Pairwise Comparison</th>
<th>Mean Difference</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>6AM-12N vs 12N-6PM</td>
<td>.1963</td>
<td>.025</td>
</tr>
<tr>
<td>6AM-12N vs 6PM-12MN</td>
<td>.4541</td>
<td>.000</td>
</tr>
<tr>
<td>6AM-12N vs 12MN-6AM</td>
<td>.2253</td>
<td>.014</td>
</tr>
</tbody>
</table>

The analysis produced three major findings that describe group differences in the circadian pattern of VLF power:

1. Visual inspection of the 24 time plots indicates that Drinkers had lower values for VLF power than Abstainers at all hours of the day except two (10:00 a.m. and 7:00 p.m.) (see Figure 11).

   Differences in VLF power between groups reached statistical significance during the time period from 12:00 a.m. - 6:00 a.m. (p = .026). Group differences approached significance for the 6:00 p.m. to 12:00 a.m. period (p = .088) (see Table 31).
Table 31: Differences in Twenty-Four Hour Spectral Analysis of [log] VLF Variability Between Abstainers and Drinkers
2. Spectral analysis of the VLF component revealed Abstainers experienced a gradual decline in VLF power that began about 8:00 a.m. and continued until approximately 10:00 p.m. A similar decline, delayed until 12:00 p.m. and continuing until approximately 11:00 p.m. was noted in Drinkers (see Figure 11).

3. Chronic moderate alcohol consumption is associated with approximately a two hour delay in the circadian pattern of HF tone (see Figure 11).

**LF/HF ratio.** For the group as a whole the LF/HF ratio was lowest during the 12:00 a.m. - 6:00 a.m. period. The only statistically significantly differences over the recording period were seen between the 12:00 a.m. - 6:00 a.m. period and the 12:00 p.m. - 6:00 p.m. period (see Table 32).
Table 32: Circadian Variation in [log] LF/HF for Abstainers and Drinkers Combined

No statistically significant differences between groups were identified for any time period (see Table 33).

Table 33: Differences in Twenty-Four Hour Spectral Analysis of [log] LF/HF Ratio between Abstainers and Drinkers
However, Abstainers tended to have lower ratios for evening and nighttime hours (9:00 p.m. to 5:00 a.m.) than Drinkers and higher ratios during the daytime hours. Drinkers had a more consistent LF/HF ratios during the 24-hour recording period and did not demonstrate the abrupt increase in LF/HF ratio that was seen in Abstainers between 6:00 a.m. and 1:00 p.m. (see Figure 12).

![Figure 12: 24-Hour Plot of Group Means for [log] LF/HF for Abstainers and Drinkers](image)
Discussion

The results of this project indicate that moderate levels of alcohol consumption are associated with a reduction in estimates of HRV and that this reduction affects all estimates of HRV except HR. Alcohol consumption in this sample independently accounted for about one-third of the sample variance in global measures of HRV when the effects of the other independent variables were held constant.

Inspection of the 24-hour plots of hourly group averages and repeated measures ANOVA revealed that subjects who consumed alcohol at rates of approximately 15 standard drinks or more a month had a tendency toward lower power in all components of the power spectrum throughout the 24-hour recording period than abstainers. These differences were statistically significant during the 12:00 a.m. - 6:00 a.m. time period and were greatest for vagal tone (i.e. HF power).

Inspection of the 24-hour plots of hourly group averages also revealed that the timing of circadian events in alcohol consumers seemed to be characterized by a phase delay. The lower absolute values of [log] HF power and [log] LF power and phase delay in circadian events noted among Drinkers blunted the abrupt morning rise in the ratio of LF/HF power seen among the Abstainers. The LF/HF ratio among Drinkers had a tendency toward higher values during the nighttime hours and lower values during the daytime hours compared to Abstainers and LF/HF ratio remained lower and more regular during the day among Drinkers compared with Abstainers. Thus, the quantitative effects of moderate alcohol consumption on the absolute measures and
rates of change in [log] HF and [log] LF power produced qualitative changes in autonomic function.

Overall, the findings of this study suggest that moderate alcohol consumption, rather than unilaterally modifying vagal tone or sympathetic activity, is associated with a resetting of autonomic balance and a decrease in the flexibility of the autonomic nervous system. The balance between vagal tone and sympathetic input is a critical factor in determining the response of the cardiovascular system to stressors (Pagani, et al., 1986). Knowledge about the flexibility of autonomic function provides more comprehensive and useful information than descriptions of isolated perturbations in either sympathetic tone or vagal tone (Malliani, Lombardi, Pagani, & Cerutti, 1994). The remainder of this discussion will support this interpretation.

Moderate Alcohol Consumption and 24-Hour Average Measures of HRV

The inverse relationship between moderate alcohol consumption rate and HRV is strongest when global estimates of HRV based on geometric measures were considered. Geometric measures are robust to the errors introduced by artifact and editing and are particularly useful when ambulatory ECG monitoring is used to measure HRV. However, the negative correlations between moderate alcohol consumption rate and all broad based measures in the time-domain and frequency-domain were also highly significant. Multiple regression analysis demonstrated that alcohol consumption explained about one third of the variance in global measures of HRV.
The finding that moderate alcohol consumption by older women is associated with lower HRV has not previously been reported. Murata, Landrigan, & Arika (1992) did not find a significant correlation between moderate alcohol consumption or smoking and either C-CV_{RSA} or C-CV_{MWSA} in either male or female subjects. The range of alcohol consumption among females subjects was restricted (1 drink/week to about 1 drink/day) compared to the subjects in this project and the earlier sample included only one female subject over the age of 60. A second study of HRV in a sample of 39 year old adults, with alcohol consumption rates comparable to those reported by the subjects in this project, found that alcohol consumption in female subjects was positively associated with HRV. However, marked age-related changes in the pharmacokinetics and pharmacodynamics of alcohol have been documented in post-menopausal women. Older women have significantly lower relative and absolute levels of vagal tone than younger women (Stein, Kleiger, & Rottman, 1997). The findings from these studies cannot be generalized to women over the age of 60.

However, findings about global measures of HRV calculated from 24-hour recordings, are available from research on clinical populations and these studies inform this discussion. A clinically significant reduction in SDNN, measured from 24 hour continuous recordings, has been reported in post-MI patients (Kleiger, et. al., 1987). Among survivors of MI, patients with SDNN values between 50 ms and 100 ms have a 1.6 time higher relative risk of all-cause mortality when compared to MI survivors whose SDNN values were greater than 100 ms. SDNN values less than 50 ms carried a 5.6 times greater relative risk of all-cause mortality.
Although the overall average SDNN reported for MI patients was lower than the average SDNN of the subjects in this project (84 ms vs 118 ms), subjects who drank 15 or more standard drinks per month had significantly lower SDNN values than Abstainers and the average SDNN for Drinkers approximated the values reported for postinfarction patients by several investigators (Kleiger, et al., 1987; Klengenhaben, Rapp, & Honloser, 1995). Approximately 50% of Drinkers had SDNN values at least as low as those associated with increased risk of mortality in post-myocardial infarction patients (≤ 98 ms). The SDNN values of subjects with the highest alcohol consumption rates approached the SDNN values associated with a high risk for mortality in post-infarction subjects (e.g., 55 ms - 67 ms).

Spectral analysis has revealed that decreases in ULF and VLF power are the strongest predictors for arrhythmic mortality in MI patients (Bigger et al., 1992). Among MI patients the ULF and VLF components of the power spectrum accounted for at least 90% of the total sample HRV for the 24 hour recording period. VLF power had the highest predictive power for arrhythmic mortality. Among the subjects of this project the correlations between VLF power and alcohol consumption rate were much stronger than the correlations between HF power and alcohol consumption rate.

Reductions in global measures of HRV have been reported in many clinical groups and can be considered as indicators of a decrease in the flexibility of the autonomic nervous system (Coumel, Maison-Blanche, & Catuli, 1994). The hypothesis that reductions in global measures of HRV are the product of a complex
interaction between both sympathetic and parasympathetic activity is supported by findings from experimental studies in animal models and clinical observations. For example, experiments in the canine model have shown that myocardial ischemia stimulates both sympathetic and vagal afferents (Mallaini, Schwartz, & Zanchetti, 1969). In humans, high level spinal anesthesia causing sympathetic blockade at T1-T4 directly decreases LF activity and indirectly decreases parasympathetic tone (Pruette, Yodlowski, Introna, Buggay, & Crumrine, 1991). The result is a loss of all spectral components of the power spectrum above .01 Hz. The authors speculate that reduced sympathetic tone rather than reduced vagal tone may be responsible for unexpected cardiac arrests during routine surgery.

Also, the release of NE at the SAN is attenuated by the release of Ach into the synapse. The inhibitory effect of vagal stimulation on automaticity and conductivity is greater when sympathetic input is high. Inversely, when vagal tone is high, sympathetic stimulation has less of an excitatory effect on automaticity and conductivity (Randell & Wurster, 1994; Urthaler, Neely, Hageman, et al, 1986; Warner & Levy, 1989). Thus, reductions in measures are the product of a complex interactions rather than isolated changes in either sympathetic or parasympathetic tone (Malliani, Lombardi, Pagani, & Cerutti, 1994).

Alcohol Consumption and Circadian Patterns Across the Power Spectrum

It was unexpected that the full correlation between alcohol consumption rate and both time-domain and frequency-domain measures of vagal tone (i.e., [log] rMSSD, [log] HF) were not as strong as the correlations between alcohol
consumption rate and other measures. This finding is surprising in view of the well documented association between higher levels of alcohol consumption and vagal neuropathy and the well documented finding that vagal neuropathy is the sentinel change in neurotoxin-related autonomic neuropathy.

There are three possible explanations for the low contribution of alcohol consumption rate to measures of vagal tone:

1. The true relationship between alcohol consumption rate and shorter-term measures of HRV is not significant.

2. The circadian variability of frequency-domain measures of HRV is well known. The averaging effects of 24-hour measures may obscure perturbations in short-term measures that are peculiar to one period of the 24-hour recording.

3. Only a small portion of the variability in HR described in this sample can be accounted for by the high frequency oscillations produced by vagal tone. Therefore, the potential treatment effect of any independent variable is less for measures of vagal tone than for measures that appraise larger portions the variability in HR. A lower treatment effect (effect size) would necessarily reduce the power of the analysis and lessen the probability of obtaining significant results.

Evaluating the circadian pattern of the spectral components of the power spectrum increased the precision and narrowed the focus of the analysis. The results of this evaluation eliminated the possibility that the true relationship between alcohol consumption and vagal tone is insignificant. An investigation of the effect of
moderate alcohol consumption on the circadian pattern of the frequency-domain measures revealed striking quantitative and qualitative between-group differences.

**Quantitative Differences.** Repeated measures analysis of variance confirmed that daytime values of the spectral components did not differ significantly between-groups, but, that estimates of [log] HF, [log] LF, and [log] VLF were all significantly lower in Drinkers compared to Abstainers during the hours of 12:00 a.m. - 6:00 a.m. Inspection of the 24-hour plots of group means indicated that the overall circadian pattern of HRV was blunted and delayed in subjects who consumed alcohol.

Altered circadian patterns of the spectral components of HRV have been reported in several clinical conditions associated with increased risk of arrhythmogenesis. Decreased HF power, increased LF power, and increased LF/HF ratios have been reported in post-MI patients (Huikuri, Linnaluoto, et al., 1992; Fallen & Kamath, 1995; Lombardi, et al., 1987). The circadian pattern of HRV is preserved in post-MI patients who have survived uncomplicated MI but, nighttime increases in HF power and the circadian variability of HF power are absent or blunted in postinfarction patients at high risk for life threatening arrhythmia (Klingenhagen, Rapp, & Honloser, 1995; Malik, Ferrell, & Camm, 1990).

Decreased HF power, decreased LF power, and increased LF/HF ratios have generally been documented in diabetic, CAD, and CHF patients (Casolo, et al., 1991, Spallone & Menzinger, 1997). Decreased LF power dissociates these chronic clinical states from the postinfarction condition. The circadian pattern of HRV has generally
been reported as preserved but blunted in CAD and CHF. These conditions are associated with a resetting of autonomic balance. The circadian pattern of HRV is generally abolished in diabetic patients and this finding is generally indicative of autonomic neuropathy (Adamopoulos, et al., 1995). In the current project attenuated LF power was noted among the Drinkers when compared to the Abstainers and the circadian pattern of HRV indices appeared blunted but preserved. These findings support the hypothesis that chronic moderate alcohol consumption is associated with a functional resetting of autonomic balance that is the result of a chronic state rather than an acute change in the condition of the heart or autonomic neuropathy.

The hypothesis that changes in HRV reported in this study are the consequence of a functional rather than an organic impairment is supported by findings from investigations of HRV in alcohol dependent subjects. While autonomic neuropathy is commonly considered to be irreversible, vagal function among alcohol dependent patients with short to moderately long drinking histories is known to improve after weeks to months of abstinence (Tan, 1984; Weise, Muller, & Krell, 1985; Yokoyama, et al., 1992). This functional impairment may involve interactions among the ANS, CNS, and/or neurohormonal mechanisms.

The relationship of alcohol consumption and VLF power in the sample is consistent with the supposition that the observed alterations in autonomic tone is the result of chronic rather than acute consumption. Moderate acute consumption has been reported to increase absolute VLF power (Gonzalez, Llorens, Novoa, & Valeriano, 1992). Since, in this project a decrease in absolute VLF power was noted,
it is reasonable to conclude that the change in VLF power was produced by chronic alcohol consumption.

**Qualitative Changes**

Visual inspection of the 24-hour plots of group difference in the LF/HF ratio and descriptive statistics showed that the mean LF/HF ratios were higher during the 6:00 p.m. - 12:00 a.m. and 12:00 - 6:00 a.m. time periods in the Drinkers compared to the Abstainers. The relationship was reversed during the 6:00 a.m. - 12:00 p.m and 12:00 p.m. - 6:00 p.m. time periods. While between-group differences in the LF/HF ratio for the entire 24 hours or any individual 6 hour time period did not reach statistical significance, if this finding is real, the pattern of the relationships is consistent with significant changes in frequency-domain measures previously described in CAD, CHF, and diabetic patients and suggests that a relative sympathetic predominance during the hours of sleep distinguishes moderate drinkers from abstainers. It has been suggested that in addition to a direct proarrhythmic effect, a relative increase in sympathetic tone during the nighttime hours may facilitate untoward cardiovascular events by increasing night time heart rate, vascular tone, blood pressure, and thrombogenesis. This change has been postulated as accounting for the increased rate of nighttime myocardial infarction documented in diabetic patients (Spallone & Menzinger, 1997).

The 24-hour average plot of the LF/HF ratio suggests that the Abstainers experienced a much more abrupt rise in the LF/HF ratio around 6:00 a.m. than the
Drinkers and that the Abstainers exhibited much greater variation (flexibility) in the LF/HF ratio throughout the day. Similar differences have been reported in normal control subjects compared to CHF and CAD patients. These differences have been interpreted as indicating that these disease states are characterized by a diminished flexibility in autonomic tone and an impaired ability to respond to changes in body posture, the demands of physical activity, and other stressors (Adamopoulos, et al., 1995; Huikuri, Niemela, et al., 1994).

Patients with CHF and CAD have a reduced response to the sleep-wake cycle and/or movement from a supine to an upright posture that may be the result of central impairment of autonomic control (Huikuri, Niemela, et al., 1994). The significance of this blunted response to arousal and assumption of the upright position is difficult to interpret. Several authors have speculated that the early morning rise in the HF/LF ratio is the trigger for the increased frequency of untoward cardiac events in the morning hours. The imputed antiarrhythmic properties of β-blockers have been attributed to central effects which decrease the abruptness of this transition. However, this rapid transition seems to be characteristic of healthy neurocardiac control mechanisms. Experiments in the canine model have demonstrated that the vagal contribution to the baroreflex is lower in dogs susceptible to ventricular fibrillation compared to resistant dogs. This is true both before and after experimentally induced myocardial infarction. Thus, the ability to reflexively increase vagal tone is diminished in susceptible dogs prior to infarction (Schwartz, Billman, & Stone, 1988). Thus, the smoothing of the night/day transition in HF/LF
power may not be a salutary event but, the result of a loss of overall flexibility of adaptation to the presence of the exogenous and endogenous stimuli common in the early morning hours. The implication of this interpretation is that moderate alcohol consumption has a negative effect on the quality of autonomic nervous system function.

Confounding Variables

As argued in the literature review, several other variables may affect neurocardiac function. Five of these were considered in this project. One of them, smoking, was significantly correlated with several measures of HRV. The strength of these correlations was comparable to those between alcohol consumption rate and measures of HRV. Since there were only 4 smokers in the study, this indicates that the negative relationship between smoking and HRV in older women is a strong one. However, because only four subjects were involved, caution must be maintained to avoid overinterpreting these findings.

The literature review indicated that age was the most likely independent variable in the analysis to have a significant negative relationship with HRV. However, this was not the case. It is possible that age associated declines in HRV are complete by age 60. On the other hand, several authors have reported that exercise can reverse age related declines in HRV. Subjects in this sample reported relatively uniform levels of exercise and were about evenly divided between the upper two daily exercise levels (mild exercise, eg., walking, bowling, golf, isometrics; strenuous exercise, eg., jogging, running tennis, swimming, exercise bike,
jazzercise). If exercise/activity levels account for the uniform levels of HRV across age groups, decreased HRV is not a universal, intrinsic, deleterious, and progressive outcome of age and therefore, and cannot be classified as a true age-related change.
CHAPTER 5
SUMMARY, LIMITATIONS, SIGNIFICANCE, RECOMMENDATIONS

Summary

The purpose of this descriptive cross-sectional study was to describe the relationship between chronic moderate alcohol consumption and various estimates of autonomic tone (HRV) in healthy community-dwelling women over the age of 60.

The sample was composed of all qualified women enrolled in a more comprehensive interdisciplinary study of 135 older women. This study extended the objectives of the parent study by introducing HRV as a dependent variable. Twenty-four hour Holter ECG recordings from every qualifying subject were re-analyzed and standard geometric, time-domain, and frequency-domain measures of HRV were calculated for the sample.

This study is the preliminary investigation of the relationship between moderate alcohol consumption and estimates of HRV in older women. The project was motivated by the lack of understanding about this relationship and the current high level of interest in the possible cardioprotective effects of alcohol. The study asked three research questions:

1. What is the direction and strength of the relationship between moderate alcohol consumption rate and heart rate variability in older women?
2. What is the unique contribution of moderate alcohol consumption to heart rate variability in older women?

3. What is the relationship between moderate alcohol consumption rate and the circadian rhythm of heart rate variability in older women?

Summary statistics (means and standard deviations, or frequencies and percents) were calculated for demographic, clinical, and behavioral characteristics of the sample. Differences in these characteristics between the alcohol abstainers and subjects who consumed 15 or more standard drinks per month were established by independent t-tests.

The strength and direction of the relationship between alcohol consumption rate and HRV was determined by bivariate correlations and univariate regression. Standard multiple regression analysis and the squared semipartial correlation coefficient ($sr^2$) derived from standard multiple regression were used to determine the unique contribution of moderate alcohol consumption and each of the other independent variables to estimates of HRV. Alcohol consumption rate, activity/exercise level, caffeine use, cardiovascular medications, and smoking were entered into the models as independent variables. Individual measures of HRV were entered into separate regression models as the dependent variable. Fast Fourier Transformation was used to calculate frequency-domain measures across the power spectrum. Repeated measures analysis of variance was used to determine the relationship between alcohol consumption rate and the circadian patterns of HF, LF, and VLF power. Right skewed variables were log transformed.
In addition, separate correlation matrices for independent variables and dependent variables were calculated and presented. Since the independent variable, alcohol consumption rate depended on self report, concurrent validity of alcohol consumption rate was established by correlational analysis and independent t-tests. Scores on a standardized screening instrument (T-ACE) and two biological markers (GGT and MCV) were used to corroborate self-report.

The results of this project indicate that moderate levels of alcohol consumption are associated with a dose dependent reduction in estimates of HRV and that this reduction affects all estimates of HRV except HR. Alcohol consumption in this sample accounts for a about one-third of the sample variance in global measures of HRV when the effects of the other independent variables are held constant.

Spectral analysis revealed that alcohol consumption among subjects who consumed 15 or more standard drinks per month alcohol consumption rate was associated with a reduction in the absolute values of HF, LF, and VLF power. Subjects who consumed alcohol at rates of 15 standard drinks a month or more had a tendency toward lower power in all components of the power spectrum throughout the day. These decrements were statistically significant during the 12:00 a.m. - 6:00 a.m. time period and greatest for vagal tone. Visual inspection of the 24-hour plots of hourly group averages revealed that the timing of circadian events in alcohol consumers was characterized by a phase delay. Among the Drinkers the lower absolute values of [log] HF power and [log] LF power combined with the phase delay and resulted in a smoothing of the abrupt morning increase in the LF/HF ratio that
characterized the abstainers. That is, the quantitatively different effects of alcohol consumption level on the absolute measures and rates of change in [log] HF and [log] LF power produced qualitative differences in autonomic balance in the drinking group. The LF/HF ratio had a tendency toward higher values during the nighttime hours and lower values during the daytime hours among Drinkers compared to Abstainers. This finding, if real, suggests a relative increase in sympathetic activity during the nighttime hours in Drinkers. The LF/HF ratio remained lower and more regular during the day among Drinkers compared to Abstainers and suggesting that overall flexibility of autonomic nervous system response to exogenous and endogenous stressors was blunted among Drinkers compared to Abstainers.

Overall, the findings of this study strongly suggest that moderate alcohol consumption, rather than unilaterally affecting either vagal tone or sympathetic activity, is associated with resetting of autonomic balance and a decrease in the flexibility of the autonomic nervous system. The balance between vagal tone and sympathetic input is a critical factor in mediating cardiovascular system response to stressors (Pagani, et al., 1986).

Limitations

This study enrolled a sample of healthy community-dwelling older women who were characterized by above average educational and socioeconomic status. Generalization to populations dissimilar to this sample may not be appropriate.

In order to exclude "sick quitters" and abstaining women with a previous history of alcohol abuse, women with common significant and/or possible alcohol-
related health problems (eg. atrial fibrillation, stroke, history of liver or pancreatic
disease) were excluded from the sample. Further, valid calculation of heart rate
variability estimates required that women be excluded from the sample if they had
conditions that may be associated with moderate alcohol consumption and potentiated
by perturbations in autonomic tone (e.g., atrial fibrillation). These exclusions could
have produced findings that underestimate the true extent of alcohol-related effects on
autonomic tone (i.e. HRV) in community dwelling women.

Heart rate variability estimates the tonic effect of the autonomic nervous
system on the SAN. Estimation of phasic autonomic effects require measures of
baroreceptor sensitivity (BRS). Phasic autonomic influences were not assessed in this
study but may have significantly contributed to the findings. Impaired baroreceptor
function may mediate the altered response to arousal and upright posture suggested by
the decreased daytime LF/HF ratio noted among subjects who consumed 15 or more
standard drinks per month.

Pharmacological studies have documented that time-domain measures based on
successive interbeat intervals (eg. rMSSD) and the HF component power spectrum are
markers for vagal tone at the SAN. Parasympathetic influences at the SAN are
assumed to be surrogate measures of vagal effects on the ventricle but, may not
definitively represent vagal influences on the ventricle.

A pure measure of sympathetic tone has not been validated and statements
about sympathetic efferent activity should only be made with great caution.

Study subjects were not requested to abstain from alcohol during the recording
period. It can be assumed that women continued to use alcohol at accustomed rates.
If this assumption is true, HRV measures were affected not only by chronic alcohol consumption, but by alcohol's acute effects. Both acute and chronic alcohol consumption decrease HF power and cause an increase in the LF/HF ratio. These findings are similar to the findings in this study. Acute alcohol dosing, as opposed to chronic alcohol abuse, is associated with an increase in absolute VLF power (Gonzalez, Llorens, Novoa, & Valeriano, 1992). This findings is in the opposite direction of the change reported in the current study. While this finding can be used to argue that the alterations in HRV in the sample are more likely to result from chronic rather than acute alcohol consumption, further research is needed to adequately dissociate the effects of chronic and acute alcohol use in older women.

Significance

Epidemiological studies have suggested that moderate alcohol use, compared to both abstinence and higher levels of consumption, is associated with lower cardiovascular mortality (Anderson, Cremona, Paxton, Turner, & Wallace, 1993; Klatsky, Armstrong, & Friedman, 1990; Klatsky, Armstrong, & Freidman, 1992; Stampfer, Graham, Willett, Speizer, Hennekens, 1988, Thun et al., 1997). Lower cardiovascular mortality rates among lighter drinkers when compared to abstainers and heavy drinkers may be partially attributable to an alcohol-related increase in high density lipoproteins (HDLs) and a subsequent reduced incidence of coronary artery disease (CAD). Other probable contributors to the cardioprotective effect of moderate alcohol consumption are: 1) alterations in cellular signaling in the vascular endothelium which inhibit the inflammatory response associated with fatty streaks in
blood vessel and, thereby, prevent atherogenesis, 2) reductions in platelet function that interfere with thrombus formation, and 3) increased fibrinolytic activity (Klatsky, Armstrong, & Friedman, 1992; Zakhari, 1997).

CAD is the most common cause of death in the United States. Approximately eighty percent of the 500,000 annual CAD deaths occur in individuals aged 65 and older (McIntosh, 1994) and CAD has a significant impact on functional status among the elderly. The majority of older adults affected by CAD are women (Vaccarino, de Leon, & Berkman, 1997). Given the high prevalence rates of CAD among the elderly, and especially women, any suggestion of an alcohol-related reduction in CAD raises important questions regarding the consumption threshold for alcohol-related cardiovascular health/harm for older adults.

The legitimacy of statements imputing a cardioprotective effect to moderate alcohol consumption has been questioned on the basis that early studies included "sick quitters" and other individuals who avoided alcohol because of illness in the abstainer groups. This error artificially raised all-cause mortality rates among non-drinkers, masked alcohol-related mortality, and increased the apparent cardioprotective effect of moderate alcohol consumption. Other studies have failed to control for the effects of smoking, diabetes, obesity, and hypertension (Arria & Van Theil, 1992). Because past research has mostly employed samples of middle-aged men, the generalizability of most findings to older adults and women is limited by sample composition. These design flaws have greatly undermined the credibility of any claim of a cardioprotective effect for alcohol among older adults and women.
A recent well designed prospective study of alcohol consumption and mortality in middle-aged and older adults raises new questions about the cardioprotective effects of alcohol consumption among older women. This study described a "slight" reduction in cardiovascular and all-cause mortality among the 490,000 study subjects (Thun et al., 1997). Unexpectedly, the greatest benefit was pinpointed among older adults with preexisting cardiovascular disease. An alcohol related cardiovascular benefit was identified for all subject subgroups delineated by the study, except healthy women over the age of 60 who were free of cardiovascular disease at the inception of the study. The lack of significant findings in this subset of 143,000 provides a strong argument that the threshold for an alcohol-related cardiovascular benefit, if there is indeed a cardiovascular benefit, is meaningfully higher in healthy older women than in other populations. Also, other researchers have noted that middle-aged and older women who drink more than three drinks per day incurred an increased risk of CAD morbidity and mortality compared to women drinking at lower levels (relative risk = 2.6, CI 1.2-5.5) (Rehm, Bondy, Sempos, & Vuong, 1997). Thus, the threshold for alcohol's imputed cardioprotective effect seems to be higher for females compared to males while the threshold for increased risk of CAD may be lower. The fact that women incur organ level alcohol-related harm after briefer and lower dose exposure to alcohol than males is well known. Since little knowledge exists concerning the effects of moderate alcohol consumption on many cardiovascular phenomena, it is possible that the therapeutic index for alcohol consumption among women may be much narrower than is currently appreciated or even that healthy women may
experience alcohol-related cardiovascular harm at consumption levels far below those that are associated with any cardioprotective effect.

Finally, little knowledge exists concerning the cardiovascular effects of long-term moderate levels of alcohol consumption on older adults, or women of all ages, despite the documented exceptional vulnerability of these groups for alcohol-related harm (Pfefferbaum, Lim, Zipursky, Mathalon, Rosenbloom, Lane, Ha, & Sullivan, 1992; U.S. Department of Health and Human Services, 1993). All these concerns motivated this dissertation project.

Possible Mechanisms

In view of the finding that moderate alcohol consumption is associated with a dose dependent decrease in HRV, the key question becomes "What is the mechanism of this effect?" Any hypotheses concerning the possible mechanisms by which moderate alcohol consumption may attenuate HRV must consider the following facts:

1. HRV is an output signal produced by the integration of intrinsic cardiac factors, medullary cardiovascular reflexes, and supramedullary factors on heart rate (McDonald, 1980) and reflects the integrity of both the central and peripheral inputs to the sinoatrial node (SAN) as well as the health of the SAN.

2. Ethanol is an alcohol comprised of a two carbon backbone and a hydroxyl group. Because of its low molecular weight and its amphophilic characteristics, alcohol can easily diffuse through any biological membrane, is miscible with both water and lipids, and equilibrates rapidly through the body.
The physical chemistry of alcohol explains its ability to effect all organ systems and prompts speculation that alcohol affects HRV at both central and peripheral cites. Chapter 2 reviewed the diverse ways that alcohol can influence HRV. The findings of this study indicate that HRV (and autonomic tone) in older women is adversely affected by moderate alcohol consumption. Findings from the current project do not afford insight into the exact mechanisms that produce this outcome. And it is likely that the actions of alcohol at all levels of the nervous system and all levels of function from membrane ionic channels to cortical function summate to produce the perturbations in HRV reported in this study. However, the attenuation of the abrupt early morning increase in the LF/HF ratio and the apparent decreased variability in this ratio during the daytime hours noted in subjects who consume alcohol compared to abstainers, suggest that one mechanism by which moderate alcohol consumption may influence HRV is by attenuation of baroreceptor function. This attenuation would interfere with the individual’s ability to respond to arousal, upright posture, and position change throughout the day. Full specification of the mechanisms of alcohol’s affect on autonomic tone will require further research.

The goal of this dissertation project was to contribute to the precise definition of the threshold level of alcohol related health/harm during later adult years by:

1. initiating specification of the relationship among aging, moderate alcohol use, perturbed autonomic tone, and cardiac rhythm disturbances.

2. contributing to clarification of the mechanism underlying alcohol-related arrhythmogenesis and SCD.
The findings of this project suggest that the relationship between chronic moderate alcohol consumption and autonomic tone is characterized by a decrease in the overall variability and flexibility of autonomic nervous system function, a marked decrease in nighttime vagal tone, a blunting of the circadian pattern of autonomic function, and a loss of the abrupt rise in the LF/HF ratio that is identified with the transition from nighttime to daytime. The first three phenomena are recognized indicators of increased risk for arrhythmic and all-cause mortality in diverse populations. The meaning of the fourth event is unclear. The abrupt increase in LF/HF ratio that accompanies morning arousal and change from the supine to the upright position is widely believed to be a trigger for the increased incidence of grave cardiovascular events at that time of day. The inverse association between β-blocker use and SCD is attributed to the ability of adrenergic antagonists to modify the steep increase in LF/HF ratio. However, it is arguable that the abrupt morning transition in LF/HF ratio is a characteristic of normal autonomic function and the loss of this characteristic is ominous. Steep increases in the LF/HF ratio have been reported in healthy normal controls but, are absent in many disease states. Dynamic equilibrium is a essential characteristic of healthy biological systems and the overall lessening of this dimension of autonomic function is likely to be of paramount importance in arrhythmogenesis. The loss of the abrupt increase in the LF/HF ratio that accompanied moderate alcohol consumption in the sample may be the result of a loss of flexibility in autonomic function and a decreased capability to respond to stress. Therefore, it is possible that the blunting of the morning increase in LF/HF is an
indicator of increased risk for cardiovascular and/or all cause morbidity and mortality. A third possibility is that smoothing of the morning increase in LF/HF tone is beneficial under certain conditions (i.e. in the presence of an arrhythmogenic substrate).

Taken as a whole, the findings of this project support the hypothesis that moderate alcohol consumption in older women is associated with perturbed autonomic nervous system function that increases the risk of cardiovascular and all-cause morbidity and mortality in the population. This hypothesis, if substantiated, will significantly contribute to the clarification of the mechanism underlying alcohol-related arrhythmogenesis and SCD.

Implications for Research

Theory Development

The findings of this dissertation project suggest several projects aimed at testing the hypothesis that moderate alcohol consumption in older women is associated with perturbations in autonomic function that increase the risk of cardiovascular and all-cause morbidity and mortality in the population:

1) A comparison of the circadian incidence of cardiovascular events in women who consume moderate amounts of alcohol and women who abstain from alcohol will help specify the impact of attenuation of the increase LF/HF that accompanies the transition from night to day. Reduced incidence of cardiovascular events between 7:00 a.m. and 11:00 a.m. accompanied by an absolute reduction in the incidence of cardiovascular events over 24 hours would indicate that the changes in LF/HF ratio
are beneficial and may partially account for the perceived cardiovascular benefit of moderate alcohol consumption. Reduced incidence of cardiovascular events in the morning hours without an absolute reduction in cardiovascular events over 24 hours would indicate that the overall loss of flexibility in the system counters any beneficial effect of smoothing the abrupt morning transition in the LF/HF ratio. Increased incidence of cardiovascular events in the morning would indicate that smoothing the abrupt morning alteration in the LF/HF ratio potentiates cardiovascular morbidity and mortality. The findings of such studies would lay the foundation for intervention studies aimed at developing guidelines that maximize the benefits of alcohol consumption while minimizing risk. Logical dependent variables in these comparison studies would be: incidence of MI, SCD, stroke, episodes of angina, ST changes, and ventricular and supraventricular arrhythmias.

2. The overall finding of this project indicates that moderate alcohol consumption is associated with a loss of flexibility in autonomic function. Biological aging is defined by a loss of the range and complexity of the physiological function that supports adaptation to stress (i.e. a loss of flexibility) (Kaplan, Furman, Pincus, Ryan, Lipsitz, & Goldberger, 1991; Strehler, 1959). This dimension of the autonomic nervous system may be best captured by the non-linear methods of chaos theory. A secondary analysis of this data using a non-linear technique may provide additional insights into the relationship of alcohol consumption to HRV in older women and the relationship between alcohol consumption and aging. Poncaire plots and approximate entropy are possible dependent variables for this analysis.
3. Cosinor analysis of the curves presented in the 24-hour plots of group averages that were constructed for the circadian analysis would quantify the phase differences noted between groups and add to our understanding of differences between women who report moderate alcohol intake and women who abstain from alcohol.

4. Replication of this project in males, across age groups, and in more diverse populations of women will yield information related to gender and age effects as well as information related to the generalizability of the findings of this project.

5. The effects of acute alcohol dosing in older women who consume moderate amounts of alcohol is unknown. Research investigating the effects of acute moderate alcohol consumption on frequency-domain measures of HRV in older women will contribute to the development of rational alcohol consumption guidelines for older adults by parsing out HF and LF responses to doses that do not recruit the cytochrome p450 system and by specifying the duration of these effects.

6. Although only four subjects in the project were cigarette smokers, smoking had a significant relationship with HRV. Further research is necessary to define the nature of the relationship among alcohol, smoking, and HRV in older women.

Research Design

The findings of this dissertation project have the following implications for research design:

1. Moderate alcohol consumption makes a significant unique contribution to autonomic function in older women. Therefore, alcohol consumption should be considered an essential covariate in HRV studies focusing on older women.
2. Broad based global measures of HRV are especially useful in HRV studies where the treatment effect is likely to be small or the amount of artifact is likely to be large (i.e. 24-hour Holter recordings of ambulant subjects). These measures are also appropriate for preliminary studies. The bivariate correlations and $sr^2$ values describing the relationship between alcohol consumption and geometric measures HRV were the stronger and more significant than correlations based on any other measure of HRV investigated. These measures are the most robust to artifact and editing errors and therefore are most likely to yield significant results.

3. Circadian analysis should be included in future investigations of the relationship between alcohol and HRV. A circadian analysis of changes in the components of the power spectrum revealed the relationship between moderate alcohol consumption and vagal tone. This approach disclosed a relative increase in the LF/HF ratio during the nighttime hours in alcohol consumers and an overall decrease in flexibility in autonomic function in this group. These findings were supported by the inverse relationship between alcohol consumption rate and broad based time-domain measures of HRV that are believed to reflect the flexibility of the autonomic nervous system.

Implications for Practice

1. The findings of this study suggest that the flexibility of autonomic nervous system function is significantly compromised among older women who self-report moderate alcohol consumption. Similar losses in flexibility have been documented in diverse populations at risk for arrhythmogenesis. As a result of these findings it is
impossible to recommend that healthy women over the age of 60 be encouraged to introduce alcohol into their diets to enhance cardiovascular health. Moreover, the effects of alcohol in the sample were far from physiologically neutral. Healthy community dwelling women over the age of 60 should be advised to restrict alcohol consumption below the current UADA/USDHHS guideline of one drink per day.

2. Recent research has documented a seemingly paradoxical decrease in cardiovascular and all-cause mortality in women with pre-existing heart disease (Thurn, et al., 1997). One possible explanation for this finding lies in the diversity of imputed cardioprotective effects of moderate alcohol consumption. In addition to decreasing cholesterol levels, alcohol may protect the cardiovascular system by: 1) producing alterations in cellular signaling in the vascular endothelium that inhibit the inflammatory response associated with fatty streaks in blood vessel and, thus, prevent atherogenesis, 2) interfering with thrombus formation and/or 3) increasing fibrinolytic activity (Klatsky, Armstrong, & Friedman, 1992; Zakhari, 1997). Although individuals with cardiovascular disease are known to be at greater risk for embolic events than healthy individuals, the cardioprotective effect of alcohol appears to be greater in women with pre-existing cardiovascular disease (Thurn et al., 1997). This suggests that the benefit of alcohol-related decreases in thrombogenesis and increases in fibrinolysis could out weigh the arrhythmogenic effects of moderate alcohol consumption and produce a net decrease in cardiovascular death in certain groups. This supposition does not support a recommendation that individuals with pre-existing cardiac disease adopt alcohol use for its cardioprotective effects. Clinical studies have
demonstrated that other modalities, such as aspirin therapy and lifestyle changes, produce similar results without incurring any increase in arrhythmogenic risk.

3. Cigarette smoking was negatively correlated with HRV and positively correlated with HR. While further studies are needed to determine the nature of the relationship between cigarette smoking, alcohol consumption, and HRV it can be postulated that this relationship is at least additive. Smoking by alcohol consumers should raise an index of concern among practitioners. Smoking cessation should be a primary emphasis in health promotion/disease prevention for alcohol consumers.

Implications for Policy

If the inverse relationship between moderate alcohol consumption and measures of HRV reported in this study is replicated in future studies, the current USDA/USDHHDS guideline of one drink a day for women should be reconsidered and separate guidelines be developed for women over the age of 60.
T-ACE

T  How many drinks does it take to make you feel high (TOLERANCE)
A  Have people ANNOYED you by criticizing your drinking?
C  Have you ever felt you ought to CUT DOWN on your drinking?
E  Have you ever had a drink first thing in the morning to steady your nerves or get rid of a hangover (EYE-OPENER)?

APPENDIX B

CHARACTERISTICS OF AMBULATORY ECG DATA PRESENTED BY CASE
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<th>% Splined Beats/ 24 Hours</th>
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APPENDIX C

CORRELATION MATRIX OF DEPENDENT VARIABLES
## Correlation Matrix of Dependent Variables

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<th>SDANN</th>
<th>ASDNN</th>
<th>IrMSSD</th>
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<th>ILF</th>
<th>IVLF</th>
<th>HR</th>
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