EFFECTS OF ANTIOXIDANT ON CARDIOVASCULAR PERFORMANCE,
EXERCISE CAPACITY, AND FUNCTIONAL STATUS
IN PATIENTS WITH CHRONIC HEART FAILURE

by

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Submitted in partial fulfillment of the requirements
For the degree of Doctor of Philosophy

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January, 2007
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Dedication

To my husband, Chuan-Chieh,

And my daughters, Chieh-Ming and Chieh-Yuan,

For their endless love and “just been there for me”
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Acknowledgements

The work described herein would not have been possible without the help of a large number of people who deserve my sincerest thanks. My sincere acknowledgement is for all my dissertation committee members: Dr. John M. Clochesy, Dr. Elizabeth A. Madigan, Dr. Chris Winkelman, Dr. Ravi Nair, and Dr. Barry Effron. I am glad to have had such distinguished professors who share their expertise with me and improved my thinking along the way.

Special thanks are given to my mentor, my academic advisor, the chairperson of my dissertation committee, and my role model, Dr. Clochesy, for his complete support, expert knowledge, patience, vision and commitment throughout the entire PhD and this research process. As I felt lost or running around in circles, he helped me visualized the path ahead. He is the person who let me find the rainbow behind every cloud.

Sincere thanks go to my friends, Kuie-Ru Chou, Gwan-Ling Lin, May-Hsien Lin, Cheng-hui, Chou, and Ru-Shen Jiang, for their emotional support, understanding, and their encouragement that helped me through this arduous process.

I would like to thank my dear parents and parents-in-law, not only for the babysitting, but also for teaching me what is really important in life and for making it all possible. A special thank to my husband, Chuan-Chieh, for always making me laugh and
always being there for me. I’m not sure exactly how I would have done this without him.

My pretty daughters, Chieh-Ming and Chieh-Yuan, they energized me along the way. To my family, thanks for everything.

Last, but certainly not the least, I would like thank all of the subjects for their participation and cooperation in my study.
Effects of Antioxidant on Cardiovascular Performance, Exercise Capacity, and Functional Status in Patients with Chronic Heart Failure

Abstract

by

CHAO-CHUNG HO

Purpose To evaluate the effects of antioxidant on cardiovascular performance, exercise capacity and functional status in chronic heart failure patients.

Methods This was a cross-over, randomized controlled trial. A total of 37 subjects were allocated to two groups randomly. Group I \((n = 19)\) received vitamin C 4000 mg daily for four weeks and followed by placebo for six weeks. Group II \((n = 18)\) received placebo for six weeks and followed by vitamin C 4000 mg daily for four weeks. The tumor necrosis factor-alpha (TNF-\(\alpha\)) was examined as an indicator of inflammatory process. The endothelial function was measured by flow-mediated vasodilation. The cardiac performance was measure by left ventricular end-diastolic diameter (LVEDD) and left ventricular ejection fraction (LVEF). Exercise capacity was evaluated by the total distance of six-minute walk test (6MWT). The functional status was assessed by three questionnaires, the Medical Outcomes Study Short Form 36-item Survey (SF-36), the
Minnesota Living with Heart Failure Questionnaire (MLHFQ) and the Center Epidemiologic study for Depression questionnaire (CES-D).

**Results**

There was a statistically significant difference in brachial diameter change percentage, baseline flow, and peak flow while using antioxidant supplementation compared to when they did not use antioxidant supplementation ($F = 10.97, 16.17, 8.57, p < .001, < .001, = .001$, respectively). There was no significant difference in LVEDD ($F = .31, p = .692$).

However, there was a significant difference in LVEF while using antioxidant supplementation ($F = 35.60, p < .001$). There was a statistically significant increase in 6MWT while using antioxidant supplementation ($F = 40.84, p < .001$). There was a statistically significant difference in the SF-36, the MLHFQ, and the CES-D while using antioxidant supplementation compared to when they did not use antioxidant supplementation ($F = 5.91, 19.53, 24.47, p = .009, < .001, < .001$, respectively).

**Conclusions**

This study provided an evidence-base of identifying the effects of vitamin C on mitigating the pro-inflammatory process, improving endothelial function, improving the cardiac contractility, augmenting exercise capacity, promoting the quality of life, and alleviating the depressive symptoms in patients with chronic heart failure.
CHAPTER 1
INTRODUCTION

Background

Almost 5 million cases of heart failure (HF) have been reported in the United States, with almost 550,000 new cases being reported each year. In a study conducted in Minnesota, the prevalence of any systolic dysfunction was 6.0%, and moderate or severe systolic dysfunction was reported in 2.0% of the total population of the U.S. Heart failure is the most common reason for hospital admission and one of leading causes of morbidity and mortality (AHA, 2005). Based on a 44-year follow up study from the Framingham Heart Study (National Heart, Lung, and Blood Institute), from 1992 to 2002, deaths from heart failure increased 35.5%, but in the same period, the overall death rate increased only 7.7% (Lloyd-Jones, Larson, Leip, Beiser, D'Agostino, Kannel, et al., 2002).

According to the health and vital statistics survey of the Department of Health, Executive Yuan, Taiwan, R. O. C. (2005), heart disease is the third leading cause of death in Taiwan. Almost 50.93 deaths per 100,000 population and 9.6% of total death were reported in 2003. Heart disease accounted for 3.98% more deaths or made a 0.35% contribution to the annual increase in the numbers of deaths from 2002 to 2003. It has been estimated that heart disease comprised nearly 7% of the total direct medical costs incurred 2003 in Taiwan. However, the related indirect expenses, such as costs due to complications or long-term home care, are not included.
Heart failure always occurs within the setting of cardiac disease. Most cases are a result of either coronary artery disease or long-term under-perfusion of myocardium. Although the incidence of acute myocardial infarction and acute coronary syndrome has decreased, and better pharmacological agents are available, the incidence and prevalence of HF is increasing. Mortality rates remain high in patients with New York Heart Association (NYHA) functional Class III-IV symptoms. The high prevalence of HF, combined with multiple complications from this condition, increase health care costs significantly. Almost one-third of these patients have NYHA functional Class III or IV heart failure, often characterized by progressive deterioration and frequent hospital admissions. Re-hospitalization rates during the 6 months after discharge are as much as 50%. It has been estimated that chronic HF cost nearly $27.9 billion in direct and indirect medical care in 2005 (AHA 2005; Aronow, 2003; Croft, Giles, Pollard, Keenan, Casper, & Anda, 1999; Nieminen, & Harjola, 2005; Zevita, 2005).

Statement of Problem

No definition of heart failure is entirely satisfactory. Heart failure may readily be described as a clinical syndrome of diverse etiologies with well-known symptoms and signs (breathlessness, fatigue, ankle swelling). It is far more difficult to provide a precise satisfactory definition. It may be better to consider the known abnormalities related to heart failure to appreciate the complexities of heart failure. Congestive heart failure develops when plasma volume increases and fluid accumulates in the lungs, abdominal
organs (especially the liver), and peripheral tissues (Beers, 2004). Packer et al. described the clinical syndrome of heart failure as being “characterized by abnormalities of left ventricular function and neurohormonal regulation, which are accompanied by effort intolerance, fluid retention and decreased longevity” (Packer, McMurray, Massie, Caspi, Charlon, Cohen-Solal, 2005). Heart failure occurs when cardiac muscle dysfunction prevents the heart from pumping adequate blood at normal cardiac pressures to meet the metabolic demands of the body, particularly during times of increased exertion (Adams, Fonarow, Emerman, LeJemtel, Costanzo, & Abraham, 2005). It is a multisystem disorder which is characterized by signs and symptoms related to abnormal contractility, fluid accumulation, activation of neurohormonal systems, limitation of peripheral circulation and end organ failure (Mann, 2005).

Chronic Heart Failure is a syndrome characterized by left ventricular dysfunction, reduced exercise tolerance, impaired quality of life, and markedly shortened life expectancy (Cohn, 1988). The ACC/AHA 2005 Guideline Update defined chronic HF as a complex clinical syndrome that can result from any structural or functional cardiac disorder that impairs the ability of the ventricle to fill with or eject blood. The cardinal manifestations of HF are dyspnea and fatigue, which limit exercise tolerance and affect fluid retention, functional capacity and quality of life (Hunt, Abraham, Chin, Feldman, Francis, Ganiats, et al., 2005; Radford, Arnold, Bennett, Cinquegrani, Cleland, Havranek, et al., 2005).

Chronic heart failure represents a major public health burden, and its prognosis is
comparable to the prognoses of other malignant diseases. Our understanding of HF has developed from the rather simplistic model of HF being mere pump failure to HF being a multisystemic disorder which affects not only the cardiovascular system but also the musculoskeletal, renal, neurohormonal, and immune systems. Therefore, the pathophysiologic process is exceedingly complex. However, the mechanisms that explain the progressive dysfunction of failing myocardium remain largely unknown.

Heart failure progresses because of the activation of neurohormones and pro-inflammatory cytokines following an initial cardiac injury or a programmed genetic mutation (Parissis, Adamopoulos, Karas, & Kremastinos, 2002). Although the potential overlap between the neurohormonal and cytokine hypotheses is rather obvious, for both cytokines and neurohormones represent classes of biologically active molecules, there are important differences in terms of both the mechanisms that initiate the over-expression of these molecules and the biologic effects that these molecules exert (Seta, Shan Bozkurt, Oral, & Mann, 1996).

The inflammatory cytokines, including Tumor Necrosis Factor, Interleukin (IL)-1β, and IL-6 are elevated in HF. They may contribute to the progression of HF by regulating growth and gene expression in cardiac myocytes. They can induce myocardial dysfunction. It is becoming increasingly apparent that inflammatory mediators play a crucial role in the development of HF, and several strategies to counterbalance different aspects of the inflammatory response have been considered (Anderson, 2000; Parissis, et al., 2002). An understanding of HF as an inflammatory process might lead to a better
overall understanding of this disease process. Several previous studies revealed that inflammatory cytokines affect the pathophysiologic process of chronic heart failure, but the number of studies which have focused on the relationships among cytokines, endothelial function, cardiac performance, exercise capacity and functional status is limited.

Functional status and health-related quality of life have emerged as important end points in treating and managing patients with HF. Despite the new and effective therapies being developed for these chronically ill patients, their health-related quality of life remains poor (Bennett, Oldridge, Eckert, Embree, Browning, & Hou, 2002; Bennett, Oldridge, Eckert, Embree, Browning, & Hou, 2003). Consequently, improving health-related quality of life has become a focus of many clinical trials and is a major goal in the treatment of patients with chronic heart failure. Therefore, the effects of antioxidant supplementation on systemic inflammatory process, endothelial function, cardiac performance, exercise capacity, and functional status will be identified in patients with HF and will be examined in this study.

Significance of This Study

There are several reasons for the current concern, as follows:

Epidemiology of Chronic Heart Failure Patients

Chronic heart failure is a worldwide issue, found not only in United States, but also in Taiwan. Approximately 3 million Americans have been diagnosed with congestive
heart failure, and approximately 400,000 cases were diagnosed in 1992 (Sullivan, 1994). By 2005, this population has grown to 5 million, and incidence of new cases has grown to 550,000 annually. Heart disease accounted for 3.98% more deaths or a 0.35% contribution to the annual increase in death from 2002 to 2003 in Taiwan. The increasing incidence and prevalence of patients with HF are remarkable. Over the past two decades, we have made significant advances in understanding the pathophysiologic process of HF and have developed effective pharmacologic therapies that can improve symptoms and exercise tolerance. However, mortality rates remain high in patients with NYHA functional Class III to IV heart failure, and quality of life also remains poor in this population. Moreover, the estimated $27.9 billion that will be spent in 2005 to manage heart failure is likely to increase markedly in the near future.

The findings of this research may provide baseline information about the inflammatory process in HF patients in Taiwan. Moreover, antioxidant supplementation may also offer a possible way to mitigate the effects of the systemic inflammatory process and improve cardiovascular performance, exercise capacity and functional status in patients with heart failure.

*Establish the Nursing Knowledge Base on Inflammatory Process in Patients with Chronic Heart Failure*

The lack of clinical trials and physiologic measures is the major problem facing the nursing domain. In 1993, the National Institute of Nursing Research (NINR) voiced
concern about the lack of physiologic variables in nursing research proposals received at the NINR. Eighty-five percent of the proposals received involved non-physiologic variables; only 114 of the 763 studies reviewed contained physiologic variables (Pugh & DeKeyser, 1995). That means, in general, there are insufficient numbers of studies by nurse researchers using biological science as a basis for nursing research. Nursing is both a discipline and a profession. It uses nursing science, related sciences, and nursing practice to promote, restore, and maintain the health of human beings. As a discipline, nurses need to develop intervention studies for clinical application (Holden, 1996).

Although many studies have been proposed to investigate health-related quality of life (QOL) in HF patients, studies which have identified the effects of antioxidant supplementation on health-related QOL in this population are limited.

The current study not only includes a nursing intervention and physiologic variables, but it also considers the connections among the biological, psychological, and social domains. Such knowledge is also beneficial to health professionals for integrating concepts of the pro-inflammatory process in HF. Understanding and recognizing the relationships among some concepts of the inflammatory process will enable nurses to identify patients who are at risk for these problems.

Nurses are the largest and most essential part of the current health care system in Taiwan. The goal of nursing is to promote the health of clients (Chinn & Kramer, 2004). Fitzpatrick (2000) defined nursing care research as “research directed to understanding the nursing care of individuals and groups and biological, physiological, social,
behavioral, and environmental mechanisms influencing health and disease that are relevant to nursing care”. An empirical approach is one of the four ways to knowledge development (Chinn & Kramer, 2004). The process of this approach involves explaining and structuring empirical phenomena, thereby creating conceptual meaning and structuring and contextualizing theory. In addition, findings from this study will be significant not only for the development of nursing interventions but also for the construction of nursing science designed to address effective inflammatory process management in patients with chronic heart failure. Because nurses provide direct care, they are in a unique position to recognize and help patients deal with their signs and symptoms when they experience illness.

Theoretical, Conceptual, and Empirical Framework

The primary theoretical-level framework guiding this study is an integrated framework of two theories: one is the inflammatory pathophysiologic process, and the other is the hypothesis of cytokines in the syndrome of heart failure.

Inflammatory Pathophysiologic Process

Inflammation is the body's basic response to injury. At the beginning of the inflammatory process, chemicals released by the body's own dying cells or by foreign microbes which attract immune cells to the injured or infected area. Among the first cells to respond are macrophages, which eliminate the dying cells and microbes by engulfing them and/or breaking them apart with destructive chemicals. Responding to the presence
of chemokines, phagocytes enter the site of inflammation within a few hours. These cells release a variety of soluble factors, many of which have potent pro-inflammatory properties. However, when the inflammatory process is either excessive or persistent it becomes harmful.

The inflammatory process plays an important role in many diseases. In many disease states, the inflammatory process spins out of control and becomes harmful. In some neuromuscular diseases (like certain muscular dystrophies), inflammation is probably a secondary response to muscle degeneration, while in others (like the inflammatory myopathies), it might be a primary cause of degeneration. In either case, the inflammation can contribute to disease progression. Elevated circulating levels of cytokines, in particular TNF-α and IL-6, have consistently been identified in patients with HF. Several studies suggest that the extent of cytokine production directly correlates to the severity of the disease process (Kubota, McNamara, Wang, Trost, McTiernan, Mann, et al., 1998; Levine, Kalman, Mayer, Fillit, & Packer, 1990; Matsumori, Yamada, Suzuki, Matoba, & Sasayama, 1994). Inappropriate or uncontrolled inflammatory responses are the basis of allergic diseases, and chronic inflammation affects the quality of life of many animals and people, especially patients with HF.

Hypothesis of Cytokines in the Syndrome of Heart Failure

Heart failure can no longer be considered a simple disease of the heart alone. Clinical manifestations are the result of changes to the heart’s cellular and molecular
components and to mediators that drive homeostatic control. The heart, circulatory system and organs, including peripheral muscles, are involved in the syndrome of heart failure. Cytokines are pathogenic factors in many disorders where inflammation etiology is suspected. Accumulating evidence indicates that inflammatory mediators play an important role not only in the pathophysiologic process of atherosclerosis, myocarditis and cardiac allograft rejection but also in the syndrome of HF (Anderson, 2000; Paulus, 1999; Seta, et al., 1996).

Cytokine biological mechanisms and molecular pathways affecting the structure and function of cardiovascular system have been identified (Parissis, et al, 2002). According to the cytokine hypothesis, HF progresses because cytokine cascades that are activated following myocardial injury exert deleterious effects on the circulation. The cytokine hypothesis holds that the progression of HF begins because cytokine cascades exacerbate hemodynamic and functional change or exert direct toxic effects on the heart. It is important to bear in mind that the cytokine hypothesis does not imply that cytokines cause heart failure, but rather that cytokines are responsible for the progression of HF (Mann, et al., 2005; Seta, et al., 1996).

Matsumori and Sasayama (2001) developed an experimental model for explaining the progress of chronic HF. They hypothesized that some aspects of HF may be mediated by reversible alterations of cardiac function and structural changes in the ventricle induced by cytokines. The effects of TNF-α on cardiac function are dependent on the amount and duration of cytokine expression. Short-term expression within the heart may
be an adaptive response to different forms of “stress,” whereas long-term expression may be maladaptive and produce cardiac decompensation (Packer, et al., 2005). Excessive TNF-α levels can produce LV dysfunction, cardiomyopathy and pulmonary edema and may be related to clinical manifestations of and the progression of HF. TNF-α may represents a biochemical mechanism that is responsible for producing symptoms in patients with HF (Levine, et al., 1990; Packer, 1995; Parissis, et al., 2002; Seta et al., 1996; Torre-Amione, Vooletich, & Farmer, 2000).

The conceptual-level framework (Figure 1-1) for the study uses a biophysiologic system approach. The major physiologic and psychosocial factors are derived and identified from a critical review of the literature, including antioxidant therapy (Ellis, Anderson, Lang, Blackman, Morris, Morris-Thurgood, et al., 2000; Ghatak, Brar, Agarwal, Goel, Rastogi, & Vaish, 1996; Hornig, Arakawa, Kohler, & Drexler, 1998; Keith, Jeejeebhoy, Langer, Kurian, Barr, & O'Kelly, 2001; Rossig, Hoffmann, Hugel, Mallat, Haase, & Freyssinet, 2001), pro-inflammatory cytokines (Adamopoulos, Parissis, & Kremastinos, 2001; Givertz, & Braunwald , 2004; Levine , et al., 1990; Mendzef & Slovinski, 2004; Torre-Amione, Kapadia, Benedict, Oral, Young, & Mann, 1996), endothelial function (Adamopoulos, Parissis, & Kremastinos, 2002; Sharma, & Davidoff, 2002; Uehara, Nomura, Ozaki, Fujinaga, Ikefuji, & Kimura, 2003), cardiac performance (Adamopoulos, et al., 2002; Remme, 2003; Tang & Francis, 2004), exercise capacity (Mancini, 1998; Willenheimer, & Erhardt, 2000), and functional status (Bennett, et al., 2002; Bennett, et al., 2003) in chronic heart failure patients.
Thus, this model provides a theoretical-level, a conceptual-level, and an empirical-level to guide the current study in identifying the effects of antioxidant supplement on endothelial function, cardiac performance, exercise capacity and functional status in patients with chronic heart failure.

**Definitions of Terms**

*Pro-inflammatory Cytokines*

Pro-inflammatory cytokines are small protein molecules that are the core of communication between immune system cells, and even between these cells and cells belonging to other tissue types. Tumor necrosis factor-alpha (TNF-α) is one of the cytokines that may cause oxidative stress in several ways. It causes a decrease in mitochondrial transmembrane potential, which precedes the induction of apoptosis. TNF-α has been shown not only to cause cachexia, inflammatory process and collagen vascular disease, but it also increases in patients with advanced HF (Ferrari, Guardigli, Mele, Percoco, Ceconi, & Curello, 2004; Levine, et al., 1990).

*Endothelial Function*

The vascular endothelium is a confluent, cellular monolayer that lines the entire vascular compartments at the interface between blood and the vessel wall. Endothelial dysfunction impairs the blood vessels' ability to dilate. Endothelial dysfunction refers to the altered vasodilatory capacity either at rest or after administration of various
endothelium-dependent factors, and it reflects the impaired release of vasodilatory chemical signals (especially nitric oxide) from the endothelium (Adamopoulos, et al., 2002). Chronic HF is a complex clinical syndrome in which abnormal vascular endothelial function has been shown to occur in both experimental animal models and in clinical models with compromised cardiac function (Sharma, & Davidoff, 2002).

Measurement of endothelial function in accessible peripheral vessels, such as in the brachial artery, is therefore a useful surrogate for gauging coronary endothelial vasomotor function and can be measured by changes in forearm blood flow induced by flow mediated dilatation (FMD) (Anderson, 1999; Doshi, Lewis, & Goodfellow, 2001). In this study, FMD was measured to assess patients’ endothelial function.

Cardiac performance

Cardiac performance or cardiac pumping function is simply the ability of the heart to do something: it is related to myocardial contractility as well as the shape of the heart chambers. It may be defined in terms of any of a number of different performance parameters depending on what our major interest is. In individual cardiac muscle cells, $V_{\text{max}}$ is a measure of contractility because it reflects changes in muscle performance which are not related to changes in preload or changes in afterload. Cardiac remodeling is best described as a process defined by structural changes in one or more cardiac chambers, in particular the ventricles (Remme, 2003), and it can also be viewed as the dynamic changes in ventricular size and shape that result from hemodynamic and
metabolic insults to the failing heart (Tang & Francis, 2004). Left ventricle remodeling is an important factor which determines the long-term prognosis in patients with myocardial infarction and HF. In current study, ejection fraction and left ventricular end-diastolic diameter (LVEDD) were measured by means of echocardiography.

**Exercise Capacity**

Exercise capacity is an indicator of the severity of HF in patients, and it can be a predictor of mortality, as well as an outcome of the syndrome in and of itself. Exercise capacity is the peak performance at a maximal level of work achieved when the heart is able to augment cardiac output and deliver oxygen to the metabolically active limbs and the capacity of skeletal muscle to extract and use oxygen (Weber & Janicki, 1986). Theoretically, exercise capacity can be defined as the body’s limit in its ability to respond to the physiologic stresses induced by prolonged physical effort. Exercise capacity is limited not only by impaired cardiac function but also by peripheral factors such as vascular tone, vasodilatory ability, and composition of muscle fiber. Besides being an important prognostic marker, exercise capacity is useful for objective assessment of functional class and response to therapy for HF. An operational definition of exercise capacity is the quantitative measurement known as the 6-minute walk distance test.

**Functional Status**

Functional status is defined by *The Merriam-Webster Dictionary* as affecting
physiological or psychological functions but not organic structure. Functional status can reflect the ability of patients to maintain their independence in the context of their daily life and encompasses dimensions of the physical, emotional, and cognitive states of individuals. Functional status data represents an important component of health outcome assessment, particularly at the level of the whole patient. Functional status is usually conceptualized as the ability to perform self-care, self-maintenance and physical activities. The majority of indices of physical health and some psychological scales build their operational definitions of health on the concept of functioning: how far is the individual able to function normally and to carry on his typical daily activities? In this study, functional status was measured using three instruments: the Medical Outcomes Study Short-Form-36 Health Survey; the Minnesota Living with Heart Failure Questionnaire; and the Center for Epidemiologic Studies Depression Scale.

**Specific Aims**

To better understand the effects of antioxidant supplementation on endothelial function, cardiac performance, exercise capacity and health-related quality of life in patients with chronic HF, the research questions in this study was to identify the effects of antioxidant supplementation on pro-inflammatory cytokines, endothelial function, cardiac performance, exercise capacity, and functional status in patients with chronic heart failure.

To achieve the specific aim of the current, five hypotheses were tested:
Hypothesis 1: Subjects will have a decrease in pro-inflammatory cytokines as measured by a decreased TNF-α level while using antioxidant supplementation compared to when they not using antioxidant supplementation.

Hypothesis 2: Subjects will have a increase in endothelial function as measured by a increased diameter change percentage and blood flow while using antioxidant supplementation compared to when they not using antioxidant supplementation.

Hypothesis 3: Subjects will have an increase in cardiac performance as measured by a decreased LVEDD and an increased LVEF on the echocardiography while using antioxidant supplementation compared to when they not using antioxidant supplementation.

Hypothesis 4: Subject will have an increase in exercise capacity as measured by an increased total distance walked on the 6MWT while using antioxidant supplementation compared to when they not using antioxidant supplementation.

Hypothesis 5: Subjects will have an increase in functional status as measured by improvements in their scores on the SF-36, MLHFQ, and CES-D while using antioxidant supplementation compared to when they not using antioxidant supplementation.

Alpha of .05 was used for each hypothesis.
CHAPTER 2
REVIEW OF THE LITERATURE

The discussion in this chapter will start with an overview of the literature on the population with chronic heart failure. Then, the systemic inflammatory process will be discussed, including pro-inflammatory cytokines, endothelial function, cardiac performance, exercise capacity, and functional status. The intervention in this study, antioxidant supplementation, will also be discussed, followed by a summary of the literature review.

Inflammatory Process

Effects of Oxidative Stress on the Heart

For more than a decade, circulating levels of pro-inflammatory cytokines have been known to be elevated in HF. Oxidative stress in living organisms occurs when there is an imbalance between free radical production and endogenous antioxidant defense, provoking tissue damage through oxidative modification of essential cellular biomolecules. However, increased oxidative stress has been demonstrated in several experimental models of chronic HF including myocardial infarction-induced HF, tachycardia-induced cardiomyopathy, and volume overload cardiac dysfunction (Korantzopoulos, Galaris, Papaioannides, & Siogas, 2003).

Several hypotheses have been proposed to explain the elevation of circulating
cytokine levels in HF patients, including endotoxin-induced cytokine production, which is related to bowel edema, myocardial cytokine production because of hemodynamic overload, or extramyocardial cytokine production because of peripheral tissue hypoperfusion and hypoxia (Paulus, 2000). Antioxidant reserves may be reduced in patients with HF, both as a primary abnormality and as a consequence of reactive oxygen species overproduction (MacCarthy & Shsh, 2003). No matter which hypothesis is considered, the formation of reactive oxygen species (ROS) is enhanced during the progression of heart failure.

Cytokines have been implicated in the syndrome of chronic heart failure for over a decade. Levine and his colleagues first described elevated plasma tumor necrosis factor-alpha (TNF-α) concentrations in non-septic patients with chronic heart failure (Adamopoulos, et al., 2001; Levine, et al., 1990). In many conditions, such as ischemic cardiomyopathy and chronic left ventricular pressure or volume overload, myocardial expression of pro-inflammatory cytokines is triggered by an elevation of left ventricular wall stress (Paulus, 2000).

*Pro-inflammatory Cytokines*

Tumor necrosis factor-alpha (TNF-α) is a cytokine that may cause oxidative stress in several ways. It is produced primarily by macrophages in a variety of infectious and inflammatory conditions. It causes a decrease in mitochondrial transmembrane potential, which precedes the induction of apoptosis. TNF-α has been shown not only to cause
cachexia, infection and collagen vascular disease, but it also increases in patients with advanced heart failure (Ferrari, et al., 2004; Levine, et al., 1990).

The first reports implicating TNF-α as a factor in heart failure appeared in 1990 when Levine showed increased levels of TNF-α in patients with advanced heart failure (Levine, et al., 1990). This case-control study recruited 33 adults with chronic HF and 33 age-matched control subjects. The patients with HF had significantly increased TNF-α levels as compared with the controls. Furthermore, patients with the most cachexia, defined as being at less than 82% of their ideal body weight, had the highest circulating TNF-α levels. This landmark study led to a paradigm shift in HF research.

In a sub-study of the Studies on Left Ventricular Dysfunction (SOLVD), it has been shown that patients who became symptomatic with respect to heart failure had progressively higher circulating TNF-α levels (Torre-Amione, Kapadia, Benedict, Oral, Young, & Mann, 1996). In addition, it was found that myocardial TNF-α was present in a failing myocardium but not in a normal myocardium (Torre-Amione, Kapadia, Lee, Durand, Bies, & Young, 1996).

Primarily, these studies have investigated the presence of pro-inflammatory cytokines and their receptors in the circulatory system or in the cardiac tissue of patients with various etiologies and severity of heart failure. The most consistent finding is that plasma concentrations of TNF-α and its circulating receptors are elevated and correlate with the severity of heart failure, as well as with poor prognosis of the patient (Baumgarten, Knuefermann, & Mann, 2000; Ferrari, et al., 2004).
Effects on Endothelial Function

Peripheral vascular function is disturbed in patients with advanced heart failure (Kubo, Rector, Bank, Williams, & Heifetz, 1991). Both at rest and during exercise, peripheral vasomotor tone is increased and vasodilatory responses are reduced (Conraads, Bosmans, & Vrints, 2002). Inflammatory changes and increased oxidative stress in the setting of chronic heart failure may play an important role in the regulation of peripheral vascular function. Oxidative stress appears to be an important feature in the development of endothelial dysfunction. Moreover, the circulating TNF-α levels correlated with impaired peak leg blood flow in heart failure patients (Anker, Volterrani, Egerer, Felton, Kox, Poole-Wilson, et al., 1998; von Haehling, Anker, & Bassenge, 2003).

Effects in the Contractile Function

At low concentrations TNF-α levels acts as a paracrine and autocrine molecule, upregulating vascular adhesion molecules, activating neutrophils and stimulating monocytes to secrete IL-1 and IL-6. At higher concentrations, TNF-α acts in an endocrine fashion and as a pyrogen, activating the clotting cascade and suppressing bone marrow stem cell development. At even higher serum concentrations TNF-α acts as a myocardial depressant and induces nitric oxide (NO) synthesis leading to decreased vascular tone (Anderson, 2000).

Oxidative stress is both increased in ischemic and non-ischemic HF patients.
Previous studies have shown that oxidative stress impairs contractile function by disrupting the excitation-contraction coupling processes, and these contractile changes can be improved by antioxidant treatment. TNF-α plays a contributory role in the progressive dysfunction of a failing myocardium and is demonstrated when myocardial cells produce TNF-α. It is known that peripheral levels of TNF-α increased in patients with symptomatic heart failure, but whether a failing myocardium contained TNF-α was not known (Torre-Amione, et al., 2000).

Although experimental evidence suggests that TNF-α induces cardiac injury in laboratory animals, there is no direct evidence that TNF-α induces cardiac injury in humans. But the mechanism by which TNF-α induced contractile abnormalities includes reduction in intracellular calcium and possibly the induction of nitric oxide, which both directly alter contractility (Yokoyama, Arai, Sekiguchi, Tanaka, Kanda, & Suzuki, 1999).

Long-term myocardial TNF-α exposure not only directly decreases contractility, but also leads to structural myocardial changes that can result in dilated cardiomyopathy over time (Bozkurt, Kribbs, Clubb, Michael, Didenko, & Hornsby, 1998; Kubota, McTiernan, Frye, Slawson, Lemster, & Koretsky, 1997). TNF-α may also induce cardiac injury by means of its ability to induce programmed cell death or apoptosis (Ferrari, 1998; Torre-Amione, et al., 2000).

Effects on Remodeling

During the process of HF, the myocardium undergoes progressive remodeling.
Local pro-inflammatory cytokines, such as TNF-α, exacerbate the process by promoting chronic inflammation in the heart. The hypothesis that the pathophysiology of HF involves, in part, an inflammatory process has been tested over the past decade (Blum, & Miller, 2001).

Although cardiac remodeling occurs in different etiologic conditions, the most important contributing factors include neurohormonal activation, mechanical stretch, cytokines, oxidative stress, ischemia, necrosis, and apoptosis. In animal models, stimulation with pathophysiologic concentrations of TNF-α leads to ventricular remodeling and prominent left ventricular dilatation (Kubota, et al., 1997; Uehara, et al., 2003). LV remodeling is an important factor which determines the long-term prognosis in patients with myocardial infarction. Therefore, the prevention of excessive LV remodeling is a very important issue.

**Endothelial Function**

*Pathophysiologic process*

A critical balance between endothelial-derived relaxing and contracting factors maintains vascular homeostasis. NO is an endogenously-synthesized free radical first characterized as a component of endothelial-derived relaxation factor. NO plays a central role in regulating vascular physiologic and cellular homeostasis as well as critical intravascular free radical and oxidant reactions. The role of NO in cytotoxic events is being recognized increasing for its significance. It does this by blocking the exocytosis of
mediators of inflammation from the endothelial cells. Endothelial dysfunction is concerned with the altered vasodilatory capacity either at rest or after administration of various endothelium-dependent factors, and it reflects the impaired release of vasodilatory chemical signals (especially NO) from the endothelium (Adamopoulos, et al., 2002; von Haehling, et al., 2003).

The vascular endothelium is deeply involved in cardiovascular physiology and various chronic diseases. The vascular endothelium plays a central role in the regulation of vascular tone, hemostasis and inflammation (Moncada, Palmer, & Higgs, 1991; Tousoulis, Charakida, & Stefanadis, 2005). One of the important pathways of influencing endothelial function is the L-arginine-nitric oxide pathway. NO is synthesized from L-arginine by the action of nitric oxide synthase (NOS), and diffuses bi-directionally into the subendothelial space and the vascular lumen (Moyna & Thompson, 2004). NO inhibits not only the adhesion of leukocytes to the endothelium but also interactions between platelets and the vessel wall. It also affects the proliferation and migration of vascular smooth muscle cells. It is well established that arterial function is impaired in heart failure, and reactive oxygen species (ROS) is believed to be an important underlying mechanism (Figure 2-1). Chronic HF is a complex clinical syndrome in which abnormal vascular endothelial function has been shown to occur in both experimental animal models and at the clinical level with compromised cardiac function (Sharma, & Davidoff, 2002).
The TNF-α induces the formation of oxygen free radicals, which rapidly scavenge and destroy NO, produced at the endothelial level. The antioxidant form of supplementation, vitamin C, improves FMD in patients with HF (Hornig, et al., 1998).

Related Factors

Endothelial function is a primary target for injury from mechanical forces and processes related to cardiovascular risks including aging (Celermajer, Sorensen, Spiegelhalter, Georgakopoulos, Robinson, & Deanfield, 1994), smoking (Celermajer,
Sorensen, Georgakopoulos, Bull, Thomas, Robinson, et al., 1993; Heitzer, & Munzel, 1996; Motoyama, T., Kawano, H., Kugiyama, K., Okumura, K., Ohgushi, M., Yoshimura, et al., 1997), serum cholesterol levels (Celermajer, 2003; von Haehling, et al., 2003), and medications, such as angiotensin-converting enzyme (ACE) inhibitors, angiotensin II antagonism, or calcium channel blocker (Anderson, Elstein, Haber, & Charbonneau, 2000; Celermajer, et al., 1994; Drexler, Kurz, Jeserich, Munzel, & Hornig, 1995).

Measurement

Endothelial function is usually assessed as a vasodilatory response to either pharmacological or mechanical stimuli, and it has been measured in both coronary and peripheral arteries. There are some invasive techniques for measuring endothelial function, but the invasive nature of these procedures makes them not only unsuitable for use in children, adolescents, and in asymptomatic adults, but they are also associated with increased clinical risk. Vascular occlusion plethysmography is a minimally invasive procedure that is commonly used to assess peripheral arterial function. The most widely employed non-invasive measure of endothelial function involves brachial artery diameter measurement using ultrasound imaging before and after several minutes of blood flow occlusion. The change in arterial diameter is a measure of flow-mediated vasodilation (FMD) (Anderson, 1999; Maltz, & Budinger, 2005; Moyna & Thompson, 2004).

Change in vessel diameter is used as an index of endothelial function in conductive vessels, and change in blood flow is used as an index of function in resistance vessel. A non-invasive technique has been developed to evaluate FMD and endothelium-dependent
function in the brachial artery. This method assesses the ability of the endothelium to
dilate in response to shear-induced increases in NO. Brachial artery diameter is measured
at baseline and after reactive hyperaemia produced by 5 minutes of upper or lower arm
occlusion. Increased blood flow during reactive hyperaemia elicits a strictly
endothelium-dependent vasodilator response by activating endothelial-derived NOS. In
principle, the vessel is stressed by increased blood flow in response to ischemia-induced
reactive hyperaemia. Relative increases in diameter from baseline are used as measures
of FMD. Diameter changes are then measured after nitroglycerin administration in order
to assess the response of the vessel to an endothelium-independent vasodilator (Moyna &

Moreover, studies showing a correlation between coronary and peripheral arterial
function suggest that FMD in peripheral arteries can be used as a surrogate for coronary
artery endothelial function (Anderson, Gerhard, Meredith, Charbonneau, Delagrange,
Creager, et al., 1995).

*Strategies for Endothelial Dysfunction*

There are several strategies that can reverse endothelial dysfunction, such as
exercise training (Maiorana, O'Driscoll, Dembo, Goodman, Taylor, & Green, 2001),
using cholesterol lowering medication (Bauersachs, & Schafer, 2004; Celermajer, 2003;
Omori, Nagashima, Tsurumi, Takagi, Ishizuka, Hagiwara, et al., 2002), ACE inhibitors
Heitzer, et al., 1996; Ting, Timimi, Boles, Creager, Ganz, & Creager, 1996), and smoking cessation (Celermajer, et al., 1993), which have all been shown to improve endothelial function in adults.

Several previous studies revealed that administering vitamin C is both able to improve brachial FMD in HF patients and to suppress the induction of endothelial cells apoptosis by TNF-α in vitro (Corretti, Anderson, Benjamin, Celermajer, Charbonneau, & Creager, 2002; Hornig, et al., 1998; Kiowski, Sutsch, Schalcher, Brunner, & Oechslin, 1998; Rossig, et al., 2001).

Epidemiologic studies indicate an association between increased intake of antioxidant vitamins and reduced risk of coronary disease (Riemersma, Wood, Macintyre, Elton, & Oliver, 1989; Rimm, Stampfer, Ascherio, Giovannucci, Colditz, & Willett, 1993), although the mechanism responsible for these observations is not completely understood. The most possible mechanism is preservation of normal endothelial function by antioxidants, since increased oxidative stress has been related to impaired action of an endothelium-derived relaxing factor, which has been defined as NO. Specifically, increased vascular ROS production is associated with inactivation of NO and loss of endothelium-dependent dilation (Keaney, Gaziano, Xu, Frei, Curran-Celentano, Shwaery, et al., 1993; Keaney, Simon, Stamler, Jaraki, Scharfstein, Vita, et al., 1993).

Meyer et al. (2005) assessed the importance of impaired endothelium-dependent FMD in patients with chronic HF. They studied 75 patients with depressed ejection fraction (LVEF < 30%). The primary endpoint for this study was a combined outcome of
either patient death or conversion to the United Network for Organ Sharing (UNOS) status 1 while awaiting cardiac transplantation. Event-free survival rate was higher in patients with FMD above the median value compared with those below. Only 19% of patients above the median cut-off value, but 63% below it, reached the combined end point. In chronic HF patients, impaired FMD is a strong independent predictor of conversion to UNOS status 1 or death.

**Cardiac Performance**

*Contractility*

The most common cause of HF is left ventricular systolic dysfunction (about 60% of patients). In this category, most cases are a result of end-stage coronary artery disease, either with a history of myocardial infarction(s) or chronically underperfused yet viable myocardium. In many patients, both processes are present simultaneously (Hobbs & Boyle, 2004). Other common causes of LV systolic dysfunction include idiopathic dilated cardiomyopathy, valvular heart disease, hypertensive heart disease, toxin-induced cardiomyopathies (ie, doxorubicin and alcohol), and congenital heart disease.

Abnormal cardiac contractility is the hallmark of heart failure and it could arise due to various causes including diabetes and hypertension. In individual cardiac muscle cells $V_{\text{max}}$ is a measure of contractility because it reflects changes in muscle performance not related to changes in preload or changes in afterload. It is the rate of shortening of unloaded muscle. This is difficult to measure clinically. The maximum rate of pressure
development in the left ventricle, \( dp/dt \text{ max} \), can be related to \( V_{\text{max}} \) on theoretical grounds, and is sometimes used as a measure of the contractile state.

Increasing contractility moves the end systolic pressure-volume relationship up and to the left. Decreasing contractility moves it down and to the right. Ejection begins from the same point as before but proceeds to the new end-systolic pressure-volume curve. Thus, increased contractility is associated with increased stroke volume and ejection fraction, while decreased contractility is associated with decreased stroke volume and ejection fraction. Regardless of etiology, changes in the pressure and volume relationship of the myocardium in heart failure result in both acute and chronic compensatory mechanisms being activated in order to preserve function (Smith & Kampine, 1990). The Frank-Starling law describes this relationship and is expressed as a curve where increasing end diastolic volume results in increasing ventricular pressure.

Ejection Fraction (EF) is the ratio of blood ejected to the total blood contained in the ventricle at end diastole. The formula is that \( EF = \text{stroke volume} / \text{end-diastolic volume} = (\text{end diastolic volume} – \text{end systolic volume}) / \text{end diastolic volume} \). It is sometimes expressed as a percentage. If the heart becomes enlarged, even if the amount of blood being pumped by the left ventricle remains the same, the relative fraction of blood being ejected decreases. EF is most commonly measured using echocardiography. This non-invasive technique provides good estimates of end-diastolic (EDV) and end-systolic volumes (ESV), and stroke volume. EF is often used as a clinical index to evaluate the inotropic status of the heart. However, it is important to note that there are
circumstances in which EF can be normal, yet the ventricle is in failure.

If the impaired function is chronic, more long-term adaptations take place. With the heart muscle, chronic volume overload results in left ventricular hypertrophy and the chambers enlarging to maintain stroke volume. While effective initially, the increase in muscle mass results in more oxygen demand, and the increased afterload further impairs the failing heart.

Remodeling

Cardiac remodeling is best described as a process defined by structural changes in one or more cardiac chambers, in particular the left ventricle (Remme, 2003), and there are also dynamic changes in ventricular size and shape that result from hemodynamic and metabolic insults to the failing heart (Tang & Francis, 2004). Cardiac remodeling has a direct relation with cardiac events and in patients with HF; the progression of LV dilation is associated with increased incidence of morbidity and mortality (Bradham, Bozkurt, Gunasinghe, Mann, & Spinale, 2002). The process of remodeling is complex, and it leads to increased end-diastolic and end-systolic volume, as well as alteration of the shape of the heart and cardiac hypertrophy, notably an increase in left ventricular mass. Alteration in endothelial function may contribute to the increased vasomotor tone and to the vascular remodeling process observed in patients with chronic HF (Adamopoulos, et al., 2002). The remodeling of the ventricular wall to a more dilated state marks the progression to end-stage HF for the patient and, at the present time, does not appear to be reversible by conventional pharmacologic therapies (Holycross, & Radin, 2002).
Despite a multitude of causes for chronic HF, a milestone in the progression of the disease process is myocardial remodeling. Recent clinical and experimental studies have provided evidence to suggest that increased release of TNF-α can contribute to the progression of LV pump dysfunction and HF. Experimental studies have documented that increased TNF-α levels can cause LV remodeling and the development of LV dysfunction (Bozkurt, et al., 1998; Bradham, et al., 2002; Norton, Woodiwiss, Gaasch, Mela, Chung, & Aurigemma, 2002). Moreover, TNF-α causes myocardial remodeling and fibrosis in myocardial infarction and chronic heart failure models. An animal trial has shown that rats that received etanercept, a soluble dimerized TNF receptor that inhibits TNF, experienced reduced leukocyte infiltration and extracellular matrix turnover and preserved cardiac function (Berry, Woo, Pirolli, Bish, Moise, Burdick, et al., 2004).

The angiotensin converting enzyme (ACE) inhibitor drug class is an essential tool in the treatment of chronic HF. Patients treated with ACE inhibitors or AII type I receptor (AT1R) antagonists have decreased concentrations of circulating TNF-α (Gurlek, Kilickap, Dincer, Dandachi, Tutkak, & Oral, 2001; Tsutamoto, Wada, Maeda, Mabuchi, Hayashi, Tsutsui, et al, 2000; Young, 1999).

**Exercise Capacity**

Patients with heart failure have marked functional impairment and reduced exercise capacity. Decreased exercise capacity in patients with HF has been traditionally attributed to a decreased cardiac output response to exercise, which leads to skeletal muscle
hypoperfusion and intramuscular lactic acidosis (Mancini, 1998).

Exercise capacity is limited not only by impaired cardiac function but also by peripheral factors such as vascular tone, vasodilatory ability, and composition of muscle fiber. Besides being an important prognostic marker, exercise capacity is useful for objective assessment of functional class and response to therapy for heart failure. Exercise intolerance in heart failure results from vasotone dysregulation caused by impairment of endothelial function. Because of its simplicity, the 6-min-walk test (6MWT) has been considered to be suitable for the routine assessment of the severity of heart failure, for prognostication, and for evaluation of therapeutic response (Willenheimer, & Erhardt, 2000). Dracup et al. (1992) demonstrated a significant correlation between the 6MWT, a self-MET level and NYHA functional class. Bittner et al. (1993) demonstrated that the 6MWT correlated with survival in patients with HF.

In patients with heart failure, exercise capacity, as measured by oxygen consumption (VO2) during walking, stationary cycling or the 6-minute walking distance test, is 30% - 40% less than that observed in age-matched normal patients (Bittner, 1994; Keteyian, Marks, Brawner, Levine, Kataoka, & Levine, 1996). These reductions in exercise capacity are due to some factors, including reduced peak cardiac output and diminished nutritive blood flow to the skeletal muscle. Decreasing exercise capacity is also correlated to cardiac output, for at peak exercise; stroke volume in patients is approximately 75% of that observed in age-matched normal patients (Sullivan, Knight, Higginbotham, & Cobb, 1989).
Other studies found different contradictory results. There is a poor correlation between exercise capacity and indices of LV dysfunction (such as ejection fraction) both at rest and during exercise (Franciosa, Park, & Levine, 1981; Szlachcic, Massie, Kramer, Topic, & Tubau, 1985). Some measures taken to acutely improve LV function, such as inotropic drugs or vasodilators, only have limited immediate impact on exercise capacity in HF (Maskin, Forman, Sonnenblick, Frishman, & LeJemtel, 1983; Massie, Simonini, Sahgal, Wells, & Dudley, 1996). And there is little close relation between exercise capacity and hemodynamic function (Clark, Volterrani, Swan, Hue, Hooper, & Coats, 1996; Clark, & Cleland, 2001; Weber, Wilson, Janicki, & Likoff, 1984).

Boffa et al. (2004) investigated the relationship between pro-inflammatory cytokines and exercise capacity in HF. This study population was composed of a series of 80 consecutive outpatients with HF and who were classified as NYHA functional class I to III and had LVEF < 45%. Each patient underwent a cardiologist’s evaluation, a standard electrocardiogram, a 2-D echocardiogram, a 6MWT and a venous blood sample on the same day. A significant correlation was found between the results of the 6MWT and plasma concentrations of TNF-α in patients with mild to moderate HF.

Several studies focused on the impact of an exercise training program on quality of life in patients with chronic HF. Part of the results of those studies indicated that an exercise training program can improve the perception of health-related QOL in patients with severe chronic HF (Collins, Langbein, Dilan-Koetje, Bammert, Hanson, Reda et al., 2004; Lavie & Milani, 1995; Quittan, Sturm, Wiesinger, Pacher, & Fialka-Moser, 1999).
Other studies concluded there was no improvement in health-related QOL after an exercise training program for patients with HF (Owen, & Croucher, 2000).

Functional Status

Functional status can reflect the ability of patients to maintain their independence in the context of their daily life, and it encompasses dimensions of the physical, emotional, and cognitive states of individuals. Functional status data represents an important component of health outcome assessment, particularly at the level of the whole patient. In a sub-study of the Studies on Left Ventricular Dysfunction (SOLVD) study, plasma levels of IL-6 and TNF-\(\alpha\) were elevated in patients in functional class I-III and were progressively elevated in relation to decreasing functional status (Torre-Amione, et al., 1996). In this study, functional status is analyzed in two ways: one is health-related quality of life, and the other is depression.

Health-Related Quality of Life

Definition of Health-Related Quality of Life

Quality of life is a broad concept which can be considered the sum of all negatively and positively valued aspects of life and can be defined in many different ways. Reviewing the numerous articles related to QOL, we can reveal that it is difficult to find a common definition of quality of life. It is because the phrase has so many different interpretations and is so complex that many authors do not define the concept at all or
measure related concepts (Haas, 1999; Meeberg, 1993). Many terms are used synonymously with QOL in the literature, such as life satisfaction, well-being or happiness; life satisfaction is most frequently used in this way (Meeberg, 1993). Most definitions of QOL are derived from the definition of health by World Health Organization (WHO), which is “a state of complete physical, mental and social well-being, and not merely the absence of disease” (Berry & McMurray, 1999; Johansson, Agnebrink, Dahlstrom & Brostrom, 2004).

Although mental and physical health status are dominant determinants of QOL, other aspects of life such as a person’s economic situation, religious convictions, vitality and social relationships may also contribute to QOL. It is because QOL is considered more than health status but can easily be influenced by changes in physical health status, that some of the research just focuses on health-related QOL to detect the influences which come from disease, such as signs, symptoms or the effects of treatment.

Health-Related Quality of Life Measurement

The choice of instrument by nurse researchers in the measurement of health-related quality of life (HRQOL) in HF patients might be made according to the objective of the study, such as the characteristics of the population, burden to subject, cost and scoring complexity and the possibility to capture the concept (Johanssonet al., 2004).

Traditional end points of clinical study in HF have been hemodynamic parameters, such as left ventricular ejection fraction or cardiac output, but it is changing at a time
when we consider there is no cure of heart failure patient. Currently, a great deal of the research focuses on quality of life rather than objective hemodynamic data as an end-point in heart failure patients. HRQOL is impaired in patients with HF in a variety of important ways. Not only is their physical health, such as dyspnea, fatigue, ankle swelling and loss of muscle strength impaired, but a person’s HRQOL is also influenced by the awareness of one’s own mortality and a negative impact on social function can contribute to depression, sleep disturbance and anxiety. When we talk about HRQOL, we have to consider many factors to understand all of the domains of one’s physical, psychological, social and interrelationships with others.

**Generic Quality of Life**

Regardless of a researcher taking a generic or disease-specific approach, several instruments have been validated in heart failure patients and are used to evaluate changes in HRQOL which are linked to disease or to treatment. Some generic instruments are usable whatever the disease or population, such as the Medical Outcome Study Short-Form-36 (SF-36), SF-20, SF-12, the Duke Health Profile (DHP), and the Sickness Impact Profile (SIP).

The SF-36 is used in this study to evaluate generic HRQOL. The SF-36 is a short form measure of generic health status in the general population. It is designed as a self-administered structured questionnaire. The SF-36 has also been used in heart disease patients, such as patients with coronary artery disease (Bengtsson, Hagman, Wahrborg, &
Wedel, 2004; Brink, Grankvist, Karlson, & Hallberg, 2005; Brown, Melville, Gray, Young, Skene, & Hampton, 2000; Muller-Nordhorn, Roll, S., & Willich, 2004), arrhythmia (Carroll, Hamilton, & McGovern, 1999), patients who have received implantable cardioverter-defibrillators (Carroll, Hamilton, & Kenney, 2002), patients who have undergone coronary bypass grafting surgery (Al-Ruzzeh, Mazrani, Wray, Modine, Nakamura, George, et al., 2004; Lindsay, Hanlon, Smith, & Belcher, 2003), patients suffering from hypertension (Aydemir, Ozdemir, & Koroglu, 2005; Bardage, & Isacson, 2001), as well as patients with HF (Brostrom, Stromberg, Dahlstrom, & Fridlund , 2004; Dempster, & Donnelly, 2000; Pihl, Jacobsson, Fridlund, Stromberg, & Martensson, 2005; Quittan, et al., 1999; Rodriguez-Artalejo, Guallar-Castillon, Pascual, Otero, Montes, Garcia,, 2005; Westlake, Dracup, Creaser, Livingston, Heywood, 2002).

Disease-Specific Quality of life

Disease-specific instruments, such as the Minnesota Living with Heart Failure questionnaire (MLHFQ), the Yale Scale, and the Quality of Life in Severe Heart Failure, Chronic Heart Failure Questionnaire, and Kansas City Cardiomyopathy Questionnaire, have been used only in heart failure patients.

The MLHFQ captures the defining attributes in the following ways: (a) QOL is an evaluation of an individual’s current life circumstances (MLHFQ asks subjects how they feel or perceive in the last one month); (b) QOL is multidimensional in nature (MLHFQ is divided into general, physical and emotional aspects); (c) QOL is value-based and
dynamic (MLHFQ can evaluate the correlation of and between different dimensions); (d) QOL comprises subjective and/or objective indicators (MLHFQ detects subjects’ feeling and also identifies their clinical symptoms); and (e) QOL is most reliably measured by subjective indicators by persons capable of self-evaluation (and acceptable validity and reliability have been derived from many previous studies).

In the clinical setting, the MLHFQ can apply to various types of study design no matter if they are descriptive (Baas, Fontana, & Bhat, 1997; Bennett, Baker, & Huster, 1998; Bennett, Perkins, Lane, Deer, Brater, & Murray, 2001; Scott, Smiddy, Schiffman, Feuer, & Pappas, 1999), randomized control trials (Gottlieb, Fisher, Freudenberger, Robinson, Zietowski, Alves, 1999; Kasper, 2002; Owen & Croucher, 2000), cross-sectional (Lewis, Johnson, Johnson, Collins, Griffin, & Stevenson, 2001; Riegel, Carlson, Glaser, & Romero, 2003) or prospective cohort studies (Varma, McElnay, Hughes, Passmore, & Varma, 1999).

And from another perspective, the MLHFQ is widely used to evaluate clinical outcomes of treatments, such as the effect(s) of clinical pharmacologic trials (Anand, Latini, Florea, Kuskowski, Rector, Masson, et al., 2005; Cowley, Wiens, Segal, Rich, Santanello, & Dasbach, 2000; Laufs, Wassmann, Schackmann, Heeschen, Bohm, & Nickenig, 2004), exercise training or performance (Arena, Humphrey, & Peberdy, 2002; von den Berg-Emons, 2004; Tyni-Lenne, Gordon, Jensen-Urstad, Dencker, Jansson, & Sylven, 1999; Vavouranakis, Lambrogiannakis, Markakis, Dermitzakis, Haroniti, & Ninidaki, 2003), antioxidant supplementation coenzyme Q-10 (Berman, Erman, Ben-Gal,
Dvir, Georghiou, Stamler, et al., 2004), concerned with differences in the races of the patients (Riegel, et al., 2003), a web-based study (Artinianm Harden, Kronenberg, & Vander, 2003), or patients implanted with pacemakers (Linde, Braunschweig, Gadler, Bailleul, & Daubert, 2003; Lupi, Brignole, Oddone, Bollini, Menozzi, & Bottoni, 2000) and so on.

Bennett and her colleagues (Bennett, et al., 2003: Bennett, et al., 2002) compared 3 instruments for evaluating HRQOL in HF patients and concluded that all three of these instruments were reliable and valid and satisfactory for measuring HRQOL, and disease-specific instruments were preferable to the generic instrument. And the MLHFQ and chronic heart failure questionnaires were more sensitive than the SF-12 in detecting clinically important changes over time. Among the numerous questionnaires available for the assessment of HRQOL, the MLHFQ appears to provide the most suitable and popular measurement of HRQOL in patients with HF (Feenstra, Lubsen, Grobbee, & Stricker, 1999; Guyatt, 1993; Johansson, et al., 2004; Rector, & Cohn, 1992).

Depressive Symptoms

A depressive symptom is defined by WHO as “a common mental disorder that presents with depressed mood, loss of interest or pleasure, feelings of guilt or low-worth, disturbed sleep or appetite, low energy, and poor concentration.” These problems can become chronic or recurrent and can lead to substantial impairments in an individual’s ability to take care of his/her everyday responsibilities. A depressive symptom is a mental
state characterized by a pessimistic sense of inadequacy and a despondent lack of activity.

The term “depression” can be confusing since it’s often used to describe normal emotional reactions. At the same time, the illness may be hard to recognize because its symptoms may be so easily attributed to other causes. Depressive symptoms include experiencing an inappropriate depressed mood. Although depressive symptoms can obviously be caused by depressive disorder (such as a medical diagnosis, major depression), there are many other possible reasons that may lead to feelings of being down, sad or depressed. In this study, depressive symptom referred to an illness that involves the body, mood, and thoughts, that affects the way a person eats and sleeps, the way one feels about oneself, and the way one values about things.

The prevalence rates of depressive symptoms in HF range from 13% to 77.5% (Freedland, Rich, Skala, Carney, Davila-Roman, & Jaffe, 2003; Friedman, & Griffin, 2001; Vaccarino, Kasl, Abramson, & Krumholz, 2001). Freedland et al. (2003) demonstrated that the prevalence of depressive symptoms in hospitalized patients with HF is similar to rates found in post-infarction patients. However, it is considerably higher in certain subgroups, such as patients with NYHA functional class III or IV.

Patients with HF have a high prevalence of depressive symptoms, which may increase their risk of adverse outcomes. Jiang et al. (2001) determined the prevalence and relationship of depression to outcomes of patients hospitalized with HF. They recruited patients aged 18 years or older with NYHA functional class II or greater. The 374
patients were screened in this study, and 35.5% had Beck Depression Inventory (BDI) scores of 10 or higher and 13.9% had a major depressive disorder. They found that major depression is common on patients hospitalized with HF and is independently associated with a poor prognosis. Depressive symptoms had been associated with increased medical morbidity, mortality, functional impairment, and occupational disability; decreased adherence to medications and to cardiovascular risk factor interventions; and worse quality of life in these patients (Freedland, Rich, Skala, Carney, Davila-Roman, & Jaffe, 2003). Depression is a graded, independent risk factor for readmission to the hospital, and it is associated with functional decline and mortality in patients with congestive HF (Jiang, Alexander, Christopher, Kuchibhatla, Gaulden, Cuffé, et al., 2001).

Depressive symptoms have been found to be significantly related to reduced functional status, higher readmission rates, and increased mortality in HF patients. Several studies have demonstrated that depression might not only have an important effect on HRQOL, but it may also be associated with more frequent hospital admissions, a decline in activities of daily living, and worse NYHA functional classification (Gottlieb, Khatta, Friedmann, Einbinder, Katzen, Baker, et al., 2004; Murberg, & Furze, 2004; Vaccarino et al., 2001). The high rates of depression in patients with HF are important because depressive symptoms have been strongly associated with increased cardiac events and mortality (Pasic, Levy, & Sullivan, 2003). Furthermore, depressive symptoms have been well established as an independent risk factor contributing to poorer outcomes and mortality in patients with coronary heart disease.
Depressive symptoms are associated with long-term decreases in multiple domains of functioning in individuals with other chronic medical conditions. Murberg and Furze (2004) demonstrated that depressive symptom in individuals with HF was strongly associated with the perception of physical limitations. On the contrary, Skotzko et al. (2000) measured depression and HRQOL using the CES-D and SF-36, respectively. They found that depressive symptoms were common but unrelated to the severity of HF.

The cytokine hypothesis of HF (Mann, et al., 2005; Seta, et al., 1996) proposes that HF progresses since the cytokine cascade activated after myocardial injury exerts deleterious effects on the heart and circulation. Several studies demonstrated that the over-expression of pro-inflammatory cytokines contributes to the progression of HF once LV dysfunction is present, but it does not mean that cytokines initiate HF. Cytokines are increasingly being considered to be a significant factor in the pathogenesis of HF and it is possible that they affect the functional status of HF. Advanced HF is correlated with a progressive increase in peripheral circulating levels of TNF-\(\alpha\). High blood levels of TNF-\(\alpha\) have been found to correspond to the severity of HF symptoms (Torre-Amione, et al., 1996).

In 1991 Smith proposed the “macrophage theory of depression,” which considers the potent brain effects of pro-inflammatory cytokines and the association between pathological states of immune alteration and depression (Smith, 1991). Cytokines are hydrophilic molecules which are too large to cross blood-brain-barrier (BBB). Four major hypotheses have been proposed for the mechanism by which peripherally released
cytokines communicate with the brain: (a) active transport of cytokines across the BBB; (b) access of cytokines to the brain in areas where the BBB is weak, such as the organum vasculosum of the lamina terminalis; (c) conversion of cytokine signals into secondary signals, such as prostaglandin or NO signals, by endothelial cells that line the blood vessels of the brain; and (d) transmission of cytokine signals (through cytokine receptor binding) along sensory afferents to the nucleus of the solitaty tract and then via ascending catacholaminergic pathways onto the relevant brain regions, including the paraventricular nucleus of the hypothalamus (Pasic, et al., 2003). Although the exact cellular targets of pro-inflammatory cytokines in the brain are still elusive, it is evident that from the animal studies, receptors for IL-1, IL-2, IL-6, and TNF-α have been localized in the rodent brain with the highest density in the hippocampus and hypothalamus (Ericsson, Liu, Hart, & Sawchenko, 1995). Major depression is accompanied by a variety if immunomodulatory processes that affect the innate responses of the immune system, including those of cellular components and soluble mediators, such as cytokines. The most consistently increased pro-inflammatory cytokines in major depression are IL-1β, IL-2, IL-6, TNF-α and so on (Pasic, et al., 2003).

Clearly the current literature has not yet addressed the role of cytokines in comorbid depression and heart failure. But cytokines may be involved in both conditions and that might be a focus of future research if cytokines are considered to be involved in their pathogenesis. A reviewed article demonstrated the evidence of cytokine involvement in major depression (Dantzer, Wollman, Vitkovic, & Yirmiya, 1999).
Therefore, it is assumed that there is some overlap of the pro-inflammatory cytokines involved in heart failure and depression (Figure 2-2).

Psychological depression has been linked to heart failure, both as an antecedent to and as a risk factor for poor outcomes among patients with existing heart failure. Ferketich et al. (2005) proposed that having elevated levels of pro-inflammatory cytokines is a physiologic link in both heart failure and depression patients. Thirty-two heart failure patients with mean EF 26.4% ($SD = 9.5$) were recruited into Ferketich et al.’s study. BDI had been administered to those patients with a cut-off point as a score of 19. The results in the multiple linear regression models controlling for age, sex, smoking,
and antidepressant medication use have shown that there was no relationship between BDI scores and circulating levels of IL-6 or IL-1β levels. However, there was a statistically significant positive relationship between the BDI score and TNF-α levels. Ferketich et al.’s study demonstrated that a depression-specific activation of pro-inflammatory cytokines may promote disease progression and mortality in patients with HF. Suarez et al. (2003) examined the relationship between severity of depressive symptoms and pro-inflammatory cytokines in non-smoking, healthy men. They found that BDI was significantly associated with TNF-α levels ($r = 0.57$). In addition, men with BDI scores of 10 or above exhibited an over-expression of TNF-α levels ($p < .05$). They concluded that compared to men with no or minimal symptoms of depression, men with mild to moderate levels of depressive symptoms showed over-expression of pro-inflammatory cytokines.

Another cohort survey identified that depressive symptoms can predict morbidity and mortality among individuals who have coronary heart disease (Ferketich, Schwartzbaum, Frid, & Moeschberger, 2000). They analyzed data from 5007 women and 2886 men enrolled in the National Health and Nutrition Examination Survey who were free of CHD in 1982-1984; subjects were interviewed and completed the CES-D. Participants were evaluated from the 1982 interview data either until the end of the study (1992) or until the occurrence of a CHD event. Cox’ proportional hazards regression models were developed to evaluate the relative risk of CHD incidence and mortality in the depressed women and men separately. The results have shown that while controlling
for possible confounding factors, depression was associated with an increased risk of CHD incidence in both men and women, as well as with CHD mortality in men. Depression had no effect on CHD mortality in women.

Most of the existing studies of depression in patients with heart failure have focused on outpatients and have relied exclusively on cutoff scores on the CES-D (Havranek, Spertus, Masoudi, Jones, & Rumsfeld, 2004; Murberg, & Furze, 2004; Skotzko, et al., 2000).

The terms “depression” and “depressive symptoms” were used interchangeably in this study. In this study, depressive symptoms were measured using the Center for Epidemiologic Studies Depression Scales.

**Antioxidant Supplementation**

Oxidants are potentially deleterious agents, and consequently, antioxidants play a role in the prevention of damage due to oxidants (Halliwell, 1996; Sies, 1997). Antioxidants protect us because they can scavenge ROS before they cause damage to the various biological molecules, or prevent oxidative damage from spreading, e.g. by interrupting the radical chain reaction of lipid peroxidation. The antioxidant defense systems in the human body are extensive and consist of multiple layers, which protect at different sites and against different types of ROS. Vitamin C is one of the most powerful natural antioxidants. Ascorbic acid is the most effective, least toxic water-soluble antioxidant “free radical scavenging” vitamin known (Bode & Vethanayagan, 1998).
Similar results were reported by other researchers investigating the effects of vitamin C infusion on acetylcholine-induced dilation of coronary arteries in diabetic patients (Ting, et al., 1996). Furthermore, these results have been confirmed and extended recently by numerous clinical studies investigating the effects of vitamin C administration on arterial function in smokers and hypertensive and hypercholesterolemia patients (Heitzer, et al., 1996; Siow, Sato, Leake, Pearson, Bannai, & Mann, 1998; Solzbach, Hornig, Jeserich, & Just, 1997; Taddei, Virdis, Ghiadoni, Magagna, & Salvetti, 1998; Ting, et al., 1997). All of these studies have used mega doses of vitamin C, either ingested or infused into brachial or coronary arteries, and they have shown very similar improvements in vasodilatory response in CAD patients (Heitzer, Baldus, von Kodolitsch, Rudolph, & Meinertz, 2005; Levin, et al., 1996).

**Antioxidant Vitamin C**

Vitamin C, ascorbate or ascorbic acid, is the main water-soluble antioxidant in human plasma. Animal studies in the guinea pig and other animals (Hornig, 1975; Keith, & Pelletier, 1974) showed that tissue concentrations are related to intake, so that higher intake results in a higher tissue concentration. Therefore, the tissue concentrations of vitamin C reported in the literature may be inaccurate (Padayatty, Daruwala, Wang, Eck, Song, Koh, et al., 2002).

Oxygen damages biological molecules by stealing electrons, a process known as oxidation. Vitamin C is called an antioxidant because, by donating its electrons, it prevents other compounds from being oxidized. However, by the very nature of this
process, vitamin C itself is oxidized in the process. Vitamin C readily scavenges reactive oxygen, nitrogen, and chlorine species, thereby effectively protecting other substrates from oxidative damage, which means, it effectively scavenges ROS and other reactive oxygen species, and it plays an important role in the regulation of intracellular redox state through its interaction with glutathione (Frei, England, & Ames, 1989; Levine, et al., 1996; Siow, et al., 1998).

Clinical Trials of Vitamin C

Many epidemiological studies and a limited number of clinical trials have indicated that dietary intake of, or supplementation with, antioxidant vitamins is associated with a reduction in the incidence of cardiovascular diseases morbidity and mortality (Gey, 1998; Weber, Bendich, & Schalch, 1996). In a placebo-controlled study, administration of 2 gm of vitamin C to CAD patients with severely impaired brachial artery dilation led to a dramatic improvement in their vasodilatory response, indicative of improved biologic action of NO. This study hypothesizes that an antioxidant, ascorbic acid, would improve FMD on in patients with CAD. The endothelial function was assessed by high-resolution vascular ultrasound before and 2 hours after oral administration of either 2 gm ascorbic acid or a placebo in a total of 46 patients with documented CAD. Ascorbic acid administration resulted in an improvement in FMD, from $6.3 \pm 1.1\%$ to $9.5 \pm 1.2\%$ (mean $\pm SEM$), whereas there was no increase in FMD with placebo administration, $4.4 \pm 1.1\%$ to $3.4 \pm 1.2\%$. The effect of ascorbic acid treatment on brachial dilation was significantly different from the effect of the placebo ($p < .05$). The researchers concluded that ascorbic
acid reverses endothelial vasomotor dysfunction in the brachial circulation of patients with CAD. Their findings suggest that increased oxidative stress contributes to endothelial dysfunction in patients with CAD and that endothelial dysfunction may respond to antioxidant therapy (Levine, et al., 1996).

The other clinical trial investigated the effects of moderate-term oral antioxidant vitamin supplementation on the endothelial function of smokers. They selected young male smokers to test the effects of a usual dosage of oral antioxidant vitamins C and E on vascular endothelial function. They studied the effects of the combined oral supplementation with vitamin C (1.0 gm/day) and vitamin E (500 mg/day) on brachial artery FMD by using high-resolution ultrasound. They performed an FMD study 3 times: before vitamin supplementation, after 25 days of vitamin supplementation, and 4 weeks after the cessation of the vitamin supplement. They concluded that the oral antioxidant vitamin supplement significantly restored FMD; however, this effect disappeared 4 weeks after the vitamin supplementations ended. The antioxidant supplementation was found to improve the endothelial function in chronic smokers (Takase, Etsuda, Matsushima, Ayaori, Kusano, Hamabe, et al., 2004).

Similar research aimed to assess the degree to which endothelial dysfunction could be corrected with acute vitamin C application in the radial artery of patients with CAD and chronic heart failure caused by either ischemic cardiomyopathy (ICM) or dilated cardiomyopathy (DCM). This study population consisted of male patients ≤ 70 years old with documented CAD and preserved left ventricular function (≥ 60%), patients with HF
caused by either ICM or DCM (LVEF ≤ 35%), and 5 healthy individuals. They concluded that acute vitamin C administration restored peripheral endothelial function in patients with CAD to normal values, whereas endothelial function remained attenuated in HF, in particular in patients with DCM. These results suggest that in patients with HF, factors other than oxidative stress (such as cytokines) contribute to pathological endothelial function (Erbs, Gielen, Linke, Mobius-Winkler, Adams, Baither, et al., 2003).

Nightingale et al. (2003) evaluated the long-term and short-term effects of vitamin C on baroreceptor sensitivity (BRS). They hypothesized that vitamin C increases NO bioavailability by directly reducing the inhibitory effect of ROS on baroreceptor nerve endings. They allocated 55 HF patients and 22 controls randomly to receive a single intravenous injection of vitamin C (2 gm) or a placebo. In addition, 45 HF patients were randomly allocated to receive a 4-week course of oral vitamin C (4 gm/day) or placebo. They found that there was no improvement in BRS in chronic HF patients given chronic oral vitamin C. Thus, acute intravenous vitamin C improved BRS in HF patients.

Raitakari et al. (2000) also suggested that oral vitamin C therapy improves endothelial dysfunction in the short term in healthy young smokers, but it has no beneficial long-term effect, despite sustained elevation of plasma ascorbate levels. Piccirillo et al. (2003) also suggested that acute administration of vitamin C at high doses improves BRS and vagal sinus modulation in patients with chronic HF.

Hornig et al. (1998) investigated both the immediate and long-term effects of antioxidant vitamin C on flow-dependent dilation in patients with chronic heart failure.
Fifteen patients with HF in NYHA functional class III with echocardiographic signs of cardiomegaly and eight healthy volunteers were studied. Vascular effects of the vitamin C and placebo were determined at rest and during reactive hyperemia before and after intra-arterial infusion of N-monomethyl-L-arginine, which inhibits endothelial synthesis of nitric oxide. In 10 patients with HF, the protocol was repeated after 4 weeks of oral therapy with vitamin C or placebo. These patients were studied 24 hours after the last oral dose of vitamin C or placebo. The results of long-term vitamin C therapy show that there was no change in the LVEF and LV end-diastolic diameter as assessed by echocardiography. However, FMD was significantly increased as compared with baseline values (11.9 ± 0.10% versus 8.2 ± 0.082%, \( p < .05 \)). The portion of FMD mediated by NO was increased as compared with baseline values. They concluded that vitamin C improved FMD in patients with HF as the result of increased availability of NO. This observation supports the concept that endothelial dysfunction in patients with HF is, at least in part, due to accelerated degradation of nitric oxide by radicals.

Ellis et al. (2000) tested the effects of short-term and long-term vitamin C therapy on oxidative stress and endothelial function in patients with HF. Fifty-five patients with NYHA functional class II to IV HF and 15 control subjects were recruited in Ellis et al.’s study. Among those HF patients, there were 15 patients who had been diagnosed with ischemic heart failure, and the other 40 heart failure patients’ illness resulted from dilated cardiomyopathy. The effects of short-term (intravenous) and long-term (oral) vitamin C therapy were assessed separately in two groups of patients with HF of non-ischemic...
etiology. To test the effects of long-term vitamin C, 10 patients were given oral vitamin C (2 gm twice daily), and 10 different patients matched for NYHA status received placebo. The results showed that FMD was significantly impaired in the patients with HF versus the control subjects, but it did not differ between patients with ischemia and those with non-ischemic etiology of HF at the baseline. With regard to long-term oral treatment with vitamin C, FMD dilation increased after oral vitamin C administration (from 3.0 ± 0.018 to 8.2 ± 1.1%). But improvements in FMD after long-term vitamin C therapy did not correlate with reductions in oxidative stress. And there was no change in NYHA status during long-term vitamin C therapy as compared with placebo.

Oral supplementation with antioxidants normalized markers of oxidative stress in heart failure patients, increased FMD, but did not appear to have any effect on functional indices or quality of life (Ellis, et al., 2000; Ghatak, et al., 1996; Keith, et al., 2001). Administration of vitamin C to chronic heart failure patients suppresses endothelial cell apoptosis in vivo, which might contribute to improvements in HF (Rossig, et al., 2001).

Steptoe et al (2004) carried out a randomized trial comparing brief behavioral counseling with nutritional education and counseling to increase vitamin C and E from vegetables and fruits. The 271 subjects were randomly recruited from the patient registers of a primary-health center in South London. The nutrition education counseling group received education about the importance of increasing consumption, emphasizing the beneficial effects of nutritional constituents and the way these act biologically to maintain health. Physical and mental health status (SF-36) and self-rated health were assessed at
baseline, 8 weeks and 12 months. Non-fasting venous samples were analyzed to determine plasma vitamin C, vitamin E, and β-carotene. They found there was a difference in fruit and vegetable consumption and in biomarkers. They suggested that increased fruit and vegetable intake and plasma vitamin levels may stimulate beneficial changes in physical health status.

Summary

This chapter reviewed research findings related to inflammatory process, endothelial function, cardiac performance, exercise capacity, and functional status, especially in health-related quality of life and depression among patients with chronic heart failure. It also mentioned findings from antioxidant supplementation in clinical trials. Therefore, the knowledge gap was identified regarding the current study’s design and potential outcomes. Earlier studies focused primarily on the pathophysiologic process of inflammation, but few demonstrated the relations among cytokines and psychosocial variables. None of the previous studies put all of the concepts together, and none of them established a bio-psycho-social model specifically for chronic heart failure patients.
CHAPTER 3
RESEARCH METHODOLOGY

The methodology described in this section includes the following elements. First, the research design is a randomized controlled trial with a crossover design to explore the effects of antioxidant supplementation on endothelial function, cardiac performance, exercise capacity, and functional status in patients with chronic heart failure. Second, the descriptors, intervention and dependent variables outlined in the framework (Figure 1-1) are defined both theoretically and operationally. Third, the sample and sampling procedures are described. The sample was composed of subjects who have been diagnosed with chronic heart failure, and who meet the inclusion criteria. In order to obtain this sample size, the subjects were recruited from a tertiary teaching hospital in Taipei County, Taiwan. Fourth, the procedures for standardizing the administration of instruments are addressed. Finally, the statistical analyses to be used are specified. The findings will be analyzed using generalized linear model (GLM) repeated measures and a combination of descriptive and inferential statistics.

Research Design

A crossover, randomized controlled trial (RCT) study was used to identify the effects of antioxidant supplementation on systemic inflammatory process, endothelial function, cardiac performance, exercise capacity, and functional status in patients with
chronic heart failure (see Figure 1-1).

The clinical trial is defined as a prospective study comparing the effect and value of intervention(s) against a control in human beings. The most definitive kind of clinical trial is the randomized controlled trial, which requires that group assignment be the result of some random process beyond the control of both the investigator and the subject (Schechtman, 2003a). A properly planned and executed clinical trial is a powerful experimental technique for assessing the effectiveness of an intervention. All the randomization procedures described avoid selection bias by not being predictable (Friedman, et al., 1998).

Crossover trials are potentially extremely efficient designs for studying the effect of treatment (Senn, 2003). In a crossover trial, subjects are randomly allocated to study arms where each arm consists of a sequence of two or more treatments given consecutively (Sibbald, & Roberts, 1998). The crossover design represents a special situation where there is not a separate comparison group. In effect, each subject serves as his/her own control. Also, since the same subject receives both treatments, there is no possibility of there being a covariate imbalance. Ideally in a crossover design, a subject is randomly assigned to a specific treatment order. In the current study, it was the simplest model AB/BA study; some subjects received the antioxidant supplementation and standard therapy first, followed by the placebo and standard therapy (AB). Others received the placebo and standard therapy first, followed by the antioxidant supplementation and standard therapy (BA). All concepts were measured using standard
tests and instruments. Removing patient variation in this way makes crossover trials potentially more efficient than similar sized, parallel group trials in which each subject is exposed to only one treatment.

A crossover design has the advantage of eliminating individual subject differences from the overall treatment effect, decreasing variance, increasing precision, and reducing costs, thereby enhancing statistical power (Kuehl, 2003; Senn, 2003). The principal drawback of the crossover trial is that the effects of one treatment may "carry over" and alter the response to subsequent treatments. If the washout period is too short, then the latter treatment period will be biased by carry-over effects from the earlier treatment (Piantadosi, 1997; Senn, 2003). Consequently, in crossover studies the washout periods between the administration of test and reference products should usually be more than 5 times the elimination half-life of the parent drug or active metabolites (Senn, 1993). The metabolic half-life of ascorbic acid has been reported to be between 60 to 206 minutes (Hickey, & Roberts, 2004; Kustermann, 1998). Vitamin C is rapidly exerted from body in urine when the plasma level exceeds renal threshold of 1.4 mg/dl (Shannon, 1995). Treatment periods were separated by a 2-week washout period (14 days), in which patients used a placebo and standard therapy, which is long enough to allow the effects of a treatment to wear off.

The causal modeling technique requires an explanation of the relationships between variables, specification and justification of the ordering of the variables, and identification of influential variables outside the model. The data were analyzed using
Generalized Linear Models (GLM) repeated-measures ANOVA (RM-ANOVA). All the hypotheses were individually examined to determine if they are statistically significant.

Selection of Subjects

Inclusion and Exclusion

The inclusion criteria were as follows: (a) documented diagnosis of HF for longer than 3 months; (b) NYHA functional II and III; (c) between 40 and 80 years of age, because most patients under 40 who suffer from HF originate from cardiomyopathy rather than ischemia; (d) literate in Chinese; and, (e) no reports of mental impairment.

The exclusion criteria were as follows: (a) being allergic to ascorbic acid; (b) regularly using a vitamin C supplement, for the principal investigator will hardly be able to identify the effects of antioxidant supplementation; (c) having serum creatinine levels greater than 3.5 mg/dl; (d) being diagnosed with severe vascular disease, uncontrolled hypertension, a history of cardiac arrest or life-threatening arrhythmia within the preceding three months; (e) having experienced an acute myocardial infarction, acute coronary syndrome, or stroke within the preceding three months; (f) having had previous coronary artery bypass grafting surgery (CABG) or valve replacement within the preceding three months or the likelihood of requiring such a procedure during the study period; (g) having a left ventricular assist device or automatic implanted cardiovertor-defibrillator device; (h) having mechanical ventilator support; and, (i) women if they are pregnant, nursing, or of childbearing age and not using an effective
method of contraception. This is because ascorbic acid will cross the placenta, and cord blood concentrations are generally 2 to 4 times the concentration in maternal blood. Also, ascorbic acid is distributed into the mother’s milk.

**Rationale for Sample Size**

The research hypothesis was that the effects of using antioxidant supplementation will be better than not using antioxidant supplementation on endothelial function in patients with chronic HF. This was a *RM-ANOVA* test at 3 different time points. In this study, the \( \alpha \) level was set as .05 because if we are claiming that there is a relationship or an effect when, in fact, there is none, type I error can mislead investigators about the evidence to be accounted for and thus seriously hamper theory development in effectiveness research.

Power analysis is an essential component of a statistical test because it represents the probability of rejecting the null hypothesis when it is false (Schechtman, 2003b). That means that power is the probability of a correct statistical conclusion when the null hypothesis is true (Lipsey, 1990). Closely related to statistical power is the false-negative rate, which is often referred to as a Type II error or \( \beta \) error. The false-negative rate equals 1 minus the power. This probability is computed under the assumption that the treatment difference or strength of association equals the minimal detectable difference. Cohen (1988) suggested \( \beta = .20 \) as a reasonable value for general use. A type II error in this effectiveness research could lead to the conclusion that an effective treatment has falsely
been discredited. In applied treatment effectiveness research it may often be desirable to keep the likelihood of such an error low even at the expense of accepting an increased probability of type I error (Lipsey, 1990). It will be serious when we fail to reject null hypothesis, so $\beta$ was set at .10 in the current study. Thus, power was determined at .90.

Effect sizes (ES) are measures of the strength of effects. ES are especially important because they allow us to compare the magnitude of experimental treatments from one experiment to another. In this study, the effect size measures how much the antioxidant supplementation will improve endothelial function. Cohen (1988) defined $d'$ as the difference between the means, $\mu_1 - \mu_2$, divided by standard deviation, $\sigma$. In practice, the pooled standard deviation, $\sigma_{pooled}$, is commonly used. Cohen (1988) also defined the pooled standard deviation as being the root mean square of the two standard deviations (Table 3-1). The ES correlation ($r_{y\lambda}$) can be computed from Cohen’s $d'$ (Becker, 2000; Lipsey, 1990). These formulas were used to compute Cohen’s $d'$ and ES from published data (Table 3-2).

The sample size is decided by $\alpha$, effect size, and statistical power. Descriptive data can be obtained from previous, similar studies which have identified the effects of vitamin C supplementation on endothelial function in cardiovascular disease patients. After the ES was determined by each previous study, the various Effect Sizes are averaged across the studies; that is, ES (average effect size) = $\Sigma$ES / N (Grissom, & Kim, 2005; Lipsey, 1990). The average ES (grand mean) is .691, following the Power Chart (Lipsey, 1990 page 92), and the estimated sample size is 76.
Table 3-1
Formulas regarding mean, SD, and effect size

<table>
<thead>
<tr>
<th>Formula 1</th>
<th>Cohen’s $d = (\mu_1 - \mu_2) / \sigma_{pooled}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\mu_1$: mean of the treatment population</td>
</tr>
<tr>
<td></td>
<td>$\mu_2$: mean of the control population</td>
</tr>
<tr>
<td></td>
<td>$\sigma_{pooled}$: the common (pooled) standard deviation of the two groups</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Formula 2</th>
<th>For $n_t = n_c$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$S_p = [(s_t^2 + s_c^2) / 2]^{1/2}$</td>
</tr>
<tr>
<td></td>
<td>$s_t^2$ and $s_c^2$ are the variances for the treatment and control groups</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Formula 3</th>
<th>For $n_t \neq n_c$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$S_p = {(n_t - 1)s_t^2 + (n_c - 1)s_c^2) / [(n_t - 1) + (n_c - 1)] }^{1/2}$</td>
</tr>
<tr>
<td></td>
<td>$s_t^2$ and $s_c^2$ are the variances for the treatment and control groups</td>
</tr>
<tr>
<td></td>
<td>$n_t$ and $n_c$ are the sample sizes for the treatment and control groups</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Formula 4</th>
<th>$r_{yx} = d / [(d^2 + 4)]^{1/2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r_{yx}$: the effect size correlation</td>
</tr>
</tbody>
</table>

Table 3-2
Power analysis for sample size justification

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>$M1 \pm SD1$</td>
<td>15.7±4.9</td>
<td>8.2±0.082</td>
<td>8.0±0.7</td>
<td>10.6±2.1</td>
<td>2.8±2.0</td>
<td>3.0±0.018</td>
</tr>
<tr>
<td>$M2 \pm SD2$</td>
<td>21.6±5.4</td>
<td>11.9±0.10</td>
<td>12.9±1.1</td>
<td>13.4±2.6</td>
<td>3.9±3.1</td>
<td>8.2±0.09</td>
</tr>
<tr>
<td>$n1$</td>
<td>8</td>
<td>15</td>
<td>16</td>
<td>9</td>
<td>20</td>
<td>55</td>
</tr>
<tr>
<td>$n2$</td>
<td>8</td>
<td>15</td>
<td>16</td>
<td>9</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>$SD (S_p)$</td>
<td>5.16</td>
<td>0.091</td>
<td>0.921</td>
<td>2.363</td>
<td>2.609</td>
<td>0.015869</td>
</tr>
<tr>
<td>Cohen’s $d$</td>
<td>-1.144</td>
<td>-40.46</td>
<td>-5.315</td>
<td>-1.185</td>
<td>-0.422</td>
<td>-82.540</td>
</tr>
<tr>
<td>$r_{yx}$ (ES)</td>
<td>-0.497</td>
<td>-0.999</td>
<td>-0.936</td>
<td>-0.510</td>
<td>-0.206</td>
<td>0.999</td>
</tr>
<tr>
<td>Average ES (Grand Mean)</td>
<td>0.691</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
On the other hand, in repeated-measures designs, N refers to the number of observations rather than the number of people (Murphy & Myors, 2004). In a two-group repeated-measures design, the real number of subjects will be less than half the number of observations. Louis et al (1984) suggested that a crossover design would require only half the number of subjects to produce the same precision as a parallel comparison. James et al. (1985) reviewed 59 crossover designs; they concluded that if the studies had been designed using parallel or non-crossover designs, 2.4 times as many participants would have been needed. Piantadosi (1997) also suggested that a crossover design needs only half as many subjects as an independent group to yield the same precision in the estimated treatment difference. Therefore, this was a crossover and repeated-measures design, and it would need 19 subjects in each group (38 total) for this study to reach a power of 0.9 when \( \alpha \) is set at 0.05 with a one-tailed test.

**Setting**

The study took place in a tertiary-care teaching hospital in Taipei, Taiwan, R.O.C. The principal investigator recruited subjects from Cardinal Tien Hospital, Taipei, Taiwan. Cardinal Tien Hospital, adjacent to the capital of Taiwan, is locally and nationally recognized for patient care, teaching and research. Cardinal Tien Hospital is a 1200-bed capacity medical complex offering 24-hour emergency care with separate facilities for adults and children. Patients were recruited in the outpatient department because their
routine follow-up appointments were likely to be on an out-patient basis.

**Empirical Indicators and Their Measurement**

A structured demographic/medical information sheet (Appendix A) was designed to collect information about age, gender, marital status, employment status, education level, smoking history and alcohol history, medical history, current medications and laboratory results. This demographic/medical information sheet was administered only at the first interviews (Time Point 1). The patients at the 4th (Time Point 2) and 10th (Time Point 3) week were given the same instruments except for the demographic/medical information sheet.

**Sample descriptors**

*Age*

Age was measured in number of years and is calculated from the date of birth of the subject as written in the informed consent to the current date. The variable for age is in the metric of years. Age was a continuous variable in this study.

*Gender*

Gender was taken from the medical chart. The variable for gender was dichotomized into male and female.

*Body Mass Index (BMI)*

The BMI was calculated as a ratio of body weight (kg) to body height square (m²).
BMI is an interval scale that reflects part of the nutrition status.

**NYHA Classification**

The New York Heart Association (NYHA) functional classification is a commonly used measure of the influence of symptoms on the functioning of cardiac patients. It is divided into four levels of functional ability and patients were asked to classify themselves in one of the four levels (Appendix B). Each subject is classified into a NYHA class based on the subject’s symptoms, observed functional capacities, and the information gathered from the medical records. It was recorded using the subject’s verbal responses regarding daily activities. The level was evaluated by a physician and the principal investigator in the cardiovascular outpatient clinic; a third expert was consulted when it is necessary. It is an ordinal scale that describes physical ability in HF patients.

**Charlson Co-morbidity Index (CCI)**

The CCI contains 19 categories of co-morbidity (Appendix C), which are primarily defined using ICD-9 diagnosis codes. Each category has an associated weight which is based on the adjusted risk of one-year mortality. The overall CCI score reflects the cumulative increased likelihood of one-year mortality; the higher the score, the more severe the burden of co-morbidity. Data about co-morbidity and the number of diseases were collected from the patients’ medical records and from questions asked directly to the patients. This index was used to determine if comorbid conditions had any influence on the outcomes of the study.
Intervention: Antioxidant Supplementation

Pathophysiologic correlations and mechanistic plausibility suggest that TNF-α contributes to the syndrome of chronic heart failure. If anti-TNF-α therapies could be shown to modify the natural history of this disease, they would constitute an important new treatment strategy. Keith et al. (1998) conducted a study that showed that oxidative stress increases in patients with heart failure. Fifty-eight patients with HF and 19 control subjects were studied. They revealed that the level of vitamin C decreased, but vitamin E levels were maintained. These data demonstrated a progressive increase in free radical injury and encroachment on antioxidant reserves with the evolution of HF.

Vitamin C is the antioxidant supplementation used in this study. A review of the two published controlled trials of long-term vitamin C administration in HF patients (Ellis, et al., 2000; Nightingale, et al., 2003), suggested that 2000 mg twice a day (4000 mg per day) can suppress endothelial cell apoptosis in vivo.

Since ascorbic acid is a water-soluble vitamin, toxic levels are not built up or stored in the body, and any excess is lost mostly through urine. While vitamin C is generally non-toxic, if mega doses (more than 2,000 mg daily) are taken, gastrointestinal problems may appear, but these will normalize when the intake is cut or reduced. To determine a level where a person might experience discomfort is difficult, since some people can easily stomach up to 25,000 mg per day, while others start having a problem at 600 or 1,000 mg (Hickey, & Roberts, 2004). Some people using mega-dose therapy of vitamin C may have side effects such as gastrointestinal complaints including diarrhea,
nausea and abdominal cramps. These side effects normally stop as soon as high potency intake is reduced or stopped. The side effects of mega doses of vitamin C supplementation were monitored in every visit. The checklist (Appendix D) was developed for assessing the side effects.

Dependent Variables

Proinflammatory Cytokines

Tumor necrosis factor-alpha (TNF-α) will be measured in this study as an indicator of the degree of systemic inflammation. TNF-α is an enzyme-amplified sensitivity immunoassay (EASIA) that is based on monoclonal antibodies against tumor necrosis factor-alpha specific sites (Immulite®, DPC, United Kingdom). At each of the three respective time points (at 0, the 4\textsuperscript{th}, and 10\textsuperscript{th} weeks), blood samples were drawn from an antecubital vein after 30 minutes of resting, and then the blood was transferred into 10-ml tubes without additives and immediately stored on ice for 30 minutes to allow clotting. Then the blood was centrifuged at 3500 rpm for 10 minutes at 4°C until the serum is frozen, and it was stored at -80°C. TNF-α levels were tested by an automated system for the measurement of cytokines. All procedures were run by laboratory director of Cardinal Tien Hospital.

Endothelial Function

Endothelial function was measured by high-frequency ultrasonographic imaging of
the brachial artery to assess endothelium-dependent flow-mediated vasodilation (FMD). This technique provokes the release of nitric oxide, resulting in vasodilation that can be quantified as an index of vasomotor function. It was performed 3 times (at 0, the 4th, and 10th weeks) by the same experienced cardiologist using a high-resolution, 7.5-MHz, vascular ultrasound transducer (Agilent/HP Sonos 7500; L7540 linear transducer, Hewlett-Packard, Andover, M.A., U.S.A.). The diameter of the right brachial artery was measured after at least 10 minutes of supine rest, and after cuff deflation completing suprasystolic compression (50 mmHg above systolic pressure) of the right arm for 5 minutes and after sublingual application of 0.8 mg nitroglycerin (NTG). The longitudinal image of the artery was recorded at baseline, continuously from 30 seconds before 2 minutes after cuff deflation. The arterial flow velocity is measured by means of a pulsed Doppler signal at a 70° angle to the vessel. Flow was determined by multiplying the arterial cross-sectional area ($\pi r^2$) by the Doppler flow velocity. Diameter and flow were measured; absolute changes and percentages of change were calculated in this study. There were interval scales that describe endothelial function.

Cardiac Performance

Cardiac performance was measured by two-dimensional echocardiography in this study. Echocardiography has been validated with radionuclide, computer tomography, and angioplasty (Goundstroem, Huikuri, Korhonen, Ikaheimo, Heikkila, & Takkunen, 1987; Rovai, Nissen, Elion, Smith, L'Abbate, Kwan, et al., 1987). Moreover,
echocardiography has been shown to be efficient for repeated-measures of ejection fraction and it has reached 99% for inter-technician reliability (Kuecherer, Kee, Modin, Cheitlin, & Schiller, 1991).

Echocardiography were performed 3 times (at 0, the 4th, and 10th weeks) using an Agilent/HP Sonos 7500 with a 2.5 MHz Doppler Probe (Hewlett-Packard, Andover, M.A., U.S.A.). Images were acquired in standard views and LVEF and other parameters were calculated through a standard echocardiogram using Simpson’s method. All 2-D echocardiography were performed by the same qualified cardiologist to maintain consistency and reliability in reading and judging the results.

**Exercise Capacity**

Exercise capacity was measured using the 6-minute distance walk test (6MWT). Because of its simplicity, the 6MWT has been considered to be suitable for the routine assessment of the severity of HF, for prognostication, and for evaluation of therapeutic responses. Patients were instructed to walk a 10-m course from end to end at a comfortable pace while attempting to cover as much ground as possible. After 6 minutes, the distance covered was measured to the nearest meter. The length of the distance that subjects walk is the interval scale that describes the subjects’ exercise capacity. All subjects were informed of the purpose, methods, and use of the 6MWT results. The method of Guyatt et al. was used to administer the 6MWT and included: (1) practicing the 6MWT walk test on the same day of the test itself; (2) informing the patient to
ambulate as far as possible in 6 minutes; (3) allowing the patient to set the pace of ambulation with rest stops as needed; (4) evaluating the patient's cardiopulmonary response by using a wheeled metabolic cart; and, (5) offering no special encouragement during the test.

The heart rate and BP were recorded before, during (at minutes 2 and 4), and after the 6MWT. The total distance ambulated in meters during the 6MWT and the number of rest stops during the test were also recorded. In this study, the 6MWT was conducted in a passageway with a length of 10 meters. Subjects were given standard instructions and told the time at each minute. All subjects were allowed to rest in either a sitting or standing position during the test period. The total distance was used in interval scales that present the ability of exercise capacity.

**Functional Capacity**

Functional status was assessed using three instruments: (a) the Medical Outcomes Study Short-Form-36 Health Survey (SF-36) was used to measure health-related quality of life; (b) the Minnesota Living with Heart Failure Questionnaire (MLHFQ) was used to measure disease-specific quality of life in patients with heart failure; and, (c) the Center for Epidemiologic Studies Depression Scales (CES-D) was used to identify depressive status in this study.

*Medical Outcomes Study Short-Form-36 Health Survey (SF-36)*
Description.

The SF-36 developed by the Medical Outcomes Study (MOS) group has been selected for use in this study. SF-36 is one of the best-known and widely used questionnaires among experts for measuring generic health status or HRQOL in the general population. During its development, extensive and thorough psychometric testing was performed not only on the general population but also on diverse disease groups, and comparisons with other established instruments were also made (McHorney, Ware, Rogers, Raczek, & Lu, 1992). The objective of the SF-36 is to satisfy the minimum psychometric standards necessary for group comparisons involving generic health concepts, and it is not specific to any age, disease, or treatment group, and can be administered to anyone over the age of 14. The SF-36 questionnaire is a 36-item, self-administered scale and the questions are designed to be easy to understand and relevant to most people’s lives. This instrument is used to obtain a comprehensive, brief, and psychometrically sound health status survey. The SF-36 questionnaire measures eight scales and two components of the health concept: physical functioning (PF), role limitations due to physical problems (RP), bodily pain (BP), general health (GH), vitality (VT), social functioning (SF), role limitations due to emotional problems (RE), and mental health (MH). The subscale scores are combined to produce 2 summary scores called the physical and mental component scores (PCS and MCS). The composite scores range from 0 to 100 with higher scores meaning better quality of life.

Validity and reliability.
From the previous research, the overall internal consistency and reliability coefficients range from 0.76 to 0.95. Most of the Cronbach’s alphas in the eight scales of the SF-36 exceed 0.8, except for social functioning. The physical functioning dimension consistently exceeded 0.90. And in the research on all of the dimensions, 91-98% of cases lay with the 95% confidence interval in Chinese version (Li, Wang, & Shen, 2003; Tseng, Lu, & Gandek, 2003; Yu, Coons, Draugalis, Ren, & Hays, 2003).

*Version and translation.*

The form only takes 5 to 10 minutes to complete. Its original language is English for the USA, and it has been translated into many languages and adapted in 29 countries. It has been translated into such languages as French, Swedish, German, Dutch, Italian, Spanish, Japanese, Korean, and so on. Even the Chinese version has Chinese for Hong-Kong, Chinese for Taiwan, Chinese for Singapore, and Chinese for the US, and the principal investigator has identified the Chinese for Taiwan version for use in this study. Furthermore, the SF-36 has been replicated across 24 different patient populations with various socio-economic situations and diagnoses (Appendix E for the English version and Appendix F for the Chinese version).

*Rationale for use.*

The SF-36 questionnaire has been selected for use in this study for the following reasons: (1) the validity of the SF-36 has been rigorously evaluated; (2) the Chinese (Taiwan) version of the SF-36 is readily available; (3) the SF-36 is suitable for the normal population, and the data from the SF-36 for a variety of groups in the normal
population are available for comparison; (4) the SF-36 provides a comprehensive health survey; (5) the data of the physical and mental components in the SF-36 could clearly demonstrate the outcome of physical and mental health; and, (6) the SF-36 can be completed at clinics in 5-10 minutes, so it will not be a large burden for HF patients.

**Minnesota Living with Heart Failure Questionnaire**

*Description.*

The Minnesota Living with Heart Failure Questionnaire (MLHFQ), developed by Dr. Thomas Rector, is a measure of the patients’ perceptions of the effects of HF on their lives. This is a validated, disease-specific, self-administered questionnaire that has 21 items related to various aspects of life focusing on signs and symptoms of HF, and it comprehensively covers physical, economic and psychological impairments that patients often relate to their HF. Subjects are asked to rate the extent to which their heart failure has prevented them from living as they wanted to during the last month using questions rated on a scale of 0 (no effect) to 5 (very much). The questionnaire is scored by summing the responses to all 21 items, thus resulting in a score from 0 to 105 with a higher scores reflecting poorer quality of life. This response format was chosen to be consistent with the concept of HRQOL and allows each individual to weigh each aspect using a common scale. Therefore, one can look at which items had the most effect, and the sum of responses reflects the overall effects of HF and treatments on the individual’s quality of life. The instrument only takes 5 to 10 minutes to complete. Its original
language is English for the USA, and it has been translated into over 30 languages.

*Validity and reliability.*

The total MLHF score is highly reliable as demonstrated by estimates of Cronbach’s alpha coefficient ($\alpha$) and the correlation ($r$) between repeated baseline assessments reported from several studies (Bennett, et al., 2002; Gorkin, Norvell, Rosen, Charles, Shumaker, McIntyre, et al., 1993; Rector, & Cohn, 1992; Rector, Johnson, Dunkman, Daniels, Farrell, Henrick, 1993; Riegel, Moser, Glaser, Carlson, Deaton, Armola, 2002). This instrument has demonstrated satisfying criterion (Bennett, et al., 2002; Gorkin, et al., 1993; Ni, Toy, Burgess, Wise, Nauman, Crispell, 2000; Rector, Kubo, & Cohn, 1993; Withman, Crighton, mcMurdo, 2006) and construct validity (Heo, Moser, Riegel, Hall, & Christman, 2005a; Heo, Moser, Riegel, Hall, & Christman, 2005b; Hulsmann, Berger, Sturm, Bojic, Woloszczuk, Bergler-Klein, 2002; Middel, Bouma, de Jongste, van Sonderen, Niemeijer, Crijns, 2001).

*Version and translation.*

Although numerous disease-specific quality of life questionnaire measures have been developed, none of them already had a Taiwanese (Chinese) version for identifying and evaluating HRQOL related to HF in Taiwan. This questionnaire was translated and adapted following Brislin’s steps for translation (Appendix G for the English version and Appendix H for the Chinese version).

*Scoring*

The questionnaire is scored by summing the responses to all 21 questions. In
addition, a physical dimension score (items 2, 3, 4, 5, 6, 7, 12, 13) and emotional
dimension score (items 17, 18, 19, 20, 21) have been identified by factor analysis and can
be scored by simple summation to further characterize the effect of heart failure on a
patient’s life. Several methods to impute missing data are discussed, and multiple
imputation using completed questions and perhaps other study variables to predict
missing responses should be considered (Fayers, 1998; Raghunathan, 2004). If a missing
response is not imputed, the item will be eliminated from that person’s.

*Center for Epidemiologic Studies Depression Scales (CES-D)*

*Description.*

The CES-D questionnaire was developed by the Center for Epidemiologic Studies
(Radloff, 1977), and it is a 20-item, self-administered depression scale developed to
identify depressive status in the general population. The object of the CES-D is to assess
the frequency and severity with which symptoms of depression are experienced in the
general population. This questionnaire covers the major components including depressed
mood, feelings of guilt and worthlessness, feelings of helplessness and hopelessness,
psychomotor retardation, loss of appetite, and sleep disorders. This scale does not contain
subscales. The items refer to the frequency of symptoms during the past week, and each
question uses a four-point Likert (0-to-3) response scale and includes: ”*Rarely, or none if
the time* (less than one day),” ”*Some or little of the time* (1-2 days),” ”*Occasionally or a
moderate amount of time* (3-4 days)” and “*Most or all the time* (5-7 days).” For each item,
the respondent selects the value that best describes how frequently the experience occurred during the previous week.

*Validity and reliability.*

In the original study, Ratloff (1977) reported a high internal consistency reliability coefficient among the items (ranging from 0.84 to 0.90). The reliability of this instrument is pretty high, and its validity had been established. Later studies also reported high internal consistency among the 20 items and highly recommended use of the total score (Miller, Malmstrom, Joshi, Andresen, Morley, & Wolinsky, 2004). A Chinese version tested for an alpha value of 0.92 (Rankin, Galbraith & Johnson, 1993).

*Version and translation.*

Its original language is English for the USA, and it has been translated into many other languages. It has been translated into such languages as French, Danish, German, Dutch, Italian, Spanish, Swedish, Japanese, Portuguese, and so on. The Chinese (Taiwan) version of the CES-D is readily available and showed good validity and reliability in previous research (Appendix I for the English version and Appendix J for the Chinese version). The Chinese version of CES-D is in the public domain.

*Scoring and interpretation.*

Four questions are phrased positively (questions 4, 8, 12, and 16), and their scores are reversed by subtracting the score from 3. Then the scores for all of the questions are summed to provide an overall score range from 0 to 60. A higher score indicates greater depression. A total score is calculated as the sum of responses to the 20 items. If more
than 10 items on a measure are missing a response, a total score is not calculated.
Measures with all responses missing are not scored.

**Procedures**

A crossover, randomized controlled trial was used in this experimental study.
Measurements occurred at three time periods, T1 (baseline) at the beginning of the study,
T2 at the 4th week, and T3 at the 10th week of the subject’s participation in the study.

**Subject Recruitment**

The principal investigator (PI) had a list of HF patients from a physician’s referrals
in the outpatient department. Individuals who meet the study inclusion criteria were
identified by the cardiologist and were referred to the PI using a prepared list. Based on
the cardiologist’s clinical schedule, the PI visited the cardiology outpatient department
four times and checked the prepared list of eligible subjects. Potential subjects meeting
the study criteria were approached by the PI in the cardiology clinic’s waiting area after
their appointment. The PI explained the purpose and procedure of this study to patients
and asked for their participation. Those patients willing to participate signed an informed
consent form (Appendix K for the English version and Appendix L for the Chinese
version). A copy of the consent form was given to all participants for their reference. The
PI asked and recorded the subject’s phone number and address and make an appointment
for a baseline evaluation day. Before each study visit, the PI contacted the subjects by
telephone to remind them of their scheduled appointment.

Data Collection

Time point 1 was considered the baseline measure. The subject’s medical chart was reviewed by the PI to collect demographic data and medical history. When subjects went back to the hospital for assessment, the PI drew blood and accompanied the subject to the echocardiography room for evaluating cardiovascular function, and then the investigator administered the exercise test in a passageway with a length of 10 meters near the echocardiography room. After finishing these tests, the PI provided a simple breakfast and let the subjects rest. The PI hand-delivered the booklet of questionnaires to these patients. After being given a brief explanation of the questionnaires, these subjects were asked to complete the questionnaires on health status.

The same measures were obtained at Time Points 2, and 3. The PI provided pens and ensured that the subjects could read the questionnaires. Before they went home, the PI gave them vitamin C or placebo in a bottle, confirmed their next scheduled visit, and made sure that they understood the dosage and schedule for the medication (Figure 3-1).
Figure 3-1. Study Design
Random Assignment

A total of 37 subjects were recruited from the cardiovascular outpatient department. All potential subjects were prospectively randomized to one of two groups (group I and group II). Permutated block randomization was performed using the SAS 9.0 software program (Cary, NC) to avoid a serious imbalance in the number of participants assigned to each group, and to prevent selection bias, avoid violating statistical theory, and increase the power of the study (Altman, 1991; Friedman, et al., 1998). In this study, blocks of 6 subjects per stratum were used (Appendix M). Eligible subjects were allocated to Group I (AB) or Group II (BA) based on the SAS generating sequence.

If a subject was allocated to Group I (AB sequence), he/she received vitamin C 2000 mg twice daily (4000 mg daily) for four weeks, followed by a placebo for six weeks. If a subject was allocated to Group II (BA sequence), he/she received a placebo for six weeks, followed by vitamin C 2000 mg twice daily (4000 mg daily) for four weeks. No matter which group subjects were assigned to, all subjects took both vitamin C and a placebo. The difference was the sequence in which the subjects received.

Protection of Human Subjects

This proposal was reviewed and granted approval by the Institutional Review Board (IRB) at University Hospital of Cleveland (# 04-05-01) and Cardinal Tien Hospital in Taiwan (CTH-94-1-2A03). The purpose and description of the study were clearly
explained to all eligible subjects, and formal informed consents were obtained. Participants were assured that their participation in this study was voluntary and that they could withdraw from the study at any time. Participants were informed that refusal to participate or withdrawal from the study did not affect or compromise any aspect of their care received by nurses or physicians.

Confidentiality was protected throughout the whole study. The group assignment and identity of each patient were kept confidential throughout the study, data analysis, and preparation of research reports. Additionally, data were reported in aggregate form and no individual will be identified in any publication or presentation of the study.

The PI kept the original consent forms in a locked file cabinet to assure the confidentiality of the participants. Hard copies of all data collection forms were stored in a locked file in the principal investigator’s office for the duration of the study. All data were converted to digital form and were entered in the computer with the use of a unique identification number. For security reasons, the computer has a set password and no one except the PI knows the password. The original medical records and questionnaires were locked in a secure place. The data stored for 5 years and then they will be destroyed completely.

Data Analysis

A total of 37 subjects were recruited for the study. All data were entered in the computer system and were checked for accuracy by visual inspection.
Descriptive statistics were used to summarize the subjects’ demographic, clinical and psychosocial characteristics. Means and standard deviations were calculated for all continuous variables, while frequencies and percentages were computed for the categorical variables. The histogram, skewness and kurtosis parameters were used to confirm normality of distribution and exam the assumptions.

Repeated-measures ANOVA were used to explore the difference between two groups and to examine the effects of antioxidant supplementation. Normality and Sphericity test were performed to ensure that relevant statistical assumptions are met. Cronbach’s alpha was calculated for the scores for each of the three questionnaires (SF-36, MLHFQ, and CES-D). All statistical analysis were done with SPSS version 13 (Chicago, IL) and SAS version 9.0.

**Summary**

This chapter presented the research design, criteria for selection of subjects, study protocol and procedures for this study. The data analysis methods were presented.
This chapter presents a description of the demographic and physiologic characteristics of the sample, psychometric evaluation of the questionnaires and the results of hypotheses testing. This chapter also presents the results of the data analyses conducted to examine the research questions for this study.

**Description of the Sample**

A total of 37 chronic heart failure patients were recruited from the cardiovascular outpatient department of Cardinal Tien Hospital, Taiwan. Forty potential subjects approached and all agreed to participate. Of the 40 subjects who signed an informed consent, 3 subjects (2 males and 1 female) had to be dropped from this study due to personal reasons yielding a completion rate of 92.5%. These remaining 37 subjects with chronic heart failure were alternately assigned to one of two treatment groups: group I (n = 19), and group II (n = 18). Subjects in group I received a daily dose of 4000 mg of vitamin C for 4 weeks, followed by a placebo for 6 weeks; subjects in group II received a placebo for 6 weeks followed by a daily dose of 4000 mg of vitamin C for 4 weeks. Measurements were occurred at three time periods, T1 (baseline) at the beginning of the study, T2 at the 4th week, and T3 at the 10th week of the patient’s participation in the study. Informed consent was obtained before participation.
Table 4-1.

*Characteristics of Subjects by Group*

<table>
<thead>
<tr>
<th>Descriptors</th>
<th>Total (n = 37)</th>
<th>Group I (n = 19)</th>
<th>Group II (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%) Mean (SD)</td>
<td>n (%) Mean (SD)</td>
<td>n (%) Mean (SD)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>24 (65%) 69 (16)</td>
<td>16 (84%) 70 (16)</td>
<td>8 (44%) 68 (16)</td>
</tr>
<tr>
<td>Female</td>
<td>13 (35%) 30 (7)</td>
<td>3 (16%) 30 (7)</td>
<td>10 (56%) 29 (6)</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>69 (16)</td>
<td>70 (16)</td>
<td>68 (16)</td>
</tr>
<tr>
<td>Body Mass Index (kg/m$^2$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time Point 1</td>
<td>30 (7)</td>
<td>30 (7)</td>
<td>29 (6)</td>
</tr>
<tr>
<td>Time Point 2</td>
<td>30 (7)</td>
<td>30 (7)</td>
<td>30 (6)</td>
</tr>
<tr>
<td>Time Point 3</td>
<td>30 (6)</td>
<td>30 (7)</td>
<td>29 (6)</td>
</tr>
<tr>
<td>NYHA Fs Classification</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>19 (51%) 2.9 (1.5)</td>
<td>8 (42%) 3.2 (1.6)</td>
<td>11 (61%) 2.4 (1.4)</td>
</tr>
<tr>
<td>III</td>
<td>18 (49%) 3.2 (1.6)</td>
<td>11 (58%) 2.4 (1.4)</td>
<td>7 (39%)</td>
</tr>
<tr>
<td>Charlson Comorbidity Index</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>22 (59%) 68 (16)</td>
<td>13 (68%) 70 (16)</td>
<td>9 (50%) 68 (16)</td>
</tr>
<tr>
<td>Prior MI</td>
<td>14 (38%) 30 (7)</td>
<td>7 (37%) 30 (7)</td>
<td>7 (39%)</td>
</tr>
<tr>
<td>Liver disease</td>
<td>6 (16%) 30 (7)</td>
<td>4 (21%) 30 (7)</td>
<td>2 (11%)</td>
</tr>
<tr>
<td>COPD</td>
<td>4 (11%) 2 (11%)</td>
<td>2 (11%)</td>
<td>2 (11%)</td>
</tr>
<tr>
<td>Current Medical Therapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diuretics</td>
<td>32 (86%) 17 (89%)</td>
<td>15 (83%) 17 (89%)</td>
<td>15 (83%)</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>17 (46%) 8 (42%)</td>
<td>9 (50%)</td>
<td></td>
</tr>
<tr>
<td>A2B</td>
<td>18 (49%) 11 (58%)</td>
<td>7 (39%)</td>
<td></td>
</tr>
<tr>
<td>CCB</td>
<td>10 (27%) 5 (26%)</td>
<td>5 (28%)</td>
<td></td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>23 (62%) 13 (68%)</td>
<td>10 (56%)</td>
<td></td>
</tr>
<tr>
<td>Digitalis</td>
<td>5 (14%) 2 (11%)</td>
<td>3 (17%)</td>
<td></td>
</tr>
<tr>
<td>Lipid-lowering agents</td>
<td>8 (22%) 5 (26%)</td>
<td>3 (17%)</td>
<td></td>
</tr>
<tr>
<td>Anticoagulants</td>
<td>14 (38%) 8 (42%)</td>
<td>6 (33%)</td>
<td></td>
</tr>
</tbody>
</table>

*Note. SD = standard deviation; NYHA = New York Heart Association; MI = myocardial infarction; COPD = chronic obstructive pulmonary disease; CCB = calcium channel blocker*
The baseline characteristics for the remaining 37 randomized subjects are presented in Table 4-1. At the beginning of the study, all subjects were classified into either NYHA Class II or III. The etiology of their heart failure was primarily ischemic heart disease. Most subjects had a number of comorbidities, including diabetes, prior myocardial infarction, and liver disease. Cardiovascular-related medical therapy was maintained throughout the study period.

**Psychometric Evaluation of Questionnaires**

*Medical Outcomes Study Short-Form-36 Health Survey (SF-36)*

Examining the validity of the SF-36 questionnaire is not necessary because a standardized Chinese (Taiwan) version is readily available. Cronbach’s alpha was used to test for internal consistency of reliability at each time points (Table 4-2, Table 4-3, and Table 4-4). The internal consistency of the SF-36 was high, as evidenced by Cronbach’s \( \alpha \) ranging from .73 to .96 over the three time points.
Table 4-2.
Reliability of Medical Outcome Study Short-form-36 (SF-36) at Time Point 1

<table>
<thead>
<tr>
<th>Scale and Subscale</th>
<th>Valid Case</th>
<th>Cronbach’s α</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Scale</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical component scores (PCS)</td>
<td>40</td>
<td>.93</td>
</tr>
<tr>
<td>Mental component scores (MCS)</td>
<td>38</td>
<td>.92</td>
</tr>
<tr>
<td>Physical function (10)</td>
<td>40</td>
<td>.93</td>
</tr>
<tr>
<td>Role function / physical (4)</td>
<td>40</td>
<td>.86</td>
</tr>
<tr>
<td>Role function / emotional (3)</td>
<td>40</td>
<td>.92</td>
</tr>
<tr>
<td>Energy / Fatigue (4)</td>
<td>39</td>
<td>.85</td>
</tr>
<tr>
<td>Emotional well-being (5)</td>
<td>39</td>
<td>.74</td>
</tr>
<tr>
<td>Social function (2)</td>
<td>40</td>
<td>.87</td>
</tr>
<tr>
<td>Body pain (2)</td>
<td>40</td>
<td>.83</td>
</tr>
<tr>
<td>General health (5)</td>
<td>40</td>
<td>.80</td>
</tr>
</tbody>
</table>

Table 4-3.
Reliability of Medical Outcome Study Short-form-36 (SF-36) at Time Point 2

<table>
<thead>
<tr>
<th>Scale and Subscale</th>
<th>Valid Case</th>
<th>Cronbach’s α</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Scale</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical component scores (PCS)</td>
<td>36</td>
<td>.92</td>
</tr>
<tr>
<td>Mental component scores (MCS)</td>
<td>37</td>
<td>.84</td>
</tr>
<tr>
<td>Physical function (10)</td>
<td>36</td>
<td>.93</td>
</tr>
<tr>
<td>Role function / physical (4)</td>
<td>37</td>
<td>.87</td>
</tr>
<tr>
<td>Role function / emotional (3)</td>
<td>37</td>
<td>.86</td>
</tr>
<tr>
<td>Energy / Fatigue (4)</td>
<td>37</td>
<td>.77</td>
</tr>
<tr>
<td>Emotional well-being (5)</td>
<td>37</td>
<td>.73</td>
</tr>
<tr>
<td>Social function (2)</td>
<td>37</td>
<td>.88</td>
</tr>
<tr>
<td>Body pain (2)</td>
<td>37</td>
<td>.80</td>
</tr>
<tr>
<td>General health (5)</td>
<td>37</td>
<td>.77</td>
</tr>
</tbody>
</table>
Table 4-4.
Reliability of Medical Outcome Study Short-form-36 (SF-36) at Time Point 3

<table>
<thead>
<tr>
<th>Scale and Subscale</th>
<th>Valid Case</th>
<th>Cronbach’s α</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Scale</td>
<td>37</td>
<td>.95</td>
</tr>
<tr>
<td>Physical component scores (PCS)</td>
<td>37</td>
<td>.91</td>
</tr>
<tr>
<td>Mental component scores (MCS)</td>
<td>37</td>
<td>.90</td>
</tr>
<tr>
<td>Subscale</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical function (10)</td>
<td>37</td>
<td>.93</td>
</tr>
<tr>
<td>Role function / physical (4)</td>
<td>37</td>
<td>.73</td>
</tr>
<tr>
<td>Role function / emotional (3)</td>
<td>37</td>
<td>.79</td>
</tr>
<tr>
<td>Energy / Fatigue (4)</td>
<td>37</td>
<td>.82</td>
</tr>
<tr>
<td>Emotional well-being (5)</td>
<td>37</td>
<td>.77</td>
</tr>
<tr>
<td>Social function (2)</td>
<td>37</td>
<td>.88</td>
</tr>
<tr>
<td>Body pain (2)</td>
<td>37</td>
<td>.78</td>
</tr>
<tr>
<td>General health (5)</td>
<td>37</td>
<td>.79</td>
</tr>
</tbody>
</table>

Minnesota Living with Heart Failure Questionnaire (MLHFQ)

The validity of the MLHFQ was established by content validity. Its content validity index (CVI) was 3.94/4. The convergent validity was also examined between the SF-36 and CES-D (Table 4-5). The total scale and subscales (physical and emotional) were highly correlated to each reference instrument. Cronbach’s alpha was used to test for reliability (Table 4-6). The internal consistency of the MLHFQ was high, as evidenced by Cronbach’s α ranging from .91 to .97 over the three time points.
Table 4-5.
*Correlation between the MLHFQ Total and Subtotal and the SF-36, CES-D*

<table>
<thead>
<tr>
<th>Scales and Subscales</th>
<th>Reference measures</th>
<th>$r$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLHFQ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical</td>
<td>SF-36 (PCS)</td>
<td>-.81***</td>
</tr>
<tr>
<td></td>
<td>NYHA Fe Classification</td>
<td>.76***</td>
</tr>
<tr>
<td></td>
<td>LVEF</td>
<td>-.68***</td>
</tr>
<tr>
<td>Emotional</td>
<td>SF-36 (MCS)</td>
<td>-.77***</td>
</tr>
</tbody>
</table>

*Note.* *p < .05, **p < .01, ***p < .000. PCS = physical component scores, MCS = mental component scores, NYHA = New York Heart Association, LVEF = left ventricular ejection fraction.

Table 4-6.
*Reliability of the Minnesota Living with Heart Failure Questionnaire (MLHFQ) at Time Points 1, 2, and 3*

<table>
<thead>
<tr>
<th>Time Points</th>
<th>Scale and Subscale (# of items)</th>
<th>Valid Case</th>
<th>Cronbach’s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time Point 1</td>
<td>Total (21)</td>
<td>40</td>
<td>.96</td>
</tr>
<tr>
<td></td>
<td>Physical (8)</td>
<td>40</td>
<td>.93</td>
</tr>
<tr>
<td></td>
<td>Emotional (5)</td>
<td>40</td>
<td>.93</td>
</tr>
<tr>
<td>Time Point 2</td>
<td>Total (21)</td>
<td>36</td>
<td>.97</td>
</tr>
<tr>
<td></td>
<td>Physical (8)</td>
<td>36</td>
<td>.91</td>
</tr>
<tr>
<td></td>
<td>Emotional (5)</td>
<td>36</td>
<td>.91</td>
</tr>
<tr>
<td>Time Point 3</td>
<td>Total (21)</td>
<td>37</td>
<td>.97</td>
</tr>
<tr>
<td></td>
<td>Physical (8)</td>
<td>37</td>
<td>.93</td>
</tr>
<tr>
<td></td>
<td>Emotional (5)</td>
<td>37</td>
<td>.95</td>
</tr>
</tbody>
</table>
Center for Epidemiologic Studies Depression Scales (CES-D)

Examining the validity of the CES-D questionnaire is not necessary because a standardized Chinese (Taiwan) version is readily available. Cronbach’s alpha was used to test for reliability (Table 4-7). The internal consistency of the CES-D was good, as evidenced by Cronbach’s $\alpha$ higher than .90 in three time points.

<table>
<thead>
<tr>
<th>Time Points</th>
<th>Valid Case</th>
<th>Cronbach’s $\alpha$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time Point 1</td>
<td>38</td>
<td>.91</td>
</tr>
<tr>
<td>Time Point 2</td>
<td>37</td>
<td>.91</td>
</tr>
<tr>
<td>Time Point 3</td>
<td>36</td>
<td>.91</td>
</tr>
</tbody>
</table>

Hypotheses Testing

Hypothesis 1

Subjects will have a decrease in pro-inflammatory cytokines as measured by a decreased TNF-$\alpha$ level while using antioxidant supplementation compared to when they not using antioxidant supplementation.

All 37 subjects had blood drawn 3 times. The mean serum levels of TNF-$\alpha$ are presented in Table 4-8. Using $t$-test, no significant difference was found between groups on time point 1 ($p = .098$). On time point 2 and 3, a significant difference was found.
between groups \((p = .005, <.001, \text{ respectively})\).

Table 4-8.

*Mean TNF-α levels (pg/mL) of Subjects at Time Points 1, 2, and 3 by Group*

<table>
<thead>
<tr>
<th>Group I ((n = 19))</th>
<th>Group II ((n = 18))</th>
<th>d.f.</th>
<th>(t)</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>S. D.</td>
<td>Mean</td>
<td>S. D.</td>
<td></td>
</tr>
<tr>
<td>TP1</td>
<td>11.6</td>
<td>2.9</td>
<td>9.7</td>
<td>4.2</td>
</tr>
<tr>
<td>TP2</td>
<td>5.8</td>
<td>2.7</td>
<td>9.5</td>
<td>4.6</td>
</tr>
<tr>
<td>TP3</td>
<td>11.5</td>
<td>3.3</td>
<td>5.0</td>
<td>2.7</td>
</tr>
</tbody>
</table>

*Note.* S.D. = standard deviation, d.f. = degree of freedom.

Hypothesis testing was done using repeated measure *ANOVA (RM-ANOVA)* for interval level data. Normality was checked by creating a histogram and inspecting skewness and kurtosis. Using *RM-ANOVA*, no significant difference was found between groups on TNF-α, \(F(1, 35) = 2.68, p = .111\). Mauchly’s \(W\) test is significant, \(W(2) = .68, p < .001\), so the sphericity assumption has not been met. The correction of degree of freedom \(d.f.\) and main effect was made using Greenhouse-Geisser Epsilon \((\epsilon = .758)\).

Using the Greenhouse-Geisser correction, the main effect was significant, \(F(1.52, 53) = 19.83, p < .001\), partial Eta squared \((\eta^2) = .36\), and observed power = .99 (Table 4-9). Eta squared is the most common designation given to \(R^2\) in the context of *ANOVA* (Kirk, 1995), and Eta is actually a correlation coefficient (Meyers, Gamst, & Guarino, 2006). The partial Eta squared is the proportion of the total variance that is attributed to an effect. It is calculated as the ratio of the effect (SS\(_{\text{effect}}\)) and error variance (SS\(_{\text{error}}\)) to the total
variance ($SS_{total}$). Because it is based on a correlation, Eta squared is interpreted as the percentage of total variance explained by a given effect. Therefore, the TNF-α change over time accounted for 36% of the total variance of TNF-α. There was a significant difference found when TNF-α levels were compared by time periods across the 3 time points.

Table 4-9.
Repeated Measures Analysis of Variance for TNF-α Level

<table>
<thead>
<tr>
<th>Source</th>
<th>$SS$</th>
<th>$d.f.$</th>
<th>$MS$</th>
<th>$F$</th>
<th>$p$</th>
<th>$\eta^2$</th>
<th>Observed power</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Between Subjects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td>70.1</td>
<td>1</td>
<td>70.1</td>
<td>2.68</td>
<td>.111</td>
<td>.07</td>
<td>.36</td>
</tr>
<tr>
<td>Within groups (Error)</td>
<td>917.3</td>
<td>35</td>
<td>26.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Within Subjects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time (TNF-α)</td>
<td>187.6</td>
<td>1.52</td>
<td>123.8</td>
<td>19.83</td>
<td>&lt;.001</td>
<td>.36</td>
<td>.99</td>
</tr>
<tr>
<td>Group*Time</td>
<td>478.1</td>
<td>1.52</td>
<td>315.4</td>
<td>50.53</td>
<td>&lt;.001</td>
<td>.59</td>
<td>.99</td>
</tr>
<tr>
<td>Group*Time (Error)</td>
<td>331.2</td>
<td>53</td>
<td>6.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note. $SS$ = sum of squares, $d.f.$ = degree of freedom, $MS$ = mean square, $\eta^2$ = partial Eta squared*

In addition, the within-subject test indicates that the interaction of TNF-α level and group is significant ($F = 50.52, p < .001$, partial $\eta^2 = .59$, observed power = .99) (Table 4-9), which indicates that the groups are changing over time and they are changing in different ways. The interaction of TNF-α and group accounted for 59% of the total variance of TNF-α. In Figure 4-1, the TNF-α level of group I first decreases between time
points 1 and 2, followed by an increase from time point 2 to 3. On the other hand, the TNF-α level of group II is nearly flat (the slopes of the line are approximately equal to zero) from time point 1 to 2, and there is obvious decreasing from time point 2 to 3. This means the effects of vitamin C do result in a decrease of TNF-α level. Hence, hypothesis 1 was supported.

![Figure 4-1](image)

**Figure 4-1.** *Mean TNF-α levels (pg/mL) of Subjects at Time Points 1, 2, and 3 by Group*

**Hypothesis 2**

Subjects will have a increase in endothelial function as measured by a increased diameter change percentage and blood flow while using antioxidant supplementation compared to when they not using antioxidant supplementation.

All 37 subjects underwent endothelial function tests at the three time points. Normality was checked. After transformation by taking logarithm, the mean diameter
change percentage (log %) is presented in Table 4-10. Using $t$-test, no significant
difference was found between groups on time point 1 and 3 ($p = .170$ and $.082$,
respectively). On time point 2, a significant difference was found between groups ($t =
4.46, p < .001$) where by Group I who received the vitamin C had larger diameter change
percentage (log %).

Table 4-10.

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th></th>
<th></th>
<th>Group II</th>
<th></th>
<th></th>
<th>d.f.</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>Mean</td>
<td>S. D.</td>
<td>n</td>
<td>Mean</td>
<td>S. D.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>.73</td>
<td>.38</td>
<td>15</td>
<td>.55</td>
<td>.36</td>
<td>29</td>
<td>1.41</td>
<td>.170</td>
<td></td>
</tr>
<tr>
<td>TP2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>1.14</td>
<td>.33</td>
<td>15</td>
<td>.66</td>
<td>.27</td>
<td>30</td>
<td>4.46</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>TP3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>.75</td>
<td>.41</td>
<td>17</td>
<td>1.00</td>
<td>.39</td>
<td>32</td>
<td>1.80</td>
<td>.082</td>
<td></td>
</tr>
</tbody>
</table>

Note. S.D. = standard deviation, d.f. = degree of freedom

RM-ANOVA was used for statistical analysis. Mauchly’s W test is significant, $W(2)
= .878, p = .203$, so the sphericity assumption has been met. Using RM-ANOVA, there is
no significant difference was found between groups in diameter change percentage (log
%), $F(1, 25) = 3.47, p = .074$. However, there was a significant difference was found
when means of diameter change percentage (log %) were compared by time period across
3 time points, $F(12, 50) = 10.97, p < .001$, partial $\eta^2 = .31$, and observed power = .99. The
interaction between groups and time was also significant different, $F(2, 50) = 12.42, p$
< .001, partial $\eta^2 = .33$, and observed power = .99 (Table 4-11).

Table 4-11.

*Repeated Measures Analysis of Variance for Mean Diameter Change Percentage (log %)*

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>d.f.</th>
<th>MS</th>
<th>$F$</th>
<th>$p$</th>
<th>$\eta^2$</th>
<th>Observed power</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Between Subjects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td>.56</td>
<td>1</td>
<td>.56</td>
<td>3.47</td>
<td>.074</td>
<td>.12</td>
<td>.43</td>
</tr>
<tr>
<td>Within groups (Error)</td>
<td>4.02</td>
<td>25</td>
<td>.16</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Within Subjects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time (Diameter)</td>
<td>1.73</td>
<td>2</td>
<td>.86</td>
<td>10.97</td>
<td>&lt;.001</td>
<td>.31</td>
<td>.99</td>
</tr>
<tr>
<td>Group*Time</td>
<td>1.95</td>
<td>2</td>
<td>.98</td>
<td>12.42</td>
<td>&lt;.001</td>
<td>.33</td>
<td>.99</td>
</tr>
<tr>
<td>Group*Time (Error)</td>
<td>3.94</td>
<td>50</td>
<td>.08</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note.* $SS =$ sum of squares, $d.f.$ = degree of freedom, $MS =$ mean square, $\eta^2 =$ partial Eta squared

In addition, the within-subject test indicates that the interaction of the diameter change percentage (log %) and the group is significant ($F = 12.42, p < .001, \text{partial } \eta^2 = .33, \text{observed power} = .99$), which indicates that the groups are changing over time and they are changing in different ways. The interaction of the diameter change percentage and the group accounted for 33% of the total variance of the diameter change percentage (log %). In Figure 4-2, the diameter change percentage (log %) of group I increase from time point 1 to time point 2, followed by a decrease from time point 2 to time point 3. On the other hand, the diameter change percentage (log %) of group II is nearly flat from time point 1 to 2, followed by markedly increasing from time point 2 to 3. These findings demonstrate that the effects of vitamin C lead to an increase of the diameter change
percentage (log %). This result supported this hypothesis.

![Graph showing mean diameter change percentage (log %) of subjects at time points 1, 2, and 3 by group]

**Figure 4-2. Mean Diameter Change Percentage (log %) of Subjects at Time Points 1, 2, and 3 by Group**

The mean baseline flow is presented in Table 4-12. Using t-test, no significant difference was found between groups on time point 1 ($p = .078$). On time point 2 and 3, a significant difference was found between groups ($p = .029$, and $p = .001$, respectively).

<table>
<thead>
<tr>
<th>Group I (n = 19)</th>
<th>Group II (n = 18)</th>
<th>d.f.</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. D.</td>
<td>S. D.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP1</td>
<td>11.6</td>
<td>9.7</td>
<td>35</td>
<td>-1.81</td>
</tr>
<tr>
<td></td>
<td>2.9</td>
<td>4.2</td>
<td></td>
<td>2.30</td>
</tr>
<tr>
<td>TP2</td>
<td>5.8</td>
<td>9.5</td>
<td>35</td>
<td>-3.80</td>
</tr>
<tr>
<td></td>
<td>2.7</td>
<td>4.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP3</td>
<td>11.5</td>
<td>5.0</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.3</td>
<td>2.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note. S.D. = standard deviation, d.f. = degree of freedom*
Statistical analysis was performed using *RM-ANOVA*. Normality was checked. Mauchly’s *W* test is significant, *W*(2) = .69, *p* = .002, so the sphericity assumption has not been met. For using *RM-ANOVA* after Greenhouse-Geisser correction, there is no significant difference was found between groups in baseline flow, *F*(1, 35) = 2.31, *p* = .138. However, there was a significant difference was found when means of baseline flow were compared by time period across 3 time points, *F*(1.53, 53) = 16.27, *p* < .001, partial $\eta^2 = .32$, and observed power = .99. The interaction between groups and time was also significantly different, *F*(1.53, 53) = 22.27, *p* < .001, partial $\eta^2 = .39$, and observed power = .99 (Table 4-13).

Table 4-13.
*Repeated Measures Analysis of Variance for Baseline Flow*

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>d.f.</th>
<th>MS</th>
<th><em>F</em></th>
<th><em>p</em></th>
<th>$\eta^2$</th>
<th>Observed power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td>37.0</td>
<td>1</td>
<td>37.0</td>
<td>2.31</td>
<td>.138</td>
<td>.062</td>
<td>.32</td>
</tr>
<tr>
<td>Within groups (Error)</td>
<td>560.4</td>
<td>35</td>
<td>16.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within Subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time (baseline flow)</td>
<td>124.2</td>
<td>1.53</td>
<td>81.3</td>
<td>16.27</td>
<td>&lt;.001</td>
<td>.32</td>
<td>.99</td>
</tr>
<tr>
<td>Group*Time</td>
<td>170.0</td>
<td>1.53</td>
<td>111.3</td>
<td>22.27</td>
<td>&lt;.001</td>
<td>.39</td>
<td>.99</td>
</tr>
<tr>
<td>Group*Time (Error)</td>
<td>267.2</td>
<td>53</td>
<td>4.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note. SS = sum of squares, d.f. = degree of freedom, MS = mean square, $\eta^2 = \text{partial Eta squared}$*
In addition, the within-subject test indicates that the interaction of baseline flow and the group is significant \( F = 22.27, p < .001, \text{partial } \eta^2 = .39, \text{observed power} = .99 \), which indicates that the groups are changing over time and they are also changing in different ways. The interaction of baseline flow and group accounted for 39% of the total variance of baseline flow. In Figure 4-3, the baseline flow of group I is increasing, followed by a decrease from time points 1 to 2 and 2 to 3, respectively. On the other hand, the baseline flow of group II is nearly flat from time point 1 to 2, followed by markedly increasing from time point 2 to 3. This demonstrates that antioxidant supplementation results in an increase of baseline flow. This result supported this hypothesis.

![Figure 4-3. Baseline Flow (mL/sec) of Subjects at Time Points 1, 2, and 3 by Group](image)
The mean peak flow change by group is presented in Table 4-14. Using \( t \)-test, no significant difference was found between groups on time points 1 and 3 (\( p = .533 \), and \(=.051 \), respectively). But there is a significant difference was found between groups on time point 2 (\( p = .013 \)).

Table 4-14.

\textit{Peak Flow (mL/sec) of Subjects at Time Points 1, 2, and 3 by Group}

<table>
<thead>
<tr>
<th></th>
<th>Group I (( n = 19 ))</th>
<th>Group II (( n = 18 ))</th>
<th>d.f.</th>
<th>( t )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean</strong></td>
<td>20.7</td>
<td>19.4</td>
<td>35</td>
<td>.63</td>
<td>.533</td>
</tr>
<tr>
<td><strong>S. D.</strong></td>
<td>5.5</td>
<td>7.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>28.0</td>
<td>20.9</td>
<td>35</td>
<td>2.63</td>
<td>.013</td>
</tr>
<tr>
<td><strong>S. D.</strong></td>
<td>9.1</td>
<td>7.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>23.1</td>
<td>27.7</td>
<td>35</td>
<td>-2.02</td>
<td>.051</td>
</tr>
<tr>
<td><strong>S. D.</strong></td>
<td>8.2</td>
<td>4.9</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textit{Note.} S.D. = standard deviation, d.f. = degree of freedom

Statistical analysis was performed using \textit{RM-ANOVA}. Normality was checked. Mauchly’s \( W \) test is significant, \( W(2) = .73, p = .005 \), so the sphericity assumption has not been met. The correction of d.f. and main effect was made by Greenhouse Epsilon (\( \epsilon = .787 \)). For using \textit{RM-ANOVA} after Greenhouse-Geisser correction, there is no significant difference was found between groups in peak flow, \( F(1, 35) = .55, p = .462 \). However, there was a significant difference was found when means of peak flow were compared by time period across 3 time points, \( F(1.57, 55) = 8.57, p = .001 \), partial \( \eta^2 = .20 \), and observed power = .92. The interaction between groups and time was also significantly different, \( F(1.57, 55) = 8.76, p = .001 \), partial \( \eta^2 = .20 \), and observed power
(.93 (Table 4-15).

Table 4-15.

_Repeated Measures Analysis of Variance for Peak Flow_

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>d.f.</th>
<th>MS</th>
<th>F</th>
<th>p</th>
<th>η²</th>
<th>Observed power</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Between Subjects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td>46.6</td>
<td>1</td>
<td>46.6</td>
<td>.55</td>
<td>.462</td>
<td>.02</td>
<td>.11</td>
</tr>
<tr>
<td>Within groups (Error)</td>
<td>2945</td>
<td>35</td>
<td>84.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Within Subjects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time (peak flow)</td>
<td>612.5</td>
<td>1.57</td>
<td>389.3</td>
<td>8.57</td>
<td>.001</td>
<td>.20</td>
<td>.92</td>
</tr>
<tr>
<td>Group*Time</td>
<td>625.9</td>
<td>1.57</td>
<td>397.8</td>
<td>8.76</td>
<td>.001</td>
<td>.20</td>
<td>.93</td>
</tr>
<tr>
<td>Group*Time (Error)</td>
<td>2500</td>
<td>55</td>
<td>45.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

_Note._ SS = sum of squares, d.f. = degree of freedom, MS = mean square, η² = partial Eta squared

In addition, the within-subject test indicates that the interaction of peak flow and group is significant \( F = 8.76, p = .001, \text{partial } η^2 = .20, \text{observed power} = .93 \), which indicates that the groups are changing over time and they are also changing in different ways. The interaction of peak flow and group accounted for 20% of the total variance of baseline flow. In Figure 4-4, the baseline flow of group I is increasing from time point 1 to 2; following this there is a decrease between time points 2 and 3. On the other hand, the baseline flow of group II is nearly flat between time points 1 and 2, and it is followed by a marked increase from time point 2 to 3. This leads to the conclusion that antioxidant supplementation results in an increase of peak flow. This result supported this hypothesis.
Hypothesis 3

Subjects will have an increase in cardiac performance as measured by a decreased LVEDD and an increased LVEF on the echocardiography while using antioxidant supplementation compared to when they not using antioxidant supplementation.

All 37 subjects had echocardiogram exams at the three time points. The mean left ventricular end-diastole diameter (LVEDD) is presented in Table 4-16. Using $t$-test, no significant difference was found between groups on time points 1, 2 and 3 ($p = .733, .663, \text{ and } .869$, respectively).

$RM-ANOVA$ was used for statistical analysis. Normality was checked. Mauchly’s $W$ test is significant, $W(2) = .78, p = .016$, so the sphericity assumption has not been met.

The correction of d.f. and main effect is made by Greenhouse Epsilon ($\epsilon = .822$). Using
the Greenhouse-Geisser correction, the main effect was significant, \( F(1.64, 58) = .31, p = .692 \). The within-subject test indicated that there is not a significant time effect; in other words, the groups did not experience changes in LVEDD over time. The interaction of LVEDD and group was not statistically significant (\( F = .80, p = .432 \)).

Table 4-16.

*Left Ventricular End-Diastolic Diameter (LVEDD)(cm) at Time Points 1, 2, and 3 by Group*

<table>
<thead>
<tr>
<th></th>
<th>Group I (n = 19)</th>
<th>Group II (n = 18)</th>
<th>d.f.</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S. D.</td>
<td>Mean</td>
<td>S. D.</td>
<td></td>
</tr>
<tr>
<td>TP1</td>
<td>5.31</td>
<td>.80</td>
<td>5.41</td>
<td>.94</td>
<td>35</td>
</tr>
<tr>
<td>TP2</td>
<td>5.24</td>
<td>1.01</td>
<td>5.38</td>
<td>.95</td>
<td>35</td>
</tr>
<tr>
<td>TP3</td>
<td>5.32</td>
<td>.96</td>
<td>5.27</td>
<td>.83</td>
<td>35</td>
</tr>
</tbody>
</table>

*Note. S.D. = standard deviation, d.f. = degree of freedom*

Table 4-17.

*Repeated Measures Analysis of Variance for Left Ventricular End-Diastolic Diameter (LVEDD)*

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>d.f.</th>
<th>MS</th>
<th>( F )</th>
<th>( p )</th>
<th>( \eta^2 )</th>
<th>Observed power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td>.11</td>
<td>1</td>
<td>.11</td>
<td>.049</td>
<td>.826</td>
<td>.00</td>
<td>.06</td>
</tr>
<tr>
<td>Within groups (Error)</td>
<td>80.25</td>
<td>35</td>
<td>2.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within Subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time (LVEDD)</td>
<td>.07</td>
<td>1.64</td>
<td>.04</td>
<td>.31</td>
<td>.692</td>
<td>.01</td>
<td>.09</td>
</tr>
<tr>
<td>Group*Time</td>
<td>.18</td>
<td>1.64</td>
<td>.11</td>
<td>.80</td>
<td>.432</td>
<td>.02</td>
<td>.17</td>
</tr>
<tr>
<td>Group*Time (Error)</td>
<td>8.07</td>
<td>58</td>
<td>.14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note. SS = sum of squares, d.f. = degree of freedom, MS = mean square, \( \eta^2 \) = partial Eta squared*
The left ventricular ejection fraction (LVEF) is presented in Table 4-18. Using $t$-test, no significant difference was found between groups on time point 1 ($p = .573$), but there is a significant difference was found between groups on time points 2 and 3 ($p = .040$, and $p = .014$, respectively).

Table 4-18.

<table>
<thead>
<tr>
<th>Time Point</th>
<th>Group I ($n = 19$)</th>
<th>Group II ($n = 18$)</th>
<th>d.f.</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S. D.</td>
<td>Mean</td>
<td>S. D.</td>
<td></td>
</tr>
<tr>
<td>TP1</td>
<td>34</td>
<td>13</td>
<td>36</td>
<td>9</td>
<td>35</td>
</tr>
<tr>
<td>TP2</td>
<td>47</td>
<td>13</td>
<td>39</td>
<td>10</td>
<td>35</td>
</tr>
<tr>
<td>TP3</td>
<td>37</td>
<td>13</td>
<td>47</td>
<td>11</td>
<td>35</td>
</tr>
</tbody>
</table>

*Note. S.D. = standard deviation, d.f. = degree of freedom*
Statistical analysis was performed using RM-ANOVA. Normality was checked.

Mauchly’s $W$ test is significant, $W(2) = .67, p = .001$, so the sphericity assumption has not been met. For using RM-ANOVA after Greenhouse-Geisser correction, there is no significant difference was found between groups in LVEF, $F(1, 35) = .16, p = .70$.

However, there was a significant difference was found when means of LVEF were compared by time period across 3 time points, $F(1.50, 53) = 35.63, p < .001$, partial $\eta^2 = .50$, and observed power = .99. The interaction between groups and time was also significantly different, $F(1.50, 53) = 41.26, p < .001$, partial $\eta^2 = .54$, and observed power = .99 (Table 4-19).

Table 4-19.
Repeate Measures Analysis of Variance for Left Ventricular Ejection Fraction (LVEF)

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>d.f.</th>
<th>MS</th>
<th>$F$</th>
<th>$p$</th>
<th>$\eta^2$</th>
<th>Observed power</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Between Subjects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td>57.3</td>
<td>1</td>
<td>57.3</td>
<td>.16</td>
<td>.70</td>
<td>.00</td>
<td>.07</td>
</tr>
<tr>
<td>Within groups (Error)</td>
<td>12966</td>
<td>35</td>
<td>370.4</td>
<td>.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Within Subjects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time (LVEF)</td>
<td>1324</td>
<td>1.50</td>
<td>881.3</td>
<td>35.63</td>
<td>&lt;.001</td>
<td>.50</td>
<td>.99</td>
</tr>
<tr>
<td>Group*Time</td>
<td>1533</td>
<td>1.50</td>
<td>1020</td>
<td>41.26</td>
<td>&lt;.001</td>
<td>.54</td>
<td>.99</td>
</tr>
<tr>
<td>Group*Time (Error)</td>
<td>1301</td>
<td>53</td>
<td>24.7</td>
<td>.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note. SS = sum of squares, d.f. = degree of freedom, MS = mean square, $\eta^2$ = partial Eta squared
In addition, the within-subject test indicates that the interaction of LVEF and group is significant ($F = 41.26, p < .001$, partial $\eta^2 = .54$, observed power = .99), which indicates that the groups are changing over time and they are also changing in different ways. The interaction of LVEF and group accounted for 54% of the total variance of LVEF. In figure 4-6, the LVEF of group I is increasing from time point 1 to 2, followed by decreasing from time point 2 to 3. On the other hand, the LVEF of group II is slightly increasing, followed by a marked increase from time point 1, 2 to 3. This result leads to the conclusion that antioxidant supplementation results in an increase of LVEF. This result supported this hypothesis.

Figure 4-6.  *Left Ventricular Ejection Fraction (LVEF) (%) of Subjects at Time Points 1, 2, and 3 by Group*
Hypothesis 4

Subject will have an increase in exercise capacity as measured by an increased total distance walked on the 6MWT while using antioxidant supplementation compared to when they not using antioxidant supplementation.

All 37 subjects completed a 6-min walk distance test (6MWT) at the three time points. The mean total distance is presented in Table 4-20. Using t-test, no significant difference was found between groups on time point 1 ($p = .456$). But there is a significant difference was found between groups on time points 2 and 3 ($p < .001$, and $p = .005$, respectively).

<table>
<thead>
<tr>
<th></th>
<th>Group I ($n = 19$)</th>
<th>Group II ($n = 18$)</th>
<th>d.f.</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S. D.</td>
<td>Mean</td>
<td>S. D.</td>
<td></td>
</tr>
<tr>
<td>TP1</td>
<td>184</td>
<td>36</td>
<td>174</td>
<td>39</td>
<td>35</td>
</tr>
<tr>
<td>TP2</td>
<td>243</td>
<td>40</td>
<td>185</td>
<td>38</td>
<td>35</td>
</tr>
<tr>
<td>TP3</td>
<td>197</td>
<td>40</td>
<td>237</td>
<td>41</td>
<td>35</td>
</tr>
</tbody>
</table>

*Note. S.D. = standard deviation, d.f. = degree of freedom*

RM-ANOVA was used for statistical analysis. Normality was checked. Mauchly’s $W$ test is significant, $W(2) = .70$, $p = .003$, so the sphericity assumption has not been met.

The correction of d.f. and main effect was made with Greenhouse Epsilon ($\varepsilon = .771$). For using RM-ANOVA after Greenhouse-Geisser correction, there is no significant difference
was found between groups in 6MWT, $F(1, 35) = .594, p = .446$. However, there was a significant difference was found when means of total distance of 6MWT were compared by time period across 3 time points, $F(1.54, 54) = 40.80, p < .001$, partial $\eta^2 = .54$, and observed power = .99. The interaction between groups and time was also significantly different, $F(1.54, 54) = 52.80, p < .001$, partial $\eta^2 = .60$, and observed power = .99 (Table 4-21).

Table 4-21.
Repeated Measures Analysis of Variance for Six-min Walk Test (6MWT)

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>d.f.</th>
<th>MS</th>
<th>$F$</th>
<th>$p$</th>
<th>$\eta^2$</th>
<th>Observed power</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Between Subjects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td>2246</td>
<td>1</td>
<td>2246</td>
<td>.594</td>
<td>.446</td>
<td>.02</td>
<td>.12</td>
</tr>
<tr>
<td>Within groups (Error)</td>
<td>132298</td>
<td>35</td>
<td>3779</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Within Subjects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time (6MWT)</td>
<td>33891</td>
<td>1.54</td>
<td>21973</td>
<td>40.84</td>
<td>&lt;.001</td>
<td>.54</td>
<td>.99</td>
</tr>
<tr>
<td>Group*Time</td>
<td>43809</td>
<td>1.54</td>
<td>28403</td>
<td>52.80</td>
<td>&lt;.001</td>
<td>.60</td>
<td>.99</td>
</tr>
<tr>
<td>Group*Time (Error)</td>
<td>29042</td>
<td>54</td>
<td>538</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note. SS = sum of squares, d.f. = degree of freedom, MS = mean square, $\eta^2$ = partial Eta squared*

In addition, the within-subject test indicates that the interaction of the 6MWT and group is significant ($F = 52.80, p < .001$, partial $\eta^2 = .60$, observed power = .99), which indicates that the groups are changing over time and they are also changing in different ways. The interaction of the 6MWT and group accounted for 60% of the total variance of
the 6MWT. In figure 4-7, the 6MWT of group I first increases, and then it decreases. On the other hand, the 6MWT of group II increases slightly between times 1 and 2, followed by a large decrease from time point 2 to 3. The longer the distance that a subject walked, the better the exercise capacity of that subject had. This result shows that antioxidant supplementation results in an increase in scores of the 6MWT. This result supported this hypothesis.

Figure 4-7. Six-min Walk Distance Test (6MWT) (m) of Subjects at Time Points 1, 2, and 3 by Group

Hypothesis 5

Subjects will have an increase in functional status as measured by improvements in their scores on the SF-36, MLHFQ, and CES-D while using antioxidant supplementation compared to when they not using antioxidant supplementation.
Functional status was assessed by means of three different questionnaires: the SF-36, MLHQF, and CES-D. All 37 subjects completed all of these three questionnaires at the three time points. The scores are shown in Tables 15, 16, and 17.

**Medical Outcomes Study Short-Form-36 Health Survey (SF-36)**

Table 4-22 presents the SF-36 scores at the three time points. For using *t*-test, no significant difference was found between groups on 3 time points (*p* = .214, .692, and .064, respectively).

<table>
<thead>
<tr>
<th></th>
<th>Group I (n = 19)</th>
<th>Group II (n = 18)</th>
<th>d.f.</th>
<th><em>t</em></th>
<th><em>p</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>S. D.</td>
<td>Mean</td>
<td>S. D.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP1</td>
<td>44</td>
<td>56</td>
<td>35</td>
<td>-1.26</td>
<td>.214</td>
</tr>
<tr>
<td>TP2</td>
<td>60</td>
<td>57</td>
<td>35</td>
<td>0.40</td>
<td>.692</td>
</tr>
<tr>
<td>TP3</td>
<td>53</td>
<td>65</td>
<td>35</td>
<td>-1.90</td>
<td>.064</td>
</tr>
</tbody>
</table>

*Note. S.D. = standard deviation, d.f. = degree of freedom*

RM-ANOVA was used for statistical analysis. Normality was checked. Mauchly’s *W* test is significant, *W*(2) = .69, *p* = .002, so the sphericity assumption has not been met. The correction of *d.f.* and main effect is made using Greenhouse Epsilon (*ε* = .761). For using RM-ANOVA after Greenhouse-Geisser correction, there is no significant difference was found between groups in SF-36 scores, *F*(1, 35) = 1.25, *p* = .272. However, there
was a significant difference was found when means of means of SF-36 scores were compared by time period across 3 time points, $F(1.52, 53) = 5.91, p = .009$, partial $\eta^2 = .14$, and observed power = .79. The interaction between groups and time was also significantly different, $F(1.52, 53) = 4.19, p = .030$, partial $\eta^2 = .11$, and observed power = .63 (Table 4-23).

Table 4-23.
Repeated Measures Analysis of Variance for SF-36 Scores

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>d.f.</th>
<th>MS</th>
<th>$F$</th>
<th>$p$</th>
<th>$\eta^2$</th>
<th>Observed power</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Between Subjects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td>1418</td>
<td>1</td>
<td>1418</td>
<td>1.25</td>
<td>.272</td>
<td>.03</td>
<td>.19</td>
</tr>
<tr>
<td>Within groups (Error)</td>
<td>39875</td>
<td>35</td>
<td>1139</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Within Subjects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time (SF-36)</td>
<td>1955</td>
<td>1.52</td>
<td>1285</td>
<td>5.91</td>
<td>.009</td>
<td>.14</td>
<td>.79</td>
</tr>
<tr>
<td>Group*Time</td>
<td>1387</td>
<td>1.52</td>
<td>911</td>
<td>4.19</td>
<td>.030</td>
<td>.11</td>
<td>.63</td>
</tr>
<tr>
<td>Group*Time (Error)</td>
<td>11583</td>
<td>53</td>
<td>217</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note. $SS$ = sum of squares, $d.f.$ = degree of freedom, $MS$ = mean square, $\eta^2$ = partial Eta squared

In addition, the within-subject test indicates that the interaction of SF-36 scores and group is significant ($F = 4.19, p = .030$, partial $\eta^2 = .11$, observed power = .63), which indicates the groups are changing over time and they are also changing in different ways. The interaction of SF-36 and group accounted for 11% of the total variance of the SF-36 scores. In Figure 4-8, the SF-36 scores of group I first increase from time point 1 to time
point 2, followed by a decrease from time point 2 to 3. On the other hand, the SF-36 scores of group II increase only slightly between time points 1 and 2, and then they increase considerably between time points 2 and 3. Higher scores indicate better quality of life. This shows that mega-doses of vitamin C lead to an increase in SF-36 scores. This result supported this hypothesis.

Figure 4-8.  *SF-36 Scores of Subjects at Time Points 1, 2, and 3 by Group*

*Minnesota Living with Heart Failure Questionnaire (MLHFQ)*

Table 4-24 presents the scores of the Minnesota Living with Heart Failure Questionnaire (MLHFQ) at the three time points. Using $t$-test, no significant difference was found between groups on time points 1 and 2 ($p = .350, .131$, respectively). But there
is a significant difference was found between groups on time point 3 ($p = .045$).

Table 4-24.

*MLHFQ Scores of Subjects at Time Points 1, 2, and 3 by Group*

<table>
<thead>
<tr>
<th></th>
<th>Group I (n = 19)</th>
<th>Group II (n = 18)</th>
<th>d.f.</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>Mean</td>
<td>S. D.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP1</td>
<td>55</td>
<td>32</td>
<td>35</td>
<td>.95</td>
<td>.350</td>
</tr>
<tr>
<td>TP2</td>
<td>29</td>
<td>21</td>
<td>35</td>
<td>-1.55</td>
<td>.131</td>
</tr>
<tr>
<td>TP3</td>
<td>46</td>
<td>31</td>
<td>35</td>
<td>2.07</td>
<td>.045</td>
</tr>
</tbody>
</table>

*Note.* S.D. = standard deviation, d.f. = degree of freedom

*RM-ANOVA* was used for statistical analysis. Normality was checked. Mauchly’s $W$ test is significant, $W(2) = .68$, $p = .001$, so the sphericity assumption has not been met.

The correction of d.f. and main effect is made by Greenhouse Epsilon ($\varepsilon = .757$). Using *RM-ANOVA* after Greenhouse-Geisser correction, there is no significant difference was found between groups in MLHFQ scores, $F(1, 35) = .29$, $p = .591$. However, there was a significant difference was found when means of MLHFQ scores were compared by time period across 3 time points, $F(1.52, 53) = 19.53$, $p < .001$, partial $\eta^2 = .36$, and observed power = .99. The interaction between groups and time was also significantly different, $F(1.52, 53) = 14.66$, $p < .001$, partial $\eta^2 = .30$, and observed power = .99 (Table 4-25).
Table 4-25.
*Repeated Measures Analysis of Variance for MLHFQ Scores*

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>d.f.</th>
<th>MS</th>
<th>F</th>
<th>p</th>
<th>(\eta^2)</th>
<th>Observed power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td>522</td>
<td>1</td>
<td>522</td>
<td>.29</td>
<td>.591</td>
<td>.01</td>
<td>.08</td>
</tr>
<tr>
<td>Within groups (Error)</td>
<td>62240</td>
<td>35</td>
<td>1777</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within Subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time (MLHFQ scores)</td>
<td>5555</td>
<td>1.52</td>
<td>3667</td>
<td>19.53</td>
<td>&lt;.001</td>
<td>.36</td>
<td>.99</td>
</tr>
<tr>
<td>Group*Time</td>
<td>4169</td>
<td>1.52</td>
<td>2752</td>
<td>14.66</td>
<td>&lt;.001</td>
<td>.30</td>
<td>.99</td>
</tr>
<tr>
<td>Group*Time (Error)</td>
<td>9953</td>
<td>53</td>
<td>188</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note.* SS = sum of squares, d.f. = degree of freedom, MS = mean square, \(\eta^2\) = partial Eta squared

In addition, the within-subject test indicates that the interaction of MLHFQ scores and group is significant \((F = 14.66, p < .001, \text{ partial } \eta^2 = .30, \text{ observed power} = .99)\), which indicates the groups are changing over time and they are also changing in different ways. The interaction of MLHFQ scores and group accounted for 30% of the total variance of the MLHFQ. In Figure 4-9, the MLHFQ scores of group I markedly decrease between time points 1 and 2; this is followed by an apparent increase from time point 2 to point 3. On the other hand, the MLHFQ scores of group II initially decrease between time points 1 and 2; this rate of decrease then gets even stronger between time points 2 and 3. Higher scores on MLHFQ reflect poorer quality of life. Consequently, it can be concluded that mega-doses of vitamin C lead to decreases on MLHFQ scores. This result supported this hypothesis.
Figure 4-9. *MLHFQ Scores of Subjects at Time Points 1, 2, and 3 by Group*

Center for Epidemiologic Studies Depression Scales (CES-D)

Table 4-26 presents the scores of the Center for Epidemiologic Studies Depression Scales (CES-D) at three time points. *RM-ANOVA* was used for statistical analysis. Using *t*-test, no significant difference was found between groups on time point 1 (*p* = .485). However, there was a significant difference was found between groups in time points 2 and 3 (*p* = .034, <.003, respectively).
Table 4-26.

<table>
<thead>
<tr>
<th></th>
<th>Group I (n = 19)</th>
<th>Group II (n = 18)</th>
<th>d.f.</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S. D.</td>
<td>Mean</td>
<td>S. D.</td>
<td></td>
</tr>
<tr>
<td>TP1</td>
<td>28</td>
<td>13</td>
<td>25</td>
<td>13</td>
<td>35</td>
</tr>
<tr>
<td>TP2</td>
<td>14</td>
<td>8</td>
<td>22</td>
<td>13</td>
<td>35</td>
</tr>
<tr>
<td>TP3</td>
<td>24</td>
<td>11</td>
<td>13</td>
<td>9</td>
<td>35</td>
</tr>
</tbody>
</table>

Note. S.D. = standard deviation, d.f. = degree of freedom

Normality was checked. Mauchly’s $W$ test is significant, $W(2) = .84$, $p = .053$, so the sphericity assumption has been met. For using RM-ANOVA, there was no significant difference was found between groups in CES-D scores, $F(1, 35) = .39$, $p = .538$. However, there was a significant difference was found when means of CES-D scores were compared by time period across 3 time points, $F(2, 70) = 24.47$, $p < .001$, partial $\eta^2 = .41$, and observed power = .99. The interaction between groups and time was also significantly different, $F(2, 70) = 22.98$, $p < .001$, partial $\eta^2 = .40$, and observed power = .99 (Table 4-27).
Table 4-27.
Repeated Measures Analysis of Variance for CES-D Scores

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>d.f.</th>
<th>MS</th>
<th>F</th>
<th>p</th>
<th>$\eta^2$</th>
<th>Observed power</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Between Subjects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td>120</td>
<td>1</td>
<td>120</td>
<td>.39</td>
<td>.538</td>
<td>.01</td>
<td>.09</td>
</tr>
<tr>
<td>Within groups (Error)</td>
<td>10885</td>
<td>35</td>
<td>311</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Within Subjects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time (CES-D)</td>
<td>1678</td>
<td>2</td>
<td>839</td>
<td>24.47</td>
<td>&lt;.001</td>
<td>.41</td>
<td>.99</td>
</tr>
<tr>
<td>Group*Time</td>
<td>1576</td>
<td>2</td>
<td>788</td>
<td>22.98</td>
<td>&lt;.001</td>
<td>.40</td>
<td>.99</td>
</tr>
<tr>
<td>Group*Time (Error)</td>
<td>2400</td>
<td>70</td>
<td>34</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note. SS = sum of squares, d.f. = degree of freedom, MS = mean square, $\eta^2$ = partial Eta squared

In addition, the within-subject test indicates that the interaction of CES-D and group was significant ($F = 22.98, p < .001$, partial $\eta^2 = .40$, observed power = .99), which indicates the groups are changing over time and they are also changing in different ways. The interaction of CES-D and group accounted for 40% of the total variance of CES-D.

In Figure 4-10, the CES-D scores of group I at first decrease between time points 1 and 2, followed by an increase from time points 2 to 3. On the other hand, the score of the CES-D for group II decreases only slightly between time points 1 and 2, and between time points 2 and 3, there is a greater decrease in scores of the CES-D. Higher scores indicate greater depressive symptoms. Hence, the effects of mega-doses of vitamin C show decreased scores on the CES-D. This result supported the hypothesis.
Figure 4-10. CES-D Scores of Subjects at Time Points 1, 2, and 3 by Group
CHAPTER 5

DISCUSSION

The discussion will be presented in four sections: (a) discussion of the hypotheses tested, (b) limitations of present study, (c) recommendations for future research, and (d) conclusions and implications.

Discussion of the Hypotheses Tested

Effects of Vitamin C on Cytokine

The first hypothesis was supported: antioxidant supplementation can mitigate the pro-inflammatory process by decreasing TNF-α levels. In the present study, heart failure patients receiving mega-doses of vitamin C experienced a decrease in TNF-α levels.

Pro-inflammatory cytokines like TNF-α and oxidative stress induce apoptotic cell death in endothelial cells. Moreover, cytokine messengers, TNF-α, and the vasoconstrictor peptide angiotensin II trigger apoptosis of endothelial cells and stimulate the generation of ROS. Systemic inflammation and increased oxidative stress in heart failure coincide with enhanced endothelial cell apoptosis and the development of endothelial dysfunction. Rossig et al. (2001) investigated the effects of antioxidant vitamin C therapy on endothelial cell apoptosis in heart failure patients. The researcher conducted a prospective, double-blind randomized controlled trial to test vitamin C treatment ($n = 17$) versus placebo ($n = 17$). The study revealed that vitamin C
administration to heart failure patients markedly reduced plasma levels of circulating apoptotic microparticles to $32 \pm 8\%$ of baseline levels, whereas placebo had no effect ($87 \pm 14\%, p < .005$). In addition, vitamin C administration suppressed the proapoptotic activity on the endothelial cells of the serum of heart failure patients. These results were in line with the findings of the current study.

*Effects of Vitamin C on Endothelial Function*

The second hypothesis was supported by the data in the current study. In the present study, heart failure patients who have received mega-doses of vitamin C tended to have better endothelial function than those who did not. This finding was consistent with those of other investigators who examined the effect of vitamin C on endothelial function in patients with HF.

Levine, et al. (1996) conducted a placebo-controlled study, which involved administration of 2 gm of vitamin C to CAD patients with severely impaired brachial artery dilation and found that it led to a dramatic improvement in their vasodilatory response, indicative of improved biologic action of NO. This study hypothesized that an antioxidant, ascorbic acid, would improve endothelium-dependent arterial dilation in patients with CAD. Endothelial function was assessed by ultrasound before and 2 hours after oral administration of either 2 gm ascorbic acid or a placebo in a total of 46 patients with documented CAD. Ascorbic acid administration resulted in an improvement in FMD, from $6.3 \pm 1.1\%$ to $9.5 \pm 1.2\%$ (mean $\pm SEM$), whereas there was no increase in FMD.
with placebo administration, 4.4 ± 1.1% to 3.4 ± 1.2%. The effect of ascorbic acid treatment on brachial dilation was significantly different from the effect of the placebo ($p < .05$). The researchers concluded that ascorbic acid reverses endothelial vasomotor dysfunction in the brachial circulation of patients with CAD. Their findings suggest that increased oxidative stress contributes to endothelial dysfunction in patients with CAD and that endothelial dysfunction may respond to antioxidant therapy.

Ellis, et al. (2001) conducted a double-blind, randomized, crossover study wherein vitamin C (2 gm) or placebo was given intravenously to 10 patients with HF. They measured FMD in the brachial artery. They found vitamin C can increase FMD and reduce plasma lipid-derived free radicals.

Nightingale, et al. (2003) evaluated the long-term and short-term effects of vitamin C on baroreceptor sensitivity (BRS). They hypothesized that vitamin C increases NO bioavailability by directly reducing the inhibitory effect of ROS on baroreceptor nerve endings. They allocated 55 heart failure patients and 22 controls randomly to receive a single intravenous injection of vitamin C (2 gm) or a placebo. In addition, 45 heart failure patients were randomly allocated to receive a 4-week course of oral vitamin C (4 gm/day) or placebo. They found that there was no improvement in BRS in HF patients given chronic oral vitamin C. Thus, acute intravenous vitamin C improved BRS in HF patients. Raitakari, et al. (2000) also suggested that oral vitamin C therapy improves endothelial dysfunction in the short term in healthy young smokers, but it has no beneficial long-term effect, despite sustained elevation of plasma ascorbic acid levels.
Takase, et al. (2004) studied the effects of the combination of oral supplementation with vitamin C (1.0 gm/day) and vitamin E (500 mg/day) on brachial artery FMD by using high-resolution ultrasound. They performed an FMD study 3 times: before vitamin supplementation, after 25 days of vitamin supplementation, and 4 weeks after the cessation of the vitamin supplement. They concluded that the oral antioxidant vitamin supplement significantly restored FMD; however, this effect disappeared 4 weeks after the vitamin supplementation ended. The antioxidant supplementation was found to improve the endothelial function in chronic smokers.

Besides those studies, Ellis, et al. (2000), Horning, et al. (1998), Erb, et al. (2003) and Ito, et al. (1998) reached similar conclusions with regard to the effects of vitamin C supplementation and endothelial function. The results of the current study are consistent with previous studies.

*Effects of Vitamin C on Cardiac Performance*

The third hypothesis can be divided to two parts. It was hypothesized that the heart’s remodeling process can be changed by means of the effects of antioxidant supplementation by an increase of LVEDD. This hypothesis was not supported. It also hypothesized that heart contractility can be improved through the effects of antioxidant supplementation by means of an increase of LVEF. There have been few previous investigations on vitamin C and cardiac performance. However, this hypothesis was supported.
Effects of Vitamin C on Exercise Capacity

The fourth hypothesis was supported: antioxidant supplementation can enhance exercise capacity by increasing the total distance walked in a 6-min walk test.

There is no previous study focusing on the relation between mega-dose vitamin C supplementation and exercise capacity. But several studies have posed the idea that there is a strong relationship between LVEF and exercise capacity, and exercise capacity could influence QOL in heart failure patients. Moreover, exercise training can improve exercise capacity as well as HRQOL.

Several studies had identified the relationship between LVEF and exercise capacity (Boriani, Fallani, Martignani, Biffi, Saporito, & Greco, 2005; DePace, et al, 1983; Hacker, Stork, Stratakis, Angermann, Huber, Hahn, et al, 2003; Meyer, Karamanoglu, Ehsani, & Kovacs, 2004). Bittner, et al. (1993) collected data during a prospective cohort study, the Studies of Levt Ventricular Dysfunction (SOLVD) Registry Substudy. A stratified random sample of 898 patients with low LVEF (less than 45%) were enrolled in the substudy and underwent a detailed clinical evaluation including a 6-min walk test. In a logistic regression model, ejection fraction and distance walked were equally strong and independent predictors of mortality and heart failure hospitalization rates during follow-up. In comparison, the present study suggests a similar direction as the SOLVD study but with a much smaller sample size. Ecochard, Colin, Rabilloud, Gevigney, Cao, and Ducreux (2001) proposed that a low ejection fraction was associated with impaired
physical mobility ($OR = 1.21$, 95% $CI = 1.05-1.39$).

The mechanism of exercise tolerance in patients with heart failure is probably multifactorial. The abnormalities of ventilation and of ventilation-perfusion matching may contribute to dyspnea or shortness of breath. Bautmans, Lambert, and Mets (2004) assessed community-dwelling elderly people (53 male, 103 female) for health status and their performance in a 6MWT. They presented the mean 6MWT as 603 m ($SD = 178$). A multiple linear regression model with health status, age and gender as independent variables explained 31% of the 6MWT’s variability. Enright, et al. (2003) determined the correlated of the total 6MWT distance in elderly adults ($\geq 68$ years old). In their study, 2,281 subjects performed the 6MWT and the mean total distance was 344 m ($SD = 88$). In the current study, the mean total distances of the 6MWT were 184 m ($SD = 36$) and 174 m ($SD = 39$) for groups I and II respectively. The total distance of both groups were much shorter than the results in Bautmans’s and Enright’s studies. This demonstrates that heart failure patients have a decrease in exercise capacity, as compared to the healthy population.

Wielenga, et al. (1998) identified the effect of exercise training on HRQOL. In this study, exercise capacity was studied in 67 patients with mild to moderate chronic heart failure (mean age was $65.6 \pm 8.3$; mean LVEF was $26.5 \pm 9.6\%$). Patients were randomly allocated to either a training group or to a control group. After intervention, a markedly significant increase in the Heart Failure Psychological Questionnaire was observed in the training group. They concluded that supervised exercise training improved both QOL and
exercise capacity and can be safely performed by HF patients. Sjoland, Wiklund, Caidahl, Albertsson, and Herlitz (1995) also presented similar results wherein significant correlations were found between HRQOL and exercise capacity \((p < .001)\). Jeng, et al. (2004) conducted a study to examine the influence of exercise tolerance on HRQOL among patients with HF in Taiwan. They concluded that both exercise duration and exercise intensity tolerance were important factors in determining HRQOL, specifically with regard to physical functioning in heart failure patients.

The evidence-base had shown that LVEF can influence exercise capacity and quality of life; and exercise capacity can influence HRQOL. So, it is possible establish a causal relationship among LVEF, exercise capacity, depression and HRQOL. Thus, future study is necessary to examine this possibility.

**Effects of Vitamin C on Functional Status**

The fifth hypothesis was supported: antioxidant supplementation can promote health status by means of an increase in SF-36 scores and decreases in MLHFQ and CES-D scores.

Recently, more and more researchers have suggested the use of health-related quality of life as an indicator to assess and evaluate the outcomes of treatments for the heart failure population. Patients with chronic HF often report poor quality of life due to their physical limitations and functional impairments. Poor HRQOL was also a univariate predictor for mortality and a strong multivariate predictor for the important outcome of
readmission (Mejhert, Kahan, Persson, & Edner, 2006). But most studies have focused on exercise training and education programs facilitating QOL, rather than the effects of antioxidant supplementation.

There have been limited studies that focus on the relationships between vitamin C supplementation and functional status, regardless of their connection with matter health-related QOL or depressive symptoms. But several studies have investigated the relationships between clinical assessments and HRQOL. Rector, et al. (2006) has shown the relationships between measures of HF-related factors and HRQOL. Using structure equation modeling, they have found that dyspnea at rest, dyspnea on exertion, paroxysmal nocturnal dyspnea, orthopnea, fatigue, and NYHA class were all significantly related to MLHFQ scores. Combined, these symptoms could explain 41% of the variance in MLHFQ scores.

Peric, et al. (2005) investigated the relation between EF and QOL after coronary artery bypass surgery. They investigated 29 patients with ejection fractions of less than 30%. They demonstrated that patients with low LVEF have worse HRQOL in the section concerning energy. Six months after CABG, HRQOL was significantly improved, and that improvement was not related to LVEF.

However, there are no previous studies which have investigated mega-dose vitamin C and functional status. In this study, subjects tended to report better HRQOL and less depressive symptoms after they received antioxidant supplementation. It is possible that clinical signs and symptoms, such as dyspnea, NYHA classification, and ejection fraction,
are the mediators or moderators between antioxidant supplementation and functional status, but it also needs future study to prove it.

**Limitations of the Present Study**

Several issues may have affected the accuracy and precision of this study because this was the first time antioxidant supplementation had been done in Taiwan.

*Theoretical limitations*

It was hard to identify whether the effects of antioxidant supplementation on exercise capacity and functional status are direct or indirect because the previous studies which had focused on the relationships among inflammation, exercise capacity and functional status were limited.

*Methodological limitations*

Generalization of the present study will be limited because of its lack of NYHA class I and IV heart failure patients and because all of the subjects in this study were recruited from a single unit. To improve this study, different units should have been used to enhance its external validity.

Second, another approach to control threats to internal validity is through sample selection by means of strict inclusion criteria; however, this also reduces the number of possible subjects available in the HF population. For example, sometimes the PI asked subjects about prior or co-existing inflammatory-related diseases (such as arthritis), but most subjects could not answer definitely. This will influence the accuracy of the
assessment of the severity of disease, and it will confound interpretation of the effects of antioxidant.

Third, regarding the health-related quality of life, all information came from a quantitative constructed questionnaire. No qualitative information was collected or analyzed.

**Recommendations for Future Research**

The research questions posed in the present study were a logical first step in exploring the effects of antioxidant therapy on cardiovascular performance, exercise capacity, and functional status in chronic heart failure patients. Future studies that can be recommended based on the present study are:

1. Studies applying the same analysis techniques with larger sample sizes could result in a more representative sample of chronic heart failure patients. In the present study, lacking NYHA I and IV HF patients will limit the study’s generalizability with regard to severity of HF.

2. Studies applying the same analysis techniques to longer periods of time with high-order crossover designs could decrease the possibility of carry-over effects.

3. Replication of this study using other empirical indicators is suggested, for this would allow greater understanding the whole pro-inflammatory process. Using more sensitive and specific biomarkers (such as cytokines family, endothelin-1 and endothelin-3) could lead to more specific evaluation of the pro-inflammatory process in
heart failure.

4. Replication of this study using a larger sample size is suggested, which would allow for analysis and identification of the relationships among cytokines, endothelial function, cardiac performance, exercise capacity and functional status in patients with chronic heart failure.

5. Studies establishing the bio-psycho-social model of chronic heart failure patients would lead to holistic perspective to HF patients.

Conclusions and Implications

Five major findings of effects of antioxidant supplementation are reported:

1. This study identified the effects of mega-dose vitamin C as mitigating the pro-inflammatory process by decreasing TNF-α level in HF patients although the response of other cytokines was not examined.

2. This study identified the effects of mega-dose vitamin C on endothelial function by increasing all of the following: diameter change percentage, baseline flow, and peak flow, which reflect improving endothelial function in chronic heart failure patients.

3. This study identified the effects of mega-dose vitamin C in terms of improving heart contractility by obviously increasing left ventricular ejection fraction. Meanwhile, there is no statistical difference in heart remodeling by changing left ventricular end-diastole diameter in chronic heart failure patients.
4. This study identified the effects of mega-dose vitamin C in terms of augmenting exercise capacity by increasing the total distance covered in a 6-minute walk test among chronic heart failure patients.

5. This study demonstrated the effects of mega-dose vitamin C on functional status in promoting both generic and disease-specific health-related quality of life by changing SF-36 and MLHFQ scores. In addition, the effects of mega-dose vitamin C were demonstrated to alleviate depressive symptoms by decreasing CES-D scores in patients with chronic heart failure.

Implications for nursing science from three perspectives:

*Implications for Nursing Practice and Nursing Policy*

Documentation of improved clinical outcomes usually provides potential for change in clinical practice. The opportunity to know the effects of antioxidant supplementation on the bio-psycho-social domain in HF patients is a good start. These findings can be used for evidence-based nursing practice. Furthermore, this study also illuminates the possible relationships between the effects of antioxidant and functional status. Most clinical nurses emphasize the effects of and responses to medications and lose sight of the potential of antioxidants. Most of the previous studies focused on patient outcomes, such as mortality or morbidity. However, the current study demonstrated that antioxidant supplementation is important for HF patients as well. Therefore, it is necessary to use these research findings as the basis for implementing antioxidant supplementation in the clinical setting.
**Implications for Nursing Education**

Results from the current study provide a new perspective to comprehend the essence of heart failure patients. In addition, traditional curricula or textbooks just talk about “medical treatment” (such as medications, or IABP) rather than the role of antioxidant supplementation in heart failure patients.

**Implication for Nursing Research**

This study contributes knowledge that identifies the effects of antioxidants on cardiovascular performance and exercise capacity in heart failure patients. First, the permuted block randomization using SAS 9.0 software that was used in the current study is recommended for future study. This method is feasible, and it can control numerous threats to internal validity. Second, this is the first crossover randomized controlled trial to provide information about the effects of antioxidant supplementation on the pro-inflammatory process, endothelial function, cardiac performance, exercise capacity, and functional status, not only in Taiwan, but also in the US. It is a good beginning and also offers a new perspective and direction for future research.
REFERENCES


Celermajer, D. S., Sorensen, K. E., Spiegelhalter, D. J., Georgakopoulos, D., Robinson, J., &


Ito, K. et al (1998) ascorbic acid heart failure. Am J Cardiol, 82,


Levine, G. N., Frei, B., Koulouris, S. N., Gerhard, M. D., Keaney, J. F., Jr., & Vita, J. A.


comparison with B-type natriuretic Peptide. *J Am Coll Cardiol, 46*(6), 1011-1018.


Comparative responsiveness of Short-Form 12 and Minnesota Living With Heart Failure Questionnaire in patients with heart failure. *J Card Fail, 6*(2), 83-91.


APPENDIX A

DEMOGRAPHIC/MEDICAL INFORMATION SHEET
Appendix A. Demographic/Medical Information Sheet

Effects of antioxidant on cardiovascular performance, exercise capacity and functional status in patients with chronic heart failure

Demographic/Medical Information Sheet

Name ____________________ No:________

Age ______________________

Gender Male / Female

Female: menopauses Yes / No, if yes, for_____year

Marital Status Single, never married / Married / Widowed / Divorced / Separated / other (specify) _________

Education Level No formal education / elementary school / junior high school / high school / college / university / graduated

Smoking Hx Current ____________________________PPY

Past _______________________________PPY

If you ceased smoking, how long? __________

And for what reason?_____________________

Alcohol Hx History of alcohol abuse: Yes / No
### Etiology of heart failure

- Ischemic cardiomyopathy /
- Idiopathic cardiomyopathy /
- Hypertension /
- Other

### Medications:

<table>
<thead>
<tr>
<th>Category</th>
<th>Blank Space</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACEI / A2B</td>
<td></td>
</tr>
<tr>
<td>Diuretics</td>
<td></td>
</tr>
<tr>
<td>β-Blocker</td>
<td></td>
</tr>
<tr>
<td>CCB</td>
<td></td>
</tr>
<tr>
<td>Digitalis</td>
<td></td>
</tr>
<tr>
<td>Anticoagulant</td>
<td></td>
</tr>
<tr>
<td>Lipid-lowering agent</td>
<td></td>
</tr>
<tr>
<td>Hormone Replacement</td>
<td></td>
</tr>
<tr>
<td>Therapy</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td></td>
</tr>
</tbody>
</table>
## Clinical Evaluation

<table>
<thead>
<tr>
<th>Date ______________</th>
<th>Body Height (cm)</th>
<th>____________________</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight (kg)</td>
<td>____________________</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>____________________</td>
<td></td>
</tr>
</tbody>
</table>

### Bedside measurement

| SBP / DBP (mmHg) | ____________________ |
| MAP (mmHg)       | ____________________ |
| HR (bpm)         | ____________________ |
| S3 gallop        | ____________________ |
| Breathing Sound  | ____________________ |
| Jugular Vein Pressure | ____________________ |
| NYHA Classification | I / II / III / IV |

### Laboratory Testing

| Hb                         | ____________________ |
| WBC                        | ____________________ |
| BUN / Serum Creatinine     | ____________________ |
| ALT / AST                  | ____________________ |
| Serum Sodium / Potassium   | ____________________ |
| Serum Calcium              | ____________________ |
| Serum Magnesium            | ____________________ |
| Serum Glucose              | ____________________ |
| T3 / TSH                   | ____________________ |
| Uric Acid                  | ____________________ |
| Total Cholesterol          | ____________________ |
| High density Lipoprotein (HDL) | ____________________ |
| Low Density Lipoprotein (LDL) | ____________________ |
| Triglyceride               | ____________________ |
| TNF-α                      | ____________________ |

### 12-lead Electrocardiogram

| ____________________ |
APPENDIX B

THE NEW YORK HEART ASSOCIATION (NYHA)

HEART FAILURE CLASSIFICATION
## Appendix B. The New York Heart Association (NYHA) Heart Failure Classification

<table>
<thead>
<tr>
<th>Classification</th>
<th>Description</th>
</tr>
</thead>
</table>
| Class I        | Diagnosed with heart failure based on cardiac changes, but having no symptoms regardless of level of activity.  
No limitations.  
Patients can perform to completion any activity up to 7 metabolic equivalents (METS) |
| Class II       | Symptoms (such as fatigue, dyspnea, palpitations, angina) with ordinary activity; slight activity limitation.  
Slight limitation of physical activity.  
Patients can perform to completion any activity up to 5 METS of activity but cannot perform to completion any activities equal to or more than 7 METS. |
| Class III      | Symptoms with less than ordinary activity; no symptoms when at a rest; marked limitation with daily tasks.  
Marked limitation of physical activity.  
Patients can perform to completion any activity up to 2 METS of activity but cannot perform to completion any activities equal to or more than 5 METS. |
| Class IV       | Symptoms with any type of physical activity, or at even a rest.  
Symptomatic at rest.  
Patients cannot perform to completion activities equal to or more than 2 METS. |
APPENDIX C

CHARLSON COMORBIDITY INDEX (CCI)
Appendix  C. Charlson Comorbidity Index (CCI)

<table>
<thead>
<tr>
<th>Comorbidities</th>
<th>Charlson Weights</th>
<th>Deyo-Charlson ICD-9-CM codes</th>
<th>Dartmouth-Manitoba ICD-9-CM codes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocardial infarction</td>
<td>1</td>
<td>410.xx, 412*</td>
<td>410.xx, 412*</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>1</td>
<td>428.x</td>
<td>402.01, 402.11, 402.91, 425.x, 428.x, 429.3</td>
</tr>
<tr>
<td>Peripheral vascular disease</td>
<td>1</td>
<td>441.x*, 443.9*, 785.4*, V43.4*, 38.48(P)</td>
<td>440.x*, 441.x*, 442.x*, 443.1-443.9*, 447.1*, 785.4*, 38.13-38.14(P)<em>, 38.16(P)</em>, 38.18(P)<em>, 38.33-38.34(P)</em>, 38.36(P)<em>, 38.38(P)</em>, 38.43-38.44(P)<em>, 38.46(P)</em>, 38.48(P)<em>, 39.22-39.26(P)</em>, 39.29(P)*</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>1</td>
<td>430-437.x, 438*</td>
<td>362.34, 430-436, 437-437.1, 437.9, 438, 781.4, 784.3, 997.0, 38.12(P), 38.42(P)</td>
</tr>
<tr>
<td>Dementia</td>
<td>1</td>
<td>290.x*</td>
<td>290.x*, 331-331.2*</td>
</tr>
<tr>
<td>Chronic pulmonary disease</td>
<td>1</td>
<td>490-496*, 500-505*, 506.4*</td>
<td>415.0*, 416.8-416.9*, 491.x-494*, 496*</td>
</tr>
<tr>
<td>Rheumatologic disease</td>
<td>1</td>
<td>710.0-710.1*, 710.4*, 714.0-714.2*, 714.81*, 725*</td>
<td>N/A</td>
</tr>
<tr>
<td>Peptic ulcer disease</td>
<td>1</td>
<td>531.4x-531.7x*, 532.4x-532.7x*, 533.4x-533.7x*, 534.4x-534.7x*, 531.0x-531.3x, 532.0x-532.3x, 531.xx-534.xx</td>
<td></td>
</tr>
<tr>
<td>Condition</td>
<td>Code Range</td>
<td></td>
<td></td>
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<tr>
<td>----------------------------------------------</td>
<td>-----------------------------------------</td>
<td></td>
<td></td>
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<tr>
<td>Mild liver disease</td>
<td>533.0x-533.3x, 531.9, 532.9, 533.9, 534.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes (mild to moderate)</td>
<td>250.0x-250.3x*, 250.7x*</td>
<td></td>
<td></td>
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<tr>
<td>Hemiplegia or paraplegia</td>
<td>342.x*, 344.1*</td>
<td>342.x, 344.x</td>
<td></td>
</tr>
<tr>
<td>Moderate or severe renal disease</td>
<td>582.x*, 583.0-583.7*, 585*, 586*, 588.x*</td>
<td>585-586*, V42.0*, V45.1*, V56.x*, 39.27(P)<em>, 39.42(P)</em>, 39.93-39.95(P)<em>, 54.98(P)</em></td>
<td></td>
</tr>
<tr>
<td>Diabetes with complications</td>
<td>250.4x-250.6x*</td>
<td>250.4x-250.9x*</td>
<td></td>
</tr>
<tr>
<td>Moderate or severe liver disease</td>
<td>572.2-582.8*, 456.0-456.2x*</td>
<td>572.2-572.4*, 456.0-456.2x*, 39.1(P)<em>, 42.91(P)</em></td>
<td></td>
</tr>
<tr>
<td>Metastatic solid tumour</td>
<td>196.x-199.x</td>
<td>196.x-199.x</td>
<td></td>
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<tr>
<td>AIDS</td>
<td>042.x-044.x</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>

* Codes with asterisks are included in the definition of comorbidity if they are listed during either index or prior hospital discharges; other codes are included only if recorded prior to the index discharge. Each asterisk applies to all codes within the indicated range.
APPENDIX D

SIDE EFFECTS OF MEGA DOSES OF VITAMIN C CHECKLIST
<table>
<thead>
<tr>
<th>Status</th>
<th>Time Point 1</th>
<th></th>
<th></th>
<th>Time Point 2</th>
<th></th>
<th></th>
<th>Time Point 3</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Y/N</td>
<td>Severity</td>
<td></td>
<td>Y/N</td>
<td>Severity</td>
<td>Relation</td>
<td>Y/N</td>
<td>Severity</td>
<td>Relation</td>
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<tr>
<td>Upset Stomach</td>
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<tr>
<td>nausea or vomiting</td>
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<td>stomach or abdominal cramps</td>
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<td>Diarrhea</td>
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<tr>
<td>Side or low back pain</td>
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<tr>
<td>dizziness or faintness</td>
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<td>flushing or redness of skin</td>
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<td></td>
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<td>headache</td>
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<td></td>
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<tr>
<td>increase in urination (mild)</td>
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<td></td>
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<tr>
<td>Kidney stone</td>
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</tbody>
</table>

Mi: mild; Mo: moderate; Se: Severe; No: not related; Pos: possible related; Def: definitely related
APPENDIX E

MEDICAL OUTCOMES STUDY SHORT-FORM-36 HEALTH SURVEY

(SF-36)

(ENGLISH VERSION)
SF-36(tm) Health Survey

Instructions for completing the questionnaire: Please answer every question. Some questions may look like others, but each one is different. Please take the time to read and answer each question carefully by filling in the bubble that best represents your response.

Patient Name: ____________________________________________

SSN#: ___________________________________  Date: _________________________________

Person helping to complete this form: ____________________________________________

1. In general, would you say your health is:
   - [ ] Excellent
   - [ ] Very good
   - [ ] Good
   - [ ] Fair
   - [ ] Poor

2. Compared to one year ago, how would you rate your health in general now?
   - [ ] Much better now than a year ago
   - [ ] Somewhat better now than a year ago
   - [ ] About the same as one year ago
   - [ ] Somewhat worse now than one year ago
   - [ ] Much worse now than one year ago

3. The following items are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

   a. Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports.
      - [ ] Yes, limited a lot.
      - [ ] Yes, limited a little.
      - [ ] No, not limited at all.

   b. Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf?
      - [ ] Yes, limited a lot.
      - [ ] Yes, limited a little.
      - [ ] No, not limited at all.
c. Lifting or carrying groceries.
   - Yes, limited a lot.
   - Yes, limited a little.
   - No, not limited at all.

d. Climbing several flights of stairs.
   - Yes, limited a lot.
   - Yes, limited a little.
   - No, not limited at all.

e. Climbing one flight of stairs.
   - Yes, limited a lot.
   - Yes, limited a little.
   - No, not limited at all.

f. Bending, kneeling or stooping.
   - Yes, limited a lot.
   - Yes, limited a little.
   - No, not limited at all.

g. Walking more than one mile.
   - Yes, limited a lot.
   - Yes, limited a little.
   - No, not limited at all.

h. Walking several blocks.
   - Yes, limited a lot.
   - Yes, limited a little.
   - No, not limited at all.

i. Walking one block.
   - Yes, limited a lot.
   - Yes, limited a little.
   - No, not limited at all.

j. Bathing or dressing yourself.
   - Yes, limited a lot.
   - Yes, limited a little.
   - No, not limited at all.
4. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

   a. Cut down the amount of time you spent on work or other activities?
      □ Yes
      □ No

   b. Accomplished less than you would like?
      □ Yes
      □ No

   c. Were limited in the kind of work or other activities
      □ Yes
      □ No

   d. Had difficulty performing the work or other activities (for example, it took extra time)
      □ Yes
      □ No

5. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

   a. Cut down the amount of time you spent on work or other activities?
      □ Yes
      □ No

   b. Accomplished less than you would like
      □ Yes
      □ No

   c. Didn't do work or other activities as carefully as usual
      □ Yes
      □ No

6. During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?

      □ Not at all
      □ Slightly
      □ Moderately
      □ Quite a bit
      □ Extremely
7. How much bodily pain have you had during the past 4 weeks?
   - Not at all
   - Slightly
   - Moderately
   - Quite a bit
   - Extremely

8. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?
   - Not at all
   - Slightly
   - Moderately
   - Quite a bit
   - Extremely

9. These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks.
   
   a. did you feel full of pep?
      - All of the time
      - Most of the time
      - A good bit of the time
      - Some of the time
      - A little of the time
      - None of the time

   b. have you been a very nervous person?
      - All of the time
      - Most of the time
      - A good bit of the time
      - Some of the time
      - A little of the time
      - None of the time

   c. have you felt so down in the dumps nothing could cheer you up?
      - All of the time
      - Most of the time
      - A good bit of the time
      - Some of the time
      - A little of the time
      - None of the time
d. have you felt calm and peaceful?
   □ All of the time
   □ Most of the time
   □ A good bit of the time
   □ Some of the time
   □ A little of the time
   □ None of the time

e. did you have a lot of energy?
   □ All of the time
   □ Most of the time
   □ A good bit of the time
   □ Some of the time
   □ A little of the time
   □ None of the time

f. have you felt downhearted and blue?
   □ All of the time
   □ Most of the time
   □ A good bit of the time
   □ Some of the time
   □ A little of the time
   □ None of the time

g. did you feel worn out?
   □ All of the time
   □ Most of the time
   □ A good bit of the time
   □ Some of the time
   □ A little of the time
   □ None of the time

h. have you been a happy person?
   □ All of the time
   □ Most of the time
   □ A good bit of the time
   □ Some of the time
   □ A little of the time
   □ None of the time
i. did you feel tired?
   - All of the time
   - Most of the time
   - A good bit of the time
   - Some of the time
   - A little of the time
   - None of the time

10. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting friends, relatives, etc.)?
   - All of the time
   - Most of the time
   - Some of the time
   - A little of the time
   - None of the time

11. How TRUE or FALSE is each of the following statements for you?

   a. I seem to get sick a little easier than other people
      - Definitely true
      - Mostly true
      - Don't know
      - Mostly false
      - Definitely false

   b. I am as healthy as anybody I know
      - Definitely true
      - Mostly true
      - Don't know
      - Mostly false
      - Definitely false

   c. I expect my health to get worse
      - Definitely true
      - Mostly true
      - Don't know
      - Mostly false
      - Definitely false

   d. My health is excellent
      - Definitely true
      - Mostly true
      - Don't know
      - Mostly false
      - Definitely false
APPENDIX  F

MEDICAL OUTCOMES STUDY SHORT-FORM-36 HEALTH SURVEY

(SF-36)

(CHINESE VERSION)
Appendix  F.  Medical Outcomes Study Short-form-36 Health Survey (SF-36)  
(Chinese version)

SF-36

本調查目的在探討您對自己健康的看法。這些資訊將能幫助您記錄您的感受，以及您在執行日常生活的能力。

敬請回答下列各問題並圈選一適當答案。如您對某一問題的回答不能確定，還是請您盡可能選一個最適合的答案。在本部份所指過去一個月內，係指從今天往前算三十天內。

1. 一般來說，您認為您目前的健康狀況是

（請僅圈選一項答案）

- 極好的................................................................................................................1
- 很好....................................................................................................................2
- 好........................................................................................................................3
- 普通....................................................................................................................4
- 不好....................................................................................................................5

2. 和一年前比較，您認為您目前的健康狀況是？

（請僅圈選一項答案）

- 比一年前好很多................................................................................................1
- 比一年前好一些...............................................................................................2
- 和一年前差不多.............................................................................................3
- 比一年前差一些.............................................................................................4
- 比一年前差很多.............................................................................................5
3. 下面是一些您日常可能从事的活动，请问您目前健康状况会不会限制您从事这些活动？如果会，到底限制有多少？

（每行请仅圈选一项答案）

<table>
<thead>
<tr>
<th>活动</th>
<th>会，受到很多限制</th>
<th>会，受到一些限制</th>
<th>不会，完全不受限制</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. 费力活动，例如跑步、提重物、参与剧烈运动</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>b. 中等程度活动，例如搬桌子、拖地板、打保龄球、或打太极拳</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>c. 提起或携带食品杂货</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>d. 爬数层楼梯</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>e. 爬一层数楼梯</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>f. 弯腰、跪下或蹲下</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>g. 走路超过1公里</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>h. 走过数个街口</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>i. 走过一个街口</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>j. 自己洗澡或穿衣</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

4. 在过去一个月内，您是否曾因为身体健康问题，而在工作上或其他日常活动方面有下列任何的问题？

（每行请仅圈选一项答案）

<table>
<thead>
<tr>
<th>问题</th>
<th>是</th>
<th>否</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. 做工作或其它活动的时间减少</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>b. 完成的工作量比您想要完成的较少</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>c. 可以做的工作或其他活动的种类受到限制</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>d. 做工作或其他活动有困难（例如，须更吃力）</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>
5. 在過去一個月內，您是否曾因為情緒問題(例如，感覺沮喪或焦慮)，而在工作上或其他日常活動方面有下列的問題？

(每行請僅圈選一項答案)

<table>
<thead>
<tr>
<th></th>
<th>是</th>
<th>否</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. 做工作或其它活動的時間減少</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>b. 完成的工作量比您想要完成的較少</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>c. 做工作或其它活動時不如以往小心</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

6. 在過去一個月內，您的健康或情緒問題，對您與家人或朋友、鄰居、社團間的平常活動的妨礙程度如何？

(請僅圈選一項答案)

完全沒有妨礙........................................................................................................1
有一點妨礙........................................................................................................2
中度妨礙........................................................................................................3
相當多妨礙........................................................................................................4
妨礙到極點........................................................................................................5

7. 在過去一個月內，您身體疼痛程度有多嚴重？

(請僅圈選一項答案)

完全不痛............................................................................................................1
非常輕微的痛....................................................................................................2
輕微的痛............................................................................................................3
中度的痛............................................................................................................4
嚴重的痛............................................................................................................5
非常嚴重的痛..............................................................................................6
8. 在過去一個月內，身體疼痛對您的日常工作(包括上班及家務)妨礙程度如何？

(請僅圈選一項答案)

<table>
<thead>
<tr>
<th></th>
<th>完全沒有妨礙</th>
<th>有一點妨礙</th>
<th>中度妨礙</th>
<th>相當多妨礙</th>
<th>妨礙到極點</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

9. 下列各項問題是關於過去一個月內您的感覺及您對周遭生活的感受，請針對每一問題選一最接近您感覺的答案。在過去一個月中有多少時候......

(每行請僅圈選一項答案)

<table>
<thead>
<tr>
<th></th>
<th>一直都 是</th>
<th>大部分 時間</th>
<th>經常</th>
<th>有時</th>
<th>很少</th>
<th>從不</th>
</tr>
</thead>
<tbody>
<tr>
<td>a.您覺得充滿活力？</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>b.您是一個非常緊張的人？</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>c.您覺得非常沮喪，沒有任何事情可以讓您高興起來？</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>d.您覺得心情平靜？</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>e.您精力充沛？</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>f.您覺得悶悶不樂和憂鬱？</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>g.您覺得筋疲力竭？</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>h.您是一個快樂的人？</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>i.您覺得累？</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
</tbody>
</table>
10. 在過去一個月內，您的身體健康或情緒問題有多少時候會妨礙您的社交活動（如拜訪親友等）？

（請僅圈選一項答案）

一直都會.................................................................................................................1
大部分時間會.........................................................................................................2
有時候會.................................................................................................................3
很少會.....................................................................................................................4
從不會.....................................................................................................................5

11.下列各個陳述對您來說有多正確？

（每行請僅圈選一項答案）

<table>
<thead>
<tr>
<th></th>
<th>完全正確</th>
<th>大部分正確</th>
<th>不知道</th>
<th>大部分不正確</th>
<th>完全不正確</th>
</tr>
</thead>
<tbody>
<tr>
<td>a.我好像比別人較容易生病</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b.和任何一個我認識的人來比，我和他們一樣健康。</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c.我想我的健康會越來越壞</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d.我的健康狀況好得很</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX G

MINNESOTA LIVING WITH HEART FAILURE QUESTIONNAIRE

(MLHFQ)

(ENGLISH VERSION)
Appendix  G.  Minnesota Living with Heart Failure Questionnaire (English version)

LIVING WITH HEART FAILURE® QUESTIONNAIRE

These questions concern how your heart failure (heart condition) has prevented you from living as you wanted during the last month. The items listed below describe different ways some people are affected. If you are not sure an item does not apply to you or is not related to your heart failure then circle 0 (No) and go on the next item. If an item does apply to you, then circle the number rating how much it prevented you from living as you wanted.

<table>
<thead>
<tr>
<th>Did your heart failure prevent you from living as you wanted during the last month by:</th>
<th>No</th>
<th>Very Little</th>
<th>Very Much</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. causing swelling in your ankles, legs, etc?</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>2. making you sit or lie down to rest during the days?</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>3. making you walking about or climbing stairs difficult?</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>4. making you working around the house or yard difficult?</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>5. making you going places away from home difficult?</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>6. making your sleeping well at night difficult?</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>7. making your relating to or doing things with your friends or family difficult?</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>8. making your working to earn a living difficult?</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>9. making your recreational pastimes, sports or hobbies difficult?</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>10. making your sexual activities difficult?</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>11. making you eat less of the foods you like?</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>12. making you short of breath</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>13. making you tired, fatigued, or low on energy?</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>14. making you stay in a hospital?</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>15. costing you money for medical care?</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>16. giving you side effects from medication?</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>17. making you feel you are a burden to your family or friends?</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>18. making you feel a loss or self-control in your life?</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>19. making you worry?</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>20. making a difficult for you to concentrate or remember things?</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>21. making you feel depressed?</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

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APPENDIX H

MINNESOTA LIVING WITH HEART FAILURE QUESTIONNAIRE

(MLHFQ)

(CHINESE VERSION)
Appendix  H.  Minnesota Living with Heart Failure Questionnaire (Chinese version)

美國明尼蘇達心臟衰竭量表

以下這些問題是要調查，在過去一個月中，是否因為您的心臟功能影響了您想要過的生活方式。這些問題描述了各種可能影響您生活方式的症狀或情形。對於不適用於您的問題，請圈 0 (不曾影響)，然後繼續答下一題;如果您也有相同情況的問題，請依生活方式受影響的程度，圈選一個您覺得最適當的數字。

<table>
<thead>
<tr>
<th>在上個月，您的心臟狀況是否曾經引發下列情形而阻礙了您想要過的生活方式：</th>
<th>不曾影響</th>
<th>些微影響</th>
<th>嚴重影響</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 引起您的腿或腳踝等處腫脹？</td>
<td>0 1 2 3 4 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. 使您白天需要坐下或躺下來休息？</td>
<td>0 1 2 3 4 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. 造成您走路或爬樓梯有困難？</td>
<td>0 1 2 3 4 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. 造成您做家務有困難？</td>
<td>0 1 2 3 4 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. 使您外出有困難？</td>
<td>0 1 2 3 4 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. 使您晚上睡不好？</td>
<td>0 1 2 3 4 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. 影響您和家人或朋友之間的往來？</td>
<td>0 1 2 3 4 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. 造成您工作謀生的困難？</td>
<td>0 1 2 3 4 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. 使您有困難從事娛樂、消遣、嗜好或做運動？</td>
<td>0 1 2 3 4 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. 影響您的性生活？</td>
<td>0 1 2 3 4 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11. 使您吃比較少您喜歡的食物？</td>
<td>0 1 2 3 4 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12. 使您喘不過氣來？</td>
<td>0 1 2 3 4 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13. 使您覺得累，精疲力竭或無精打采？</td>
<td>0 1 2 3 4 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14. 造成您要住院？</td>
<td>0 1 2 3 4 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15. 造成您醫療上的花費？</td>
<td>0 1 2 3 4 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16. 造成因使用藥物而產生的副作用？</td>
<td>0 1 2 3 4 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17. 使您覺得您是家人或朋友的累贅？</td>
<td>0 1 2 3 4 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18. 使您覺得失去生活上的自主能力？</td>
<td>0 1 2 3 4 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19. 使您擔憂？</td>
<td>0 1 2 3 4 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20. 造成您注意力不集中或健忘？</td>
<td>0 1 2 3 4 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21. 使您覺得沮喪？</td>
<td>0 1 2 3 4 5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

明尼蘇達州大學著作權 1986
APPENDIX I

THE CENTER FOR EPIDEMIOLOGIC STUDIES DEPRESSION SCALE

(CES-D)

(ENGLISH VERSION)
Appendix I. The Center for Epidemiologic Studies Depression Scale (CES-D)

(English version)

Mood Scale (CES-D)

Below is a list of some of the ways you may have felt or behaved. Please indicate how often you have felt this way during the past week by checking the appropriate space.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>Rarely or none of the time (less than 1 day)</th>
<th>Some or a little of the time (1-2 days)</th>
<th>Occasionally or a moderate amount of the time (3-4 days)</th>
<th>All of the time (5-7 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>I was bothered by things that usually don't bother me.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>I did not feel like eating; my appetite was poor.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>I felt that I could not shake off the blues even with help from my family.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>I felt that I was just as good as other people.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>I had trouble keeping my mind on what I was doing.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>I felt depressed.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>I felt that everything I did was an effort.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>I felt hopeful about the future.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>I thought my life had been a failure.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td>I felt fearful.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11.</td>
<td>My sleep was restless.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.</td>
<td>I was happy.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13.</td>
<td>I talked less than usual.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15.</td>
<td>People were unfriendly.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16.</td>
<td>I enjoyed life.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17.</td>
<td>I had crying spells.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18.</td>
<td>I felt sad.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19.</td>
<td>I felt that people disliked me.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20.</td>
<td>I could not get &quot;going.&quot;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX J

THE CENTER FOR EPIDEMIOLOGIC STUDIES DEPRESSION SCALE

(CES-D)

(CHINESE VERSION)
Appendix J. The Center for Epidemiologic Studies Depression Scale (CES-D)

(Chinese version)

以下的問題是要瞭解過去一週內對自己情緒感受或是行為的主觀感覺，請勾選出最適合描述您感受程度的答案。

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>原來不介意的事，最近竟然會困擾我</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>我的胃口不好，不想吃東西</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>即使有親友幫忙，我還是無法拋開煩惱</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>我覺得我和別人一樣好</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>我做事無法集中精神</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>我覺得悶悶不樂</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>我做任何事都覺得費力</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>我對未來充滿希望</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>我認為我的人生是失敗的</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>我覺得恐懼</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>11</td>
<td>我睡得不安寧</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>12</td>
<td>我是快樂的</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>13</td>
<td>我比平日不愛講話</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>14</td>
<td>我覺得寂寞</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>15</td>
<td>人們是不友善的</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>16</td>
<td>我享受了生活的樂趣</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>17</td>
<td>我曾經痛哭</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>18</td>
<td>我覺得悲傷</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>19</td>
<td>我覺得別人不喜歡我</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>20</td>
<td>我缺乏幹勁</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>
APPENDIX K

INFORMED CONSENT

(ENGLISH VERSION)
UNIVERSITY HOSPITALS OF CLEVELAND
CONSENT FOR INVESTIGATIONAL STUDIES

Project Title: Effects of Antioxidant on Cardiovascular Performance, Exercise Capacity, and Functional Status in Patients with Chronic Heart Failure

Principal Investigator: Chao-Chung Ho

Introduction/Purpose

You are being asked to participate in a research study of supplemental antioxidants (Vitamins C) because you have been diagnosed of heart failure.

The purpose of this study is to see if taking supplemental antioxidants will increase your heart function, exercise ability and health status. Sixty (60) patients will participate in this study.

Study Procedures

After agreeing to participate in this study, you will be assigned to one of two groups by chance using a process similar to the flip of a coin. This process is called randomization. Neither you nor the study researcher will select the group to which you will be assigned. No matter to which group you are assigned, you will take Vitamin C and placebo (a placebo is an inactive substance containing no medication). The difference is the sequence in which you will take them.

The two groups to which you may be assigned are:

Group 1: Vitamin C 2000 mg twice daily followed by placebo
Group 2: Placebo followed by Vitamin 2000 mg twice daily

Your pills, whether placebo or vitamin C, will be provided in a bottle. You take them at 9 a.m. and 9 p.m. everyday. Other than the vitamin C or
placebo, management of your heart failure will be your usual care. Your participation in this study will last for 10 weeks and involve 3 visits. If you feel any discomfort might be related to vitamin C or placebo, please do not hesitate to contact with Chao-Chung Ho or Dr. Chuan-Chieh Liu.

As a participant in this study, you will be asked to come to the echocardiogram room, which is located in the Building C 2nd floor at 8 a.m. on three days. You can not eat anything after midnight on these days until after your test.

Some medications will be held on your physician’s direction so that accurate measurement of endothelial function can be made. You will take your medications after you complete your test. Some food may influence results of exam, such as alcohol, caffeine, or using tobacco. You will not be able to use them for at least 4 to 6 hours before the study. In the first visit, the physician and principal investigator will conduct a couple of tests that include:

(1) Biochemical test: You will have 10 ml (less than 1 tablespoon) of blood withdrawn 3 times (before the study, the 4th and 10th week after you begin participation). The total amount of blood drawn for the entire study will be 30 ml (2 tablespoon).

(2) Echocardiogram and endothelial function test: They are not invasive examinations to evaluate your heart and blood vessel function. While echocardiogram is performed, you are in either a supine, left lateral, or semi-Fowler’s position. Which position is used depends on your clinical condition and on which position can provides the best view of the structures examined. A transducer will be placed on your chest for imaging the heart. After echocardiogram, the endothelial function test will be performed. You will maintain in the same position. Initially a blood pressure cuff is placed on your forearm and then the cuff is inflated for 5 minutes and the transducer will be placed in your antecubital fossa. The sublingual NTG will be administrated in this procedure.

(3) Exercise ability test: You will be asked walking as long as you can in 6 minutes, if you feel tired or any discomfort, you can stop in anytime.

(4) Questionnaires: You will be asked to complete 3 questionnaires regarding health status and mood status, they will take about 30 minutes to complete.
The same procedures will be performed at visit 2 (4th week) and visit 3 (10th week).

If you withdraw from the study prior to its completion, you will be asked to return all study medication and, for your safety, come in for a final clinical visit.

The study drug must be taken only by you. It must be kept in a safe place out of reach of children and other people who cannot read well or understand that they should not take it.

**Risks**

Your participation in this study may involve the following risks:

*Vitamin C*
At dose greater than 1000 mg per day, some individuals develop diarrhea, kidney stone, and change of menstrual cycle. Some people who suddenly stop taking high dose of Vitamin C may develop rebound scurvy including sores in their mouths.

*Biochemical test (blood drawn)*
The insertion of the needle to draw blood is painful; however, this discomfort is brief. For most people, needle punctures to get blood samples do not cause any serious problems; however, they may cause bleeding, bruising, discomfort, infections, dizziness, or fainting.

*Questionnaires*
Some of the questions may be upsetting, or you may feel uncomfortable answering them. If you do not wish to answer a question, you may skip it and go to the next question.

We cannot predict all risks or potential side effects.

**Benefits**

There will be no direct benefit to you by your participation in this research study. Your participation may contribute to the development of better clinical care of patients with heart failure in the future.
Alternatives to Participation

If you do not participate in this study, you will receive standard treatment as prescribed by your physician.

Financial Information

You will not be paid for your participation in this study. The study will pay for all the cost of the echocardiogram, exercise ability test, and supplemental antioxidants and placebo that you will receive.

Confidentiality

The confidentiality of participants will be protected by putting all data in a locked file. The data will be entered in the computer. Nobody except the researcher will know the password. The original questionnaire will be locked in a secure place. The data will be in storage for 5 years and then will be destroyed.

Summary of your rights as a participant in a research study

Your participation in this research study is voluntary. Refusing to participate will not alter your usual health care or involve any penalty or loss of benefits to which you are otherwise entitled. If you decide to join the study, you may withdraw at any time and for any reason without penalty or loss of benefits. If information generated from this study is published or presented, your identity will not be revealed. In the event new information becomes available that may affect the risks or benefits associated with this study or your willingness to participate in it, you will be notified so that you can decide whether or not to continue participating. If you experience physical injury or illness as a result of participating in this research study, medical care is available at University Hospitals of Cleveland (UHC) or elsewhere; however, UHC will not provide free care or compensation for lost wages.

Disclosure of your study records

You authorize the Principal Investigator and other investigators, Cardinal
Tien Hospital, UHC, Case Western Reserve University and their employees
to use and disclose information concerning you and your identity, medical
history and information collected during this study for the following purpose:
For audits of this research project. Such information may also be disclosed
or used by others involved in or overseeing the study including the UHC
Institutional Review Board, the study sponsor and its agents, as well as U.S.,
European, your and other governmental, regulatory and accrediting agencies.
Foreign laws governing privacy, use and disclosure of health information
may provide less protection than the laws of your country. Once disclosed
your information may be redisclosed by others who are not required to
maintain the privacy of your information. You may withdraw authorization
to collect additional information about you at any time by writing to the
local Principal Investigator, but information already collected may be
continue to be used and disclosed. This authorization has no expiration
date.

Efforts will be made to keep the personal information in your research
record private and confidential, but absolute confidentiality cannot be
guaranteed. The University Hospitals of Cleveland Institutional Review
Board may review your study records. If this is a treatment study, the
records must be available to the Food and Drug Administration, the study
sponsor, and possibly foreign regulatory agencies. If your records are
reviewed your identity could become known.

Contact information

Chao-Chung Ho, Dr. Chuan-Chieh Liu and Dr. John M. Clochesy have
described to you what is going to be done, the risks, hazards, and benefits
involved. If you have any other question, please contact Chao-Chung Ho &
Dr. Chuan-Chieh Liu. (Cell Phone: 0920-552-522 or Cath Lab
+886-2-2219-3391x65252)

Further information with respect to illness or injury resulting from a research
procedure as well as a research subjects' rights is available from the Office
of the Chief Medical Officer at (216) 844-3695. If you would like to talk to
someone other than the researcher(s) about; (1) concerns regarding this
study, (2) research participant rights, (3) research-related injuries, or (4)
other human subject issues, please contact Cardinal Tien Hospital’s
Institutional Review Board at 886-2-2219-3391 or write: Cardinal Tien
Hospital; Institutional Review Board; No. 362, Chung-Jeng Rd., Hsintein, Taipei 231, Taiwan.

Signature

Signing below indicates that you have been informed about the research study in which you voluntarily agree to participate; that you have asked any questions about the study that you may have; and that the information given to you has permitted you to make a fully informed and free decision about your participation in the study. By signing this consent form, you do not waive any legal rights, and the investigator(s) or sponsor(s) are not relieved of any liability they may have. A copy of this consent form will be provided to you.

________________________________    Date :____________________
Signature of Participant

________________________________    Date :____________________
Signature of Person Obtaining Consent

Printed Name of Person Obtaining Consent  Chao-Chung Ho

Date :____________________

Date :
Signature of Principal Investigator (Affirming subject eligibility for then study and that informed consent has been obtained.)
APPENDIX L

INFORMED CONSENT

(CHINESE VERSION)
人體試驗受試者說明及同意書

您被邀請參與此研究。本表格提供您有關本研究之相關資訊，研究主持人或其他協同主持醫師將會為您說明研究內容並回答您的任何疑問。

計畫名稱：抗氧化藥物對心衰竭病患心臟血管功能運動能力以及健康狀況的影響

醫院名稱：天主教耕莘醫院
電話：(02) 2219-3391

計畫主持人：劉傳捷
職稱：主治醫師
電話：分機65252

緊急聯絡人：何昭中
職稱：護理師
電話：0920552522

受試者姓名：
性別：
年齡：
病歷號碼：

通訊地址：
電話：

法定代理人姓名：
性別：
年齡：

通訊地址：
電話：

（醫療法第五十七條規定：受試者為無行為能力或限制行為能力人，應得其法定代理人之同意）

一、試驗目的及方法：本研究的目的是在探討抗氧化劑對心衰竭病患心臟血管功能運動能力以及健康狀況的影響。如您同意參加本研究，則您在心臟科約診時，會被要求進行以下的步驟：(1) 在參加本研究的開始及第四週與第十週（共三次）均需空腹抽血約10 ml；有部份與心臟有關的藥物可能需依醫師指示先暫時停用，待檢查完畢再服用，以免影響檢查的結果；(2) 研究者會陪同您進行心臟及血管超音波檢查，並同時記錄您的資料。此二項檢查會同時進行，是為非侵入性的檢查，會依您的狀況採左側臥或半坐臥姿勢，醫師會將超音波儀器探頭放在您的前胸及手臂，並將血壓計的壓脈帶綁上，充氣5分鐘，並在過程中讓您在舌下服用一顆硝化甘油（NTG），以評估您心臟的情形以及血流的狀況。在此過程中，您不會感到任何的不適；(3) 研究者會陪同您進行運動功能檢查，並同時記錄您的資料；(4) 填寫有關健康狀況的量表，大約需要20分鐘；(5) 您將會被隨機分到不同的組別，一組是先服用維生素C，接下來再服用安慰劑，另一組
為先使用安慰劑一段時間後，再使用維生素C，無論在那一組都有機會服用維生素C及安慰劑，只是順序先後的不同而已。在本研究中會持續評估您的心臟功能、運動能力以及健康狀況，一次在參與本研究後的第四週，另一次在本研究結束的第十週。除了維生素C及安慰劑外，您的所有的有關心臟的藥物都與未參加本研究之前相同，並不因本研究而有所改變。

二、預期醫療效能：促進心臟血管功能、增強運動能力以及提昇生活品質

三、可能產生之併發症、副作用、危險及其處理方法：本研究的可能危險性如下：
(1) 維生素C在高劑量的使用下，有可能會出現腹瀉、腎結石或是女性可能會影響到月經週期；
(2) 抽血會造成輕微的疼痛，或是偶會有血腫出現；
(3) 運動檢查可能會讓您覺得疲累；
(4) 心臟超音波是一種非侵入性的檢查，至目前為止並無已知的副作用。如果在研究期間有副作用或疑似藥物副作用發生，請隨時與劉傳捷醫師或何昭中護理師連絡，連絡電話於同意書首頁。

四、其他可能之治療方法及說明：無

五、試驗經費來源及所有參與試驗之機構：本研究所有的項目都不需您額外的花費
六、受試者應注意事項：在受檢該日禁食早餐，包含藥物在內，在受檢完即可服用早餐及補服該日的藥物。

七、基因治療人體試驗特別注意事項：
（一）為了下一代的健康，貴受試者需於試驗期間或醫院告知之期間，採行避孕措施。
（二）為評估基因治療的長期安全性和醫療效能，請貴受試者提供聯絡方式，以便安排長期後續追蹤，並請惠予配合相關追蹤檢查（如抽血、病理檢查等）。
（三）其他：無。

八、本試驗受試者之權益將受到下列保護：
（一）醫院將盡力維護貴受試者在試驗施行期間之權益，並善盡醫療上必要之注意。
（二）本試驗已經得到醫院人體試驗審議委員會審查通過，該委員會的審查重點即對受試者是否有適當的保護。
（三）試驗所獲得資料之使用或發表，醫院將對受試者之隱私（例如：姓名、得以辨識受試者身分之照片等資料）絕對保密。
（四）貴受試者於試驗施行期間中，可隨時無條件撤回同意，退出試驗。但退出試驗後，仍得要求醫院提供與受試者已接受之試驗相關之必要追蹤檢查。
（五）受試者退出試驗，將不影響醫病關係或任何醫療上的正當權益。
（六）施行試驗期間之相關醫療費用，均為免費；該項人體試驗於行政院衛生署公告開放為常規醫療前之追蹤檢查費用，亦為免費。但與試驗有關之常規醫療，並經事先告知受試者，取得其同意者，不在此限。
（七）使用醫療器材或製品所致傷害，將由廠商或製造人負責；因醫療行為所致傷害，由醫院負責。

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九、其他與試驗有關之重要事項：

十、經本試驗計畫主持人或其代理人向本人說明上列事項後，本人已明瞭其內容；有關本試驗之疑問，亦得到詳細解答，本人係在完全自主，未被詐欺、脅迫或利誘之情形下，同意參加本試驗，並知悉本人在試驗期間有權隨時無條件退出試驗，且試驗內容如有變更，將先取得本人同意。

受試者簽名：

日期：

（法定代理人簽名：

日期：）

計畫主持人或其代理人簽名：

十一、為研究或學術之需要，本人□同意，□不同意身後接受病理解剖。

受試者簽名：

日期：

（法定代理人簽名：

日期：）

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APPENDIX M

PERMUTED BLOCK RANDOMIZATION
Appendix  M.  Permuted Block Randomization

Effects of Antioxidant in Patients with Chronic Heart Failure

1:1 allocation, block randomization

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Effects of Antioxidant in Patients with Chronic Heart Failure

1:1 allocation, block randomization

07:46 Tuesday, September 27, 2005

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